

# Abnormal contraction caused by expression of G<sub>i</sub>-coupled receptor in transgenic model of dilated cardiomyopathy

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Departments of Radiology,<sup>1</sup> Medicine,<sup>2</sup> and Pharmacology,<sup>3</sup> Cardiovascular Research Institute,<sup>4</sup>  
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**Baker, Anthony J., Charles H. Redfern, Mark D. Harwood, Paul C. Simpson, and Bruce R. Conklin.** Abnormal contraction caused by expression of G<sub>i</sub>-coupled receptor in transgenic model of dilated cardiomyopathy *Am J Physiol Heart Circ Physiol* 280: H1653–H1659, 2001.—Although increased G<sub>i</sub> signaling has been associated with dilated cardiomyopathy in humans, its role is not clear. Our goal was to determine the effects of chronically increased G<sub>i</sub> signaling on myocardial function. We studied transgenic mice that expressed a G<sub>i</sub>-coupled receptor (Ro1) that was targeted to the heart and regulated by a tetracycline-controlled expression system. Ro1 expression for 8 wk resulted in abnormal contractions of right ventricular muscle strips *in vitro*. Ro1 expression reduced myocardial force by >60% (from 35 ± 3 to 13 ± 2 mN/mm<sup>2</sup>, *P* < 0.001). Nevertheless, sensitivity to extracellular Ca<sup>2+</sup> was enhanced. The extracellular [Ca<sup>2+</sup>] resulting in half-maximal force was lower with Ro1 expression compared with control (0.41 ± 0.05 vs. 0.88 ± 0.05 mM, *P* < 0.001). Ro1 expression slowed both contraction and relaxation kinetics, increasing the twitch time to peak (143 ± 6 vs. 100 ± 4 ms in control, *P* < 0.001) and the time to half relaxation (124 ± 6 vs. 75 ± 6 ms in control, *P* < 0.001). Increased pacing frequency increased contractile force three-fold in control myocardium (*P* < 0.001) but caused no increase of force in Ro1-expressing myocardium. When stimulation was interrupted with rests, postrest force increased in control myocardium, but there was postrest decay of force in Ro1-expressing myocardium. These results suggest that defects in contractility mediated by G<sub>i</sub> signaling may contribute to the development of dilated cardiomyopathy.

signaling; calcium; G proteins; mouse

G PROTEIN-COUPLED RECEPTORS are a large class of cell-surface receptors that activate specific G protein pathways. The best studied G protein pathways are G<sub>i</sub>, G<sub>s</sub>, and G<sub>q</sub>, which inhibit adenylyl cyclase, stimulate adenylyl cyclase, and stimulate phospholipase C, respectively. In the heart, altered signaling through each of these G protein pathways has been implicated in cardiac disease (1, 10, 21, 24).

Dilated cardiomyopathy (DCM) is a major cause of heart failure and is characterized by cardiac dilation

and reduced systolic function (17). Idiopathic DCM accounts for almost half of all DCM cases (12). Despite being a major source of morbidity and mortality, the molecular basis of DCM is unclear. Idiopathic DCM in humans is associated with increased G<sub>i</sub> protein levels (22, 23), increased G<sub>i</sub> signaling (5, 9, 22), and autoantibodies that activate signaling by G<sub>i</sub>-coupled receptors (6, 8, 14). These findings suggest that increased G<sub>i</sub> signaling may play a role in DCM. Consistent with this, we recently found that conditional expression of a G<sub>i</sub>-coupled receptor in the adult transgenic mouse heart resulted in a lethal DCM (21). Use of pertussis toxin (to inhibit G<sub>i</sub> signaling) indicated specific effects due to G<sub>i</sub> signaling rather than nonspecific effects of receptor expression. Taken together, these findings implicate increased G<sub>i</sub> signaling as a key factor mediating the development of a form of DCM.

Our new mouse model of DCM induced by expression of a G<sub>i</sub>-coupled receptor offers an opportunity to understand the mechanisms by which increased G<sub>i</sub> signaling can lead to cardiac disease. One mechanism by which increased G<sub>i</sub> signaling in the heart may be deleterious is through inducing abnormalities of myocardial contractility. In addition, this model also leads to ventricular conduction delay, a common feature of cardiomyopathy and associated with a poor prognosis (21). Therefore, to better understand the interaction between increased G<sub>i</sub> signaling and the function of the myocardium, the goal of this study was to determine the influence of expression of this G<sub>i</sub>-coupled receptor on the intrinsic contractile properties of the myocardium. The experimental approach was to study *in vitro* the contraction of small papillary muscles and trabeculae isolated from the right ventricle (RV). Contractile function was evaluated before the appearance of overt heart failure. We found that expression of the G<sub>i</sub>-coupled receptor (RO1) was associated with several major abnormalities of contraction and relaxation. These findings suggest that increased G<sub>i</sub> signaling leads to several intrinsic defects in myocardial contractility. These findings also suggest that with increased

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G<sub>i</sub> signaling impaired myocardial contractility may be an important factor in the development of DCM.

## METHODS

**Transgenic mouse model.** The transgenic mouse model of DCM has been recently described (21). Briefly, conditional expression of a cardiac-targeted G<sub>i</sub>-coupled receptor (termed Ro1) was induced in the adult mouse heart by using a tetracycline-controlled expression system (26). Mice were generated with the cardiac-specific  $\alpha$ -myosin heavy chain ( $\alpha$ -MHC) promoter driving the tetracycline transactivator ( $\alpha$ -MHC-tTA) and tetO-Ro1 transgene constructs (3, 20). Doxycycline (200  $\mu$ g/ml) added to the drinking water suppressed tTA-dependent transactivation; withdrawal of doxycycline induces Ro1 expression. All  $\alpha$ -MHC-tTA/tetO-Ro1 mice were backcrossed for at least seven generations into an FVB/N background.

Chronic Ro1 expression results in a lethal DCM (>90% mortality at 16 wk) with clinical signs of cardiomyopathy commonly occurring a few days before death (21). However, before the appearance of end-stage clinical signs of distress, mice appear otherwise healthy. In the present and previous study, we assessed myocardial function by using mice at 8 wk of Ro1 expression. Although 8 wk of Ro1 expression is associated with 40% mortality, the mice that were used in this study had not yet developed clinical signs of end-stage cardiomyopathy. The role of G<sub>i</sub> signaling in the cardiomyopathy caused by Ro1 expression was demonstrated by blocking the cardiomyopathy with an antagonist to the receptor and by inhibiting G<sub>i</sub> signaling with pertussis toxin (21).

**Muscle preparation and solutions.** All experiments were performed at 22°C, because a recent study found mouse muscle strips to be stable in vitro for several hours at 22°C but to deteriorate at 37°C (7). Male mice (~35 g,  $n = 18$ ) were anticoagulated with heparin sodium (1,000 U/kg ip) and deeply anesthetized with pentobarbital sodium (100 mg/kg ip). Hearts were removed and were exsanguinated by aortic perfusion for several minutes with a dissection solution. The dissection solution consisted of a modified Krebs-Henseleit solution containing (in mM) 116 NaCl, 5 KCl, 1.2 MgCl<sub>2</sub>, 1.2 Na<sub>2</sub>SO<sub>4</sub>, 2 NaH<sub>2</sub>PO<sub>4</sub>, 20 NaHCO<sub>3</sub>, 10 glucose, and CaCl<sub>2</sub>, which was varied as indicated in the text. Solutions were equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> to obtain a pH of 7.4. To minimize spontaneous contractions during dissection, the dissection solution also contained 10 mM butane-dione monoxime, an additional 20 mM KCl, and [Ca<sup>2+</sup>] was 0.1 mM (whereas residual effects of anesthetics and drugs are a concern, these effects would impact both control and experimental groups).

The RV was opened and a cube of myocardium containing a papillary muscle was removed. RV papillary muscles were available in all hearts studied (an important consideration given the effort generating binary transgenic mice with timed doxycycline dosing). The muscle dimensions were measured with an ocular micrometer in the dissection microscope. The dimensions of RV papillary muscles from control and Ro1-expressing hearts were not significantly different ( $P > 0.05$ ): length  $1.32 \pm 0.3$  mm (control) vs.  $1.28 \pm 0.11$  mm (Ro1); width  $0.49 \pm 0.02$  mm (control) vs.  $0.54 \pm 0.03$  mm (Ro1); thickness  $0.25 \pm 0.03$  mm (control) vs.  $0.32 \pm 0.03$  mm (Ro1). For two control experiments, trabeculae were used. As previously noted, trabeculae occurred infrequently (7). Trabeculae (control,  $n = 2$ ) were smaller 0.075–0.078 mm wide by 0.071–0.078 mm thick, but the muscle properties were similar to those of control RV papillary muscles.

The cube of ventricle was held in a basket made of platinum wire (36 gauge) attached to a strain gauge (AE801, SensoNor; Horten, Norway). The other end of the muscle, a remnant of the tricuspid valve, was mounted on a stainless steel pin (100  $\mu$ m diameter) attached to a position-sensitive motor (model 308B, Cambridge Technology; Watertown, MA). Muscles were mounted in a muscle bath ( $3 \times 3 \times 15$  mm) and superfused with Krebs-Henseleit solution ( $\approx 5$  ml/min).

**Experimental protocol.** The mechanical properties of muscle strips from transgenic mouse hearts were determined after 8 wk of Ro1 expression ( $n = 9$ ). Controls consisted of muscle strips from mice that contained the tetracycline transactivator but lacked the Ro1 transgene and that received the same doxycycline treatments as Ro1-expressing mice ( $n = 9$ ).

Muscles were initially superfused with Krebs-Henseleit solution with [Ca<sup>2+</sup>] set to 0.1 mM. The [Ca<sup>2+</sup>] was gradually raised to 0.5 mM over 15 min. Muscles were field stimulated using platinum wire electrodes in the side walls of the chamber. Stimulation consisted of 4-ms stimulus pulses with the voltage set to 50% above that giving maximum tension; stimulation frequency was 1 Hz. Muscle length was adjusted to maximize the twitch tension, and stimulated muscles were allowed to equilibrate for 1 h. After equilibration at 0.5 mM [Ca<sup>2+</sup>], the mechanical properties were assessed over a range of extracellular Ca<sup>2+</sup> levels, stimulation frequencies, and rest intervals. Signals from the tension transducer were digitized at 1,000 Hz and stored in a laboratory computer.

**Data analysis and statistics.** Pooled data are presented as means  $\pm$  SE of control and experimental groups ( $n = 9$  in each group). The statistical significance of differences between results from control and Ro1-expressing mice was determined by *t*-test and ANOVA. The level of significance was set at  $P < 0.05$ .

## RESULTS

The goal of this study was to determine the influence of expression of the G<sub>i</sub>-coupled receptor Ro1 on the intrinsic contractile properties of the myocardium in a transgenic mouse model of DCM. Contractile function was assessed in vitro by using small isolated RV papillary muscles and trabeculae.

**Force development.** Figure 1A shows superimposed isometric twitches recorded from RV papillary muscles from control mice and mice expressing the G<sub>i</sub>-coupled receptor Ro1 (1.5 mM extracellular [Ca<sup>2+</sup>]). Ro1 expression resulted in a profound decrease in force devel-

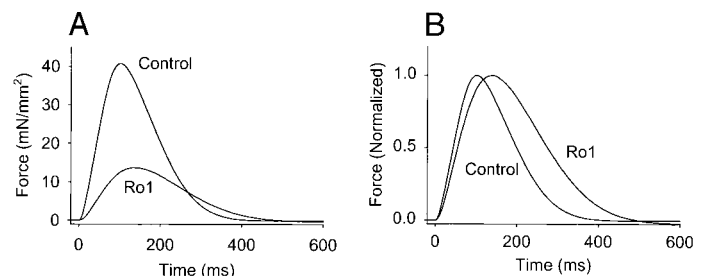


Fig. 1. Contractile force of mouse right ventricular papillary muscles from control and Ro1-expressing mice (1.5 mM extracellular [Ca<sup>2+</sup>]). A: expression of Ro1 markedly reduced developed force. B: twitch contractions, normalized to peak force. Ro1 expression markedly slowed twitch kinetics.

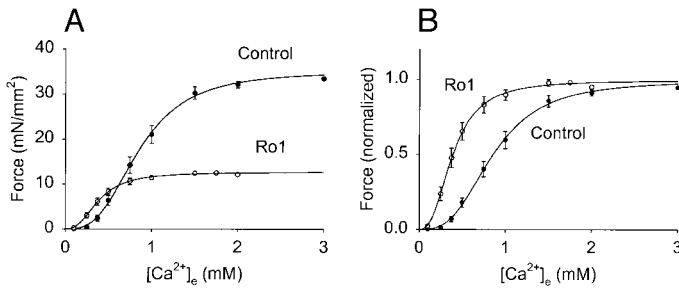


Fig. 2. A: relationship between force and extracellular [Ca<sup>2+</sup>]<sub>e</sub> ([Ca<sup>2+</sup>]<sub>e</sub>). Ro1 expression reduced maximum force at high [Ca<sup>2+</sup>]<sub>e</sub>. B: expressing force relative to maximum reveals that Ro1 expression enhanced the sensitivity to [Ca<sup>2+</sup>]<sub>e</sub> (force-[Ca<sup>2+</sup>]<sub>e</sub> relationship is left-shifted with Ro1 expression). Pooled data ( $n = 9$  per group). Solid lines are fits of force ( $F$ ) to the Hill equation:  $F = F_{\max} [Ca^{2+}]^{n_H} / (Ca_{50}^{n_H} + [Ca^{2+}]^{n_H})$ , where  $F_{\max}$  is the maximum force,  $n_H$  is the Hill coefficient, and  $Ca_{50}$  is the [Ca<sup>2+</sup>]<sub>e</sub> at half  $F_{\max}$ .

oped per unit area of myocardium during a twitch contraction. For all experiments, the myocardial twitch force with Ro1 expression was reduced >60% compared with control.

To facilitate comparison of twitch kinetics of myocardium from control and transgenic mice, Fig. 1B shows twitches normalized to the same amplitude. From Fig. 1B it is clear that Ro1 expression resulted in considerable slowing of the kinetics of the twitch contraction; both the rise of systolic force and diastolic relaxation were slower in Ro1-expressing myocardium than in control myocardium (see *Twitch kinetics*).

Thus Ro1 expression significantly impaired both the magnitude of force development and the kinetics of contraction and relaxation. This suggests that increased G<sub>i</sub> signaling causes deleterious effects on myocardial function. This is consistent with the cardiomyopathy that results from chronic Ro1 expression.

To investigate force development over a range of levels of muscle activation, we varied the level of extracellular [Ca<sup>2+</sup>]<sub>e</sub>. Figure 2A shows the relationship between developed force and extracellular [Ca<sup>2+</sup>]<sub>e</sub> for RV muscle strips from mouse hearts from control mice and transgenic mice expressing Ro1. Extracellular [Ca<sup>2+</sup>]<sub>e</sub> was varied over the full range of muscle activation from negligible force development at 0.1 mM [Ca<sup>2+</sup>]<sub>e</sub> to near-maximal force development at 2–3 mM [Ca<sup>2+</sup>]<sub>e</sub> (we did not investigate higher Ca<sup>2+</sup> levels to preclude the possibility of injury). Figure 2A shows that for myocardium from control and Ro1-expressing mice there was a sigmoidal relation between developed force and extracellular [Ca<sup>2+</sup>]<sub>e</sub> that was closely fit by the Hill equation (solid lines). Figure 2A shows that the maximum developed force of myocardium from Ro1-expressing mice (open symbols) was considerably lower compared with that for myocardium from control mice (closed symbols). For myocardium from control mice, the developed force extrapolated to a maximum of  $35 \pm 3$  mN/mm<sup>2</sup>. In contrast, the maximum force of myocardium from Ro1-expressing mice was reduced >60% to  $13 \pm 2$  mN/mm<sup>2</sup> (means  $\pm$  SE,  $n = 9$ ,  $P < 0.001$ ). These data demonstrate that at saturating levels of extracellular [Ca<sup>2+</sup>]<sub>e</sub> there was much lower twitch force devel-

opment associated with Ro1 expression. This suggests that increased G<sub>i</sub> signaling in this transgenic mouse model results in marked impairment of maximal twitch force development.

**Ca<sup>2+</sup> sensitivity.** Figure 2A also shows that despite lower maximal force with Ro1 expression, at several submaximal levels of extracellular [Ca<sup>2+</sup>]<sub>e</sub>, force development was actually greater compared with control. This indicates that Ro1 expression resulted in greater sensitivity to extracellular [Ca<sup>2+</sup>]<sub>e</sub> compared with control. This difference in sensitivity to levels of extracellular [Ca<sup>2+</sup>]<sub>e</sub> is readily apparent in Fig. 2B where force development has been expressed relative to the maximum values for myocardium from control and for Ro1-expressing mice. Figure 2B shows that Ro1 expression resulted in considerably greater sensitivity to extracellular [Ca<sup>2+</sup>]<sub>e</sub> compared with control as evidenced by higher relative force at submaximal Ca<sup>2+</sup> levels. The extracellular [Ca<sup>2+</sup>]<sub>e</sub> that resulted in half-maximal activation of the twitch ( $Ca_{50}$ ) was  $0.88 \pm 0.05$  mM for control myocardium. In contrast, with Ro1 expression  $Ca_{50}$  was much lower at  $0.41 \pm 0.05$  mM (means  $\pm$  SE,  $n = 9$ ,  $P < 0.001$ ).

**Twitch kinetics.** As noted in Fig. 1B, the time course of contraction and relaxation was appreciably slower with Ro1 expression compared with control. The twitch kinetics were assessed by measuring the time from the start to the peak of the twitch and the time from peak of the twitch to 50% relaxation. Figure 3 shows these timing parameters at different extracellular Ca<sup>2+</sup> levels for all experiments. Figure 3A shows that at all Ca<sup>2+</sup> levels, the time to peak twitch was greater with Ro1 expression ( $P < 0.001$  two-way repeated measures ANOVA); furthermore, for both control and Ro1-expressing myocardium there was a slight reduction in the time to peak at higher Ca<sup>2+</sup> levels ( $P < 0.01$  one-way repeated measures ANOVA).

Figure 3B shows that with Ro1 expression, the time to half relaxation was much greater than for control at all Ca<sup>2+</sup> levels ( $P < 0.001$  two-way repeated measures ANOVA). However, the relaxation time was not appreciably affected by extracellular [Ca<sup>2+</sup>]<sub>e</sub> for either control or Ro1-expressing myocardium ( $P > 0.05$  one-way repeated measures ANOVA).

Taken together, Fig. 3, A and B, demonstrates that expression of Ro1 resulted in appreciable prolongation of the twitch contraction time at all levels of activation.

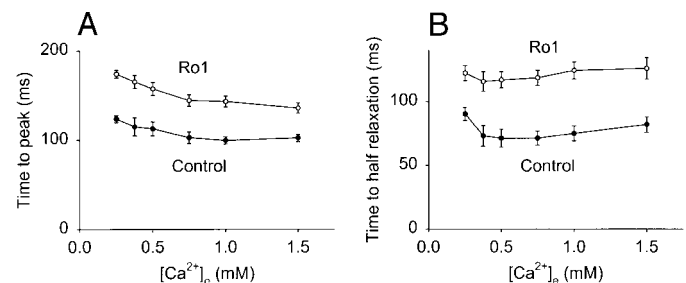


Fig. 3. Kinetics of the twitch contraction. Ro1 expression increased both the time to peak of the twitch (A) and the time from peak to half relaxation (B) at all levels of [Ca<sup>2+</sup>]<sub>e</sub>. Pooled data,  $n = 9$  per group.

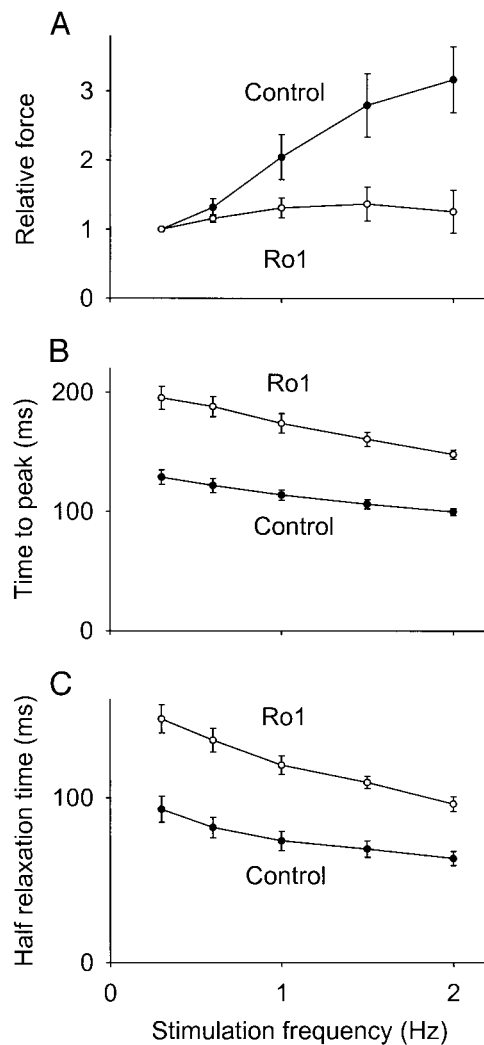


Fig. 4. Effect of stimulation frequency on contraction. A: at higher stimulation frequency contractile force increased in control myocardium ( $P < 0.001$ ), but not in Ro1-expressing myocardium ( $P > 0.3$ ). B and C: twitch time to peak and half relaxation time were similarly influenced by stimulation frequency. Pooled data,  $n = 9$  per group.

Slowed contraction and relaxation may contribute to impaired myocardial function and the cardiomyopathy that develops in this animal model.

**Force-frequency relationship.** Mouse myocardium resembles that of many species in displaying an increase of force with increased pacing frequency (7) (treppe). A positive treppe is an important mechanism contributing to augmentation of contractile function at increased work rates. This mechanism is often absent or blunted with cardiomyopathy (11, 18). Therefore, we assessed the effect of pacing frequency on contractile force (to minimize the possibility of injury, pacing was performed over a restricted frequency range: 0.3–2 Hz). For both control and Ro1-expressing myocardium, submaximal activation was achieved by setting the extracellular  $[Ca^{2+}]$  close to the  $Ca_{50}$ ; the influence of pacing frequency was then determined.

Figure 4A shows the relationship between developed force and stimulation frequency for control and Ro1-

expressing myocardium. Force was normalized to the force developed at the lowest stimulation rate (0.3 Hz). There was a significant difference between the response of control versus Ro1-expressing myocardium to increased pacing frequency ( $P = 0.013$ ). Consistent with previous studies, there was a marked increase of force for control myocardium at increased pacing frequency ( $P < 0.001$ ). However, with Ro1-expressing myocardium, the treppe response was abolished and increased stimulation frequency had no significant effect on developed force ( $P > 0.3$ ). Thus Ro1 expression resulted in a loss of the ability of the myocardium to augment contractile function at increased pacing rates.

Despite the absence of a treppe response with Ro1 expression, there was an effect of pacing rate on twitch kinetics. Figure 4 shows for both control and Ro1-expressing myocardium there was a significant reduction in the time to peak (Fig. 4B) and half relaxation (Fig. 4C) with increased pacing frequency ( $P < 0.001$ ). Thus, notwithstanding the loss of a positive treppe with Ro1 expression, the acceleration of contraction and relaxation with increased pacing frequency was preserved. At all pacing frequencies the timing of contraction and relaxation was slower with Ro1 expression compared with control ( $P < 0.001$ ) (see also Figs. 1 and 3).

**Postrest contractions.** The amplitude of the twitch contraction after periods of rest is thought to reflect on loading of  $Ca^{2+}$  into the sarcoplasmic reticulum (SR) during the rest period (19). Postrest force was assessed at submaximal extracellular  $[Ca^{2+}]$  (0.375–0.75 mM), close to the  $Ca_{50}$  for each group. Figure 5 shows the relationship between the postrest force versus the duration of the rest for control and Ro1-expressing myocardium. Figure 5A shows that for control myocardium the postrest force increased progressively with increasing periods of rest. In contrast, with Ro1 expression, Fig. 5B shows that force rose to the maximum with shorter rest intervals than control, and then force declined with longer rest intervals. There is a small but statistically significant fall of postrest force with 80-s rest intervals compared with 10-s rest intervals ( $P < 0.01$ , paired  $t$ -test); in contrast for control muscles, postrest force increased over these rest intervals ( $P = 0.01$ , paired  $t$ -test). With Ro1 expression, the decline of

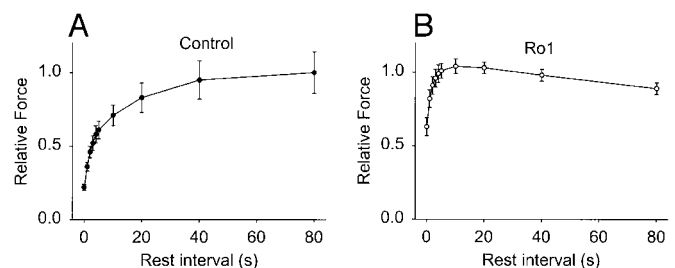


Fig. 5. Relationship of postrest force (normalized to the maximum twitch force) to the duration of the rest. A: in control myocardium, force rose with increasing rest duration. B: in Ro1-expressing myocardium, force rose to a maximum and then declined with increasing rest duration. Pooled data,  $n = 9$  per group.

force at longer rest intervals suggests a decline of SR Ca<sup>2+</sup> loading.

## DISCUSSION

This study determined the physiological effects of expression of a G<sub>i</sub>-coupled receptor in a transgenic mouse model of DCM. The major finding was that expression of the receptor was associated with marked abnormalities of contraction and relaxation. Specifically, there was a decrease of twitch force, enhanced responsiveness to extracellular Ca<sup>2+</sup>, slowing of twitch kinetics, abolition of the treppe response, and development of postrest decay of force. These findings suggest that increased G<sub>i</sub> signaling may result in altered myocardial function, which contributes to the cardiomyopathic phenotype in this model of DCM.

*Role of G<sub>i</sub> signaling in DCM.* Some forms of human idiopathic DCM have been indirectly linked to increased G<sub>i</sub> signaling. G<sub>i</sub> protein and mRNA levels are increased in the ventricles of patients with idiopathic DCM (5, 9, 22, 23). Hearts of patients with idiopathic DCM have decreased adenylyl cyclase activity (a known effect of G<sub>i</sub> signaling) (9, 22). Up to 40% of patients with idiopathic DCM have autoantibodies that bind to and cause signaling by the G<sub>i</sub>-coupled M<sub>2</sub> muscarinic receptor (6, 8, 14).

The results of the present study provide a more direct link between increased G<sub>i</sub> signaling and major abnormalities of myocardial contraction and relaxation. Thus this study provides a further rationale to connect increased G<sub>i</sub> signaling to some forms of cardiac disease.

In this study, contractile function with Ro1 expression was evaluated before the appearance of overt congestive heart failure. Thus the appearance of abnormalities of contractile function with Ro1 expression may be an early indicator of the eventual progression to heart failure. Therefore, this study suggests that increased G<sub>i</sub> signaling leads to abnormalities of contraction and relaxation, which in turn may lead to the progression to heart failure.

*Effects of Ro1 expression on force development.* Ro1 expression substantially reduced the twitch force at maximal activation. The decreased force with Ro1 expression thus could not be offset by raising extracellular [Ca<sup>2+</sup>]. This finding suggests that impaired force development is not due to incomplete muscle activation and therefore reflects a reduction in the intrinsic force-generating capacity of the myocardium (e.g., due to loss of myofilaments or decreased cross-bridge function).

Despite the reduction of maximum force with Ro1 expression, the sensitivity of force development to extracellular Ca<sup>2+</sup> was enhanced. Because the amplitude of the intracellular Ca<sup>2+</sup> transient is related to the level of extracellular [Ca<sup>2+</sup>], the enhanced sensitivity to extracellular [Ca<sup>2+</sup>] may reflect enhanced sensitivity to intracellular [Ca<sup>2+</sup>]. Previously enhanced myofilament Ca<sup>2+</sup> sensitivity was also found in human DCM (25), and furthermore, has been implicated in human familial hypertrophic cardiomyopathy (4, 15).

In this transgenic mouse model of DCM, expression of the G<sub>i</sub>-coupled receptor Ro1 could lead to increased G<sub>i</sub> signaling, reduced cAMP levels, and thus to enhanced myofilament sensitivity. With Ro1 expression, increased myofilament Ca<sup>2+</sup> sensitivity may tend to compensate for the decreased intrinsic force-generating capacity of the myocardium. However, more direct measures are required to determine whether myofilament Ca<sup>2+</sup> sensitivity is fundamentally altered in this model or whether intracellular Ca<sup>2+</sup> regulation is altered.

Finally, Ro1 expression slowed the kinetics of myocardial contraction and relaxation. This finding is consistent with an effect of G<sub>i</sub> signaling on Ca<sup>2+</sup> handling. Specifically, the lowering of cAMP levels with increased G<sub>i</sub> signaling would retard Ca<sup>2+</sup> reuptake by the SR through inhibition of the SR Ca<sup>2+</sup> pump. Increased myofilament Ca<sup>2+</sup> sensitivity due to increased G<sub>i</sub> signaling could also contribute to the slowing of twitch kinetics.

In summary, expression of Ro1 leads to several major abnormalities of force development, which have some features consistent with the effects of increased G<sub>i</sub> signaling. These abnormalities may play important roles in the development of cardiomyopathy in this model of disease. Future experiments using pertussis toxin will be important to determine whether the mechanical deficits with Ro1 expression are ameliorated by inhibiting G<sub>i</sub> signaling, as has been shown for conduction abnormalities after in vivo treatment with pertussis toxin (21).

*Effects of Ro1 expression on the force-frequency relation.* In normal myocardium, an increase of pacing frequency results in considerable augmentation of force (treppe response). In marked contrast, the treppe response was completely abolished with Ro1 expression. This suggests that increased G<sub>i</sub> signaling leads to loss of the treppe response. With heart failure in humans the treppe response is also abolished (11, 18). Thus abolition of the treppe response with Ro1 expression provides further support for the view that important features of human cardiac disease are manifest with Ro1 expression.

The mechanism for the loss of the treppe response in Ro1-expressing mice is unclear. In normal myocardium, the increase of force with increased pacing rate is due to both a rise of the Ca<sup>2+</sup> transient and to Ca<sup>2+</sup> sensitization of the myofilaments. Indirect evidence suggests that both Ca<sup>2+</sup> handling and Ca<sup>2+</sup> sensitization may be abnormal in this mouse model of cardiac disease. Specifically, with Ro1 expression the twitch kinetics are slower, which may involve impaired SR uptake. Furthermore, the increased responsiveness to extracellular [Ca<sup>2+</sup>] with Ro1 expression suggests that the myofilaments may already be more sensitive to Ca<sup>2+</sup>, thus further augmentation of Ca<sup>2+</sup> sensitivity may be limited.

In control muscle, increased stimulation frequency has both inotropic and lusitropic effects (i.e., both increased force and accelerated twitch kinetics). Interestingly, despite the lack of a positive treppe response

with Ro1 expression, increased stimulation frequency did accelerate the kinetics of the twitch contraction in a manner that was similar to that found for control muscle. Thus with Ro1 expression, the lusitropic effect of increased pacing remained intact in the absence of an inotropic response to increased pacing. This indicates that the inotropic and lusitropic responses to increased pacing, which normally occur in parallel, have become uncoupled in this transgenic cardiomyopathy model. Faster relaxation with faster pacing is thought to involve acceleration of the decline of the Ca<sup>2+</sup> transient. However, the mechanism causing faster Ca<sup>2+</sup> transient decline remains unclear. Whereas Ca/calmodulin-dependent protein kinase II is involved in some forms of activity-dependent acceleration of relaxation (2), a recent study suggests it is not involved in the acceleration of relaxation induced by faster pacing (13).

*Effects of Ro1 expression on postrest contractions.* With increasing periods of rest, postrest force was increased in control myocardium. This is consistent with increased Ca<sup>2+</sup> loading of the SR (19). In contrast, with Ro1 expression, increased periods of rest eventually resulted in postrest decay of force. This is consistent with loss of Ca<sup>2+</sup> from the SR, suggesting that increased G<sub>i</sub> signaling impairs Ca<sup>2+</sup> loading of the SR. Increased G<sub>i</sub> signaling could impair SR Ca<sup>2+</sup> loading through inhibitory effects of lowered cAMP levels on the function of both L-type Ca<sup>2+</sup> channels and the SR Ca<sup>2+</sup> pump (16).

*Relation to myocardial function in vivo.* We previously found that expression of Ro1 resulted in impaired myocardial function in vivo (21). Specifically, electrocardiograms of ambulatory mice expressing Ro1 displayed marked arrhythmias characterized by wide QRS complexes. These arrhythmias were abolished by administration of pertussis toxin, which specifically blocks G<sub>i</sub> signaling. This suggested that the arrhythmias induced by Ro1 expression were mediated by G<sub>i</sub> signaling (21).

In addition, transthoracic echocardiography revealed that Ro1 expression also resulted in several functional abnormalities compared with controls: a 50% increase of left ventricular end-systolic chamber diameter; and ≈40% reduction in both left ventricular fractional shortening and aortic peak flow velocity (21).

Thus the present findings of impaired function of cardiac muscle strips in vitro are consistent with our previous findings of impaired function in vivo. The present study extends the previous findings in several directions. The present study demonstrates that abnormal myocardial function with Ro1 expression exists independent of effects due to electrocardiogram abnormalities. The in vitro studies reveal major functional abnormalities with Ro1 expression that were not apparent from in vivo studies.

In conclusion, expression of the G<sub>i</sub>-coupled receptor Ro1 in transgenic mice causes marked abnormalities of myocardial contractile function and eventually leads to a lethal DCM. This suggests that increased G<sub>i</sub> signaling causes impaired contractile function, which con-

tributes to development of DCM. These findings have important implications for some forms of human dilated cardiomyopathy where increased G<sub>i</sub> signaling has been suggested to play a role.

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