Biophysical Journal, Volume *113*

Supplemental Information

Omecamtiv Mecarbil Abolishes Length-Mediated Increase in Guinea Pig Cardiac Myofiber Ca^{2+} Sensitivity

Sampath K. Gollapudi, Sherif M. Reda, and Murali Chandra

MATERIALS AND METHODS

Determination of phosphorylation status of sarcomeric proteins in untreated and OM-treated fibers: Untreated and 0.3 μM OM-treated left ventricular papillary muscle fibers were solubilized using a muscle protein extraction buffer (2.5% SDS, 10% glycerol, 50 mM tris base (pH 6.8 at 4ºC), 1 mM Dithiothreitol, 4 mM benzamidine HCl, and a cocktail of phosphatase/protease inhibitors). The final concentrations of all samples were adjusted to 2 mg/ml using protein loading dye (125 mM Tris-HCl (pH 6.8), 20% glycerol, 2% SDS, 0.01% bromophenol blue, and 50 mM β-mercaptoethanol). Equal quantities (10 μg) of standardized protein samples were loaded and ran on a 12.5% small SDS gel. The gel was fixed in a solution containing 50% methanol and 10% acetic acid, and then treated with Pro-Q diamond stain and destain (P33300 and P33310, Life Technologies, Grand Island, NY), as described in the Life Technologies manual. Phosphoproteins were visualized by imaging the gel using UV transillumination on a BioRad ChemiDoc XRS camera.

Preparation of detergent-skinned cardiac muscle fibers: Left ventricular papillary muscle fibers from guinea pigs were prepared using methods described previously (1-3). Briefly, guinea pigs were deeply anesthetized using isoflurane, and hearts were quickly excised and placed into an ice-cold high-relaxing (HR) solution containing the following (in mM): 20 2,3-butanedione monoxime (BDM), 50 *N*,*N*-bis (2-hydroxyethyl)-2-amino-ethane-sulfonic acid (BES), 30.83 potassium propionate (K-prop), 10 sodium azide (NaN_3), 20 ethylene glycol tetra-acetic acid (EGTA), 6.29 magnesium chloride ($MgCl_2$), 6.09 Na_2ATP, 4.0 benzamidine HCl, 1.0 of dithiothreitol (DTT), and a cocktail of protease inhibitors. Left ventricular papillary muscle bundles were quickly removed in HR solution, dissected into smaller sections measuring 2.0–2.5

mm in length and 150–200 μm in thickness, and were detergent-skinned overnight in HR solution containing 1% Triton-X-100.

Measurements of pCa-tension relationship: Steady-state isometric tension measurements in muscle fibers were made at various pCa ($-\log_{10}$ of $[Ca^{2+}]_{free}$), as described before (2,4-6). In brief, muscle fibers were attached between a motor arm (322C, Aurora Scientific Inc., Ontario, Canada) and a force transducer (AE 801, Sensor One Technologies, Sausalito, CA) using T-shaped aluminum clips. The sarcomere length (SL) of muscle fibers was adjusted to either 1.9 or 2.3 μm in HR solution using He-Ne laser diffraction technique (5). Following two cycles of maximal activation (pCa 4.3) and relaxation (pCa 9.0), SL was readjusted to the desired SL if necessary. The muscle length (ML) and cross-sectional area (CSA) were measured and then muscle fibers were exposed to various solutions with pCa ranging from 4.3 to 9.0 in a constantly-stirred chamber. Force responses were recorded on a computer at a sampling rate of 1 kHz. Isometric steady-state tension values were plotted against pCa to construct the pCa-tension relationship. The Hill equation was fitted to the normalized pCa-tension relationship to estimate two parameters, n_H (myofilament cooperativity) and pCa_{50} (myofilament Ca^{2+} sensitivity). All measurements are made at 20ºC.

pCa solutions and their compositions: Compositions of pCa 9.0 and pCa 4.3 solutions were calculated based on the program by Fabiato and Fabiato (7). pCa 9.0 solution contained the following (in mM): 50 BES, 5 NaN_3, 10 phosphoenol pyruvate (PEP), 10 EGTA, 0.024 calcium chloride ($CaCl_2$), 6.87 $MgCl_2$, 5.83 Na_2ATP, and 51.14 K-Prop, while pCa 4.3 solution contained the following (in mM): 50 BES, 5 NaN_3, 10 PEP, 10 EGTA, 10.11 $CaCl_2$, 6.61 $MgCl_2$, 5.95

Na_2ATP, and 31 K-Prop. Both pCa 9.0 and pCa 4.3 solutions included a cocktail of protease inhibitors ((in μM): 10 leupeptin, 1000 pepstatin, 100 PMSF, 20 diadenosine penta-phosphate, 10 oligomycin). The pH and ionic strength of pCa solutions were adjusted to 7.0 and 180 mM, respectively. All other intermediate pCa solutions were made by mixing appropriate amounts of pCa 9.0 and pCa 4.3 solutions, which were based on the program by Fabiato and Fabiato (7).

Dynamic muscle fiber stiffness: A series of various amplitude stretch/release perturbations (±0.5%, ±1.0%, ±1.5%, and ±2.0% of the initial ML) was applied on muscle fibers and the corresponding force responses were recorded (8). A non-linear recruitment-distortion (NLRD) model was fit to force responses, as described previously (8), to estimate four model parameters: the magnitude of the instantaneous muscle fiber stiffness caused by a sudden change in ML (E_D); the rate by which the strain within bound XBs dissipates to a steady-state level (c); the rate by which new XBs are recruited into the force-bearing state due to a change in ML (b); and the magnitude of increase in the muscle fiber stifness due to ML-mediated recruitment of additional force-bearing XBs (E_R). Below, we explain the physiological significance of various model parameters using 2% sudden stretch (Fig. 1 *A* in the main article) and the elicited force response (Fig. 1 *B*) from an untreated guinea pig cardiac muscle fiber.

***E*$_D$**: In phase 1, a sudden increase in ML (Fig. 1 *A*) causes an instantaneous increase in force from the isometric steady state value (F_{ss}) to F_1 (Fig. 1 *B*). F_1 results from the distortion of elastic elements within strongly-bound XBs. Thus, F_1 increases when the number of strong XBs in the steady state (prior to ML change) is higher and vice versa. Because E_D is estimated as the slope of the linear relationship between changes in F_1-F_{ss} and the imposed changes in ML (ΔL), it is an approximate measure of the number of strongly-bound XBs (8,9).

***c*:** In phase 2, when the muscle fiber is held at the increased ML (Fig. 1 *A*), force decays exponentially at a characteristic rate, *c* (Fig. 1 *B*). This rapid decay results from the detachment of distorted XBs, followed by their equilibration into the non-force bearing state. We have previously demonstrated that *c* is an approximate measure of XB detachment rate, *g* (9).

***b*:** In phase 3, force begins to rise gradually in an exponential fashion at a characteristic rate, *b* (Fig. 1 *B*). This gradual rise in force results from the recruitment of additional XBs into the force-bearing state.

***E*$_R$:** The steady rise in force during phase 3 levels off to a new steady-state value (F_{nss}) that is higher than F_{ss} (Fig. 1 *B*). The magnitude of increase, from F_{ss} to F_{nss}, is proportional to the number of additional force-bearing XBs recruited for a given increase in ML. Because E_R is derived as the slope of the linear relationship between changes in F_{nss}-F_{ss} and ΔL, it is an approximate measure of the magnitude of ML-mediated XB recruitment.

Rate constant of tension redevelopment (k_{tr}): k_{tr} was estimated using a modified version of the large slack/restretch maneuver originally described by Brenner and Eisenberg (10). The modified version is described in our earlier published works (11-13). Briefly, fully activated muscle fiber was rapidly slackened by 10% of the initial ML using a servo motor and was held for 25 ms at the reduced length. The muscle fiber was then rapidly (0.5 ms) stretched past the initial ML by 10%, brought back to the initial ML and allowed to redevelop force. k_{tr} was estimated by fitting the following mono-exponential function to the rising phase of the resulting force response:

$$F = (F_{ss} - F_{res})(1 - e^{-k_{tr}t}) + F_{res}$$

where F_{ss} is the steady-state isometric force and F_{res} is the residual force from which the fiber starts to redevelop force.

SUPPORTING REFERENCES

1. Ford, S. J., R. Mamidi, J. Jimenez, J. C. Tardiff, and M. Chandra. 2012. Effects of R92 mutations in mouse cardiac troponin T are influenced by changes in myosin heavy chain isoform. *J Mol Cell Cardiol* 53:542-551.

2. Gollapudi, S. K., J. C. Tardiff, and M. Chandra. 2015. The functional effect of dilated cardiomyopathy mutation (R144W) in mouse cardiac troponin T is differently affected by alpha- and beta-myosin heavy chain isoforms. *Am J Physiol Heart Circ Physiol* 308:H884-893.

3. Mamidi, R. and M. Chandra. 2013. Divergent effects of alpha- and beta-myosin heavy chain isoforms on the N terminus of rat cardiac troponin T. *J Gen Physiol* 142:413-423.

4. Chandra, M., M. L. Tschirgi, S. J. Ford, B. K. Slinker, and K. B. Campbell. 2007. Interaction between myosin heavy chain and troponin isoforms modulate cardiac myofiber contractile dynamics. *Am J Physiol Regul Integr Comp Physiol* 293:R1595-1607.

5. de Tombe, P. P. and G. J. Stienen. 1995. Protein kinase A does not alter economy of force maintenance in skinned rat cardiac trabeculae. *Circ Res* 76:734-741.

6. Stienen, G. J., R. Zaremba, and G. Elzinga. 1995. ATP utilization for calcium uptake and force production in skinned muscle fibres of Xenopus laevis. *J Physiol* 482 (Pt 1):109-122.

7. Fabiato, A. and F. Fabiato. 1979. Calculator programs for computing the composition of the solutions containing multiple metals and ligands used for experiments in skinned muscle cells. *J Physiol (Paris)* 75:463-505.

8. Ford, S. J., M. Chandra, R. Mamidi, W. Dong, and K. B. Campbell. 2010. Model representation of the nonlinear step response in cardiac muscle. *J Gen Physiol* 136:159-177.

9. Campbell, K. B., M. Chandra, R. D. Kirkpatrick, B. K. Slinker, and W. C. Hunter. 2004. Interpreting cardiac muscle force-length dynamics using a novel functional model. *Am J Physiol Heart Circ Physiol* 286:H1535-1545.

10. Brenner, B. and E. Eisenberg. 1986. Rate of force generation in muscle: correlation with actomyosin ATPase activity in solution. *Proc Natl Acad Sci USA* 83:3542-3546.

11. Ford, S. J. and M. Chandra. 2013. Length-dependent effects on cardiac contractile dynamics are different in cardiac muscle containing alpha- or beta-myosin heavy chain. *Arch Biochem Biophys* 535:3-13.

12. Gollapudi, S. K., C. E. Gallon, and M. Chandra. 2013. The tropomyosin binding region of cardiac troponin T modulates crossbridge recruitment dynamics in rat cardiac muscle fibers. *J Mol Biol* 425:1565-1581.

13. Gollapudi, S. K., R. Mamidi, S. L. Mallampalli, and M. Chandra. 2012. The N-terminal extension of cardiac troponin T stabilizes the blocked state of cardiac thin filament. *Biophys J* 103:940-948.

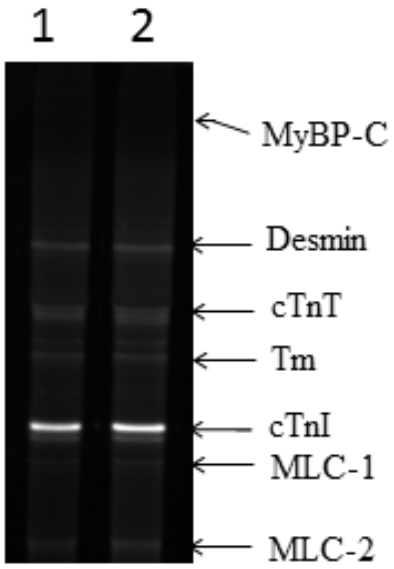

Figure S1 Pro-Q Diamond-stained gel showing the levels of phosphorylated proteins in untreated and 0.3 µM OM-treated fibers. Fibers were solubilized in 2.5% SDS solution and ran on a 12.5% SDS-gel. The gel was fixed in a solution containing 50% methanol and 10% acetic acid and then treated with Pro-Q diamond stain and destain, as described in Materials and Methods. A visual examination of the Pro-Q stained gel shows that the phosphorylation levels of various proteins are similar in untreated (*lane* 1) and OM-treated fibers (*lane* 2). MyBP-C, myosin binding protein-C; cTnT, cardiac troponin T; Tm, tropomyosin; cTnI, cardiac troponin I; MLC-1, myosin light chain 1; MLC-2, myosin light chain 2.

Table S1. Effects of 3.0 μM OM on SL-dependency of various contractile parameters.

Parameter	Untreated fibers			OM (3.0 μM)-treated fibers		
	1.9 μm	**2.3 μm**	**Δ**	**1.9 μm**	**2.3 μm**	**Δ**
Tension ($mN \cdot mm^{-2}$)	37.5±1.2	57.9±1.3	$+20.5^{***}$	42.1±0.72	55.6±1.1	$+13.5^{***}$
E_D ($mN \cdot mm^{-3}$)	813±27	1006±29	$+193^{***}$	955±26	1109±36	$+154^{**}$
pCa_{50}	5.63±0.01	5.71±0.01	$+0.08^{***}$	6.18±0.01	6.19±0.02	$+0.01^{NS}$
n_H	3.05±0.09	2.44±0.09	-0.61^{***}	1.09±0.05	0.96±0.06	-0.13^{NS}
c (s^{-1})	11.1±0.6	7.8±0.5	-3.30^{***}	2.45±0.27	1.75±0.20	-0.70^{NS}
b (s^{-1})	3.67±0.12	4.02±0.10	$+0.35^{NS}$	1.34±0.12	1.06±0.13	-0.28^{NS}
k_{tr} (s^{-1})	2.06±0.11	1.66±0.12	-0.40^{***}	0.28±0.01	0.26±0.01	-0.02^{NS}
E_R ($mN \cdot mm^{-3}$)	140±6	255±9	$+115^{***}$	141±7	225±11	$+84^{***}$

'Δ' represents the change in contractile parameter in response to an increase in SL from 1.9 to 2.3 μm ('+' indicates increase and '-' indicates decrease). Statistical differences were analyzed by two-way ANOVA and subsequent post-hoc Fisher's LSD method. Asterisks indicate significant difference when compared to the data at 1.9 μm (*P<0.05; **P<0.01; ***P<0.001; NS, not significant). A separate set of fibers from three hearts was used for each group. The number of fibers measured for untreated and 3.0 μM OM groups at short SL were 12 and 11, while those at long SL were 12 and 11, respectively. Data are expressed as Mean±SEM.

Table S2. Effects of 0.3 μM OM on SL-dependency of various contractile parameters at submaximal activation (pCa 5.8).

Parameter	Untreated fibers			OM (0.3 μM)-treated fibers		
	1.9 μm	**2.3 μm**	**Δ**	**1.9 μm**	**2.3 μm**	**Δ**
Tension ($mN \cdot mm^{-2}$)	8.3±0.7	22.9±1.8	$+14.6^{***}$	21.5±1.5	29.0±1.7	$+7.5^{***}$
E_D ($mN \cdot mm^{-3}$)	299±39	488±28	$+189^{***}$	540±27	676±35	$+136^{**}$
c (s^{-1})	11.1±0.8	8.1±0.5	-3.0^{**}	5.7±0.6	6.0±0.7	$+0.3^{NS}$
b (s^{-1})	2.0±0.2	2.1±0.1	$+0.1^{NS}$	1.4±0.1	1.6±0.1	$+0.2^{NS}$
E_R ($mN \cdot mm^{-3}$)	106±15	205±10	$+115^{***}$	172±13	206±17	$+34^{NS}$

'Δ' represents the change in contractile parameter in response to an increase in SL from 1.9 to 2.3 μm ('+' indicates increase and '-' indicates decrease). Statistical differences were analyzed by two-way ANOVA and subsequent post-hoc Fisher's LSD method. Asterisks indicate significant difference when compared to the data at 1.9 μm (**P<0.01; ***P<0.001; NS, not significant). A separate set of fibers from three hearts was used for each group. The number of fibers measured for untreated and 0.3 μM OM groups at short SL were 10 and 9, while those at long SL were 10 and 11, respectively. Data are expressed as Mean±SEM.