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ORIGINAL ARTICLE

Frequency-dependent effects of omecamtiv mecarbil on cell shortening of isolated canine ventricular cardiomyocytes

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Abstract Omecamtiv mecarbil (OM) is a myosin activator agent developed for the treatment of heart failure. OM was reported to increase left ventricular ejection fraction and systolic ejection time, but little is known about the effect of heart rate on the action of OM. The present study, therefore, was designed to investigate the effects of OM on unloaded cell shortening and intracellular Ca^{2+} ($[Ca^{2+}]_i$) transients as a function of the pacing frequency. Isolated cardiomyocytes were stimulated at various frequencies under steady-state conditions. Cell length was monitored by an optical edge detector and changes in $[Ca^{2+}]_i$ were followed using the Ca^{2+}-sensitive dye Fura-2. At the pacing frequency of 1 Hz, OM (1–10 μM) significantly decreased both diastolic and systolic cell length, however, fractional shortening was augmented only by 1 μM OM. Time to peak tension and time of 90% relaxation were progressively increased by OM. At the frequency of 2 Hz, diastolic cell length was reduced by 10 μM OM to a larger extent than systolic cell length, resulting in a significantly decreased fractional shortening under these conditions. OM

had no effect on the parameters of the $[Ca^{2+}]_i$ transient at any pacing frequency. The results suggest that supratherapeutic concentrations of OM may decrease rather than increase the force of cardiac contraction especially in tachycardic patients.

Introduction

Heart failure is a leading cause of mortality in the twenty-first century, and this tendency is not likely to be terminated in the future. Beyond the conventional inotropic therapies, used to support the impaired cardiac contractility, recently, a novel strategy of enhancing the systolic performance of the heart, based on elevation of the number of active force-generating cross-bridges, has been developed (Teerlink et al. 2009; Malik and Morgan 2011; James and Robbins 2011). Omecamtiv mecarbil (OM), the lead molecule of this myosin motor activator group, acts directly on the β-myosin heavy chain without interacting with the troponin-tropomyosin system (Teerlink 2009; Malik et al. 2011; Liu et al. 2015). According to results of recent phase II studies, OM displayed favorable effects in acute and chronic heart failure patients, including the decrease of episodes with dyspnea, elevation of cardiac output and the systolic ejection time, and reduction of left ventricular end-systolic as well as end-diastolic diameters (Teerlink et al. 2016a; Teerlink et al. 2016b).

However, in line with its known mechanism of action, OM was shown to increase the duration of cardiac contractions significantly (Shen et al. 2010; Malik et al. 2011), which may limit the diastolic filling of the heart causing diastolic stiffness and anginal attack in some patients at concentrations

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higher than the usual therapeutic range (Cleland et al. 2011; Teerlink et al. 2011). The development of such problems may be more prominent at higher heart rates when the diastolic function is relatively compromised. The present study was designed to investigate the frequency dependence of the OM effect in isolated myocytes, where the problem of the compromised diastolic filling is irrelevant. The effects of supratherapeutic OM concentrations (1 and 10 μM) on unloaded cell shortening, action potential morphology, and changes in $[Ca^{2+}]_i$ were studied in a frequency-dependent manner. In absence of healthy human ventricular preparations, canine myocytes were studied since they resemble the human ventricular cells regarding their physiological properties (Szabó et al. 2005; Szentandrássy et al. 2005) and because dog hearts are more sensitive to OM than the hearts of small rodents (Malik et al. 2011). Previous studies revealed that OM had little effect on the electrophysiological properties, like ion currents and action potential characteristics, in canine cardiomyocytes except for high concentrations of OM (Szentandrássy et al. 2016).

Materials and methods

Isolation of single canine ventricular cardiomyocytes

Adult mongrel dogs of either sex were anesthetized with intramuscular injections of 10 mg/kg ketamine hydrochloride (Calypsol, Richter Gedeon, Hungary) and 1 mg/kg xylazine hydrochloride (Sedaxylan, Eurovet Animal Health BV, The Netherlands). The hearts were quickly removed and placed in Tyrode solution containing (in mM) NaCl, 144; KCl, 5.6; CaCl₂, 2.5; MgCl₂, 1.2; HEPES, 5; and dextrose, 11 at pH = 7.4. Single cardiomyocytes were obtained by enzymatic dispersion using the segment perfusion technique, as described previously (Magyar et al. 2004; Szabó et al. 2013; Váczi et al. 2015). Briefly, a wedge-shaped section of the left ventricular wall supplied by the left anterior descending coronary artery was dissected, cannulated, and perfused with oxygenized Tyrode solution. After removal of blood, the perfusion was switched to a nominally Ca^{2+}-free Joklik solution (Minimum Essential Medium Eagle, Joklik Modification; Sigma-Aldrich Co. St. Louis, MO, USA) for 5 min. This was followed by 35-min perfusion with Joklik solution supplemented with 1 mg/ml collagenase (type II, Worthington Biochemical Corporation, Lakewood, NJ, USA) and 0.2% bovine serum albumin (Fraction V., Sigma) containing 50 μM Ca^{2+}. The full transmural section of the middle portion of the left ventricular wall was cut into small pieces and the cell suspension was washed with Joklik solution. These tissue chunks contained dominantly midmyocardial myocytes. After gradually restoring the normal external Ca^{2+} concentration, the cells were stored in Minimum Essential Medium Eagle until use.

Recording of action potentials and cell shortening

All electrophysiological measurements were performed at 37 °C as previously described (Magyar et al. 2002; Simkó et al. 2010; Ruzsnavszky et al. 2014). Rod-shaped viable ventricular cardiomyocytes, showing clear striation, were placed in a 1-ml-volume experimental chamber on the stage of an inverted microscope and superfused with oxygenized Tyrode solution. After sedimentation, the cardiomyocytes were superfused continuously at a rate of 2 ml/min and were impaled with 3 M KCl-filled sharp glass microelectrodes (tip resistance 20–40 MΩ). The cells were paced through these intracellular electrodes using 1–2-ms-wide rectangular current pulses having amplitudes of twice the diastolic threshold. The simultaneously recorded action potentials were used for monitoring the condition of the cell. Myocytes were allowed to equilibrate in the dimethyl sulfoxide (DMSO)-containing Tyrode solution for 10 min before taking the control records. Then, the cells were exposed to either 1 or 10 μM of OM for at least 10 min. In frequency-dependent measurements, the cardiomyocytes were paced first at 0.5 Hz and recording was performed after equilibration. Then, the frequency was increased to 1 Hz and recording was repeated following 100 cycles at the new frequency. Similar transition was made between 1- and 2-Hz frequencies as well.

Cell shortening was measured using a video-edge detector (VED-105, Crescent Electronics, Sandy, UT, USA) system as previously described (Kistamás et al. 2015). The analogue signal was amplified (DC amplifier, Főnixcomp Ltd., Hungary), digitized at 240 Hz (Digidata 1440A, Molecular Devices, Sunnyvale, CA, USA), and recorded with pClamp 10 software (Molecular Devices, Sunnyvale, CA, USA). During subsequent off-line analysis, 10 consecutive contractile curves were averaged and compared before and after drug application. Systolic and diastolic cell lengths were determined and fractional shortening was expressed as a percent of diastolic cell length.

Recording of intracellular Ca^{2+} transients

Myocytes were loaded with 5 μM Fura-2 AM for 30 min at room temperature in Tyrode solution in the presence of Pluronic F-127. Twenty-five milligrams Pluronic F-127 was dissolved in 1 ml DMSO and this solvent was used to make a Fura-2 AM stock solution of 1 mM. After loading, cardiomyocytes were allowed to rest for 30 min at room temperature to de-esterify the dye, then the cells were stored at 15 °C before the experiments. Intracellular Ca^{2+} transients were recorded from cardiomyocytes paced through the microelectrode allowing continuous monitoring of an action

potential morphology. Ca^{2+} transients were measured using an alternating dual beam excitation fluorescence photometry set-up (RatioMaster; Photon Technology International, New Brunswick, NJ, USA). Excitation wavelengths of 340 and 380 nm were used to monitor the fluorescence signals of Ca^{2+}-bound and Ca^{2+}-free Fura-2 dye, respectively. Fluorescent emission was detected at 510 nm with a R1527P photomultiplier tube (Hamamatsu Photonics Deutschland GmbH., Herrsching am Ammersee, Germany). These traces were digitized at 200 Hz using the FeliX Software (Photon Technology International, New Brunswick, NJ, USA) and stored for off-line analysis. Background fluorescence was measured by moving the cell out of the field of view, and it was subtracted from total fluorescence in order to obtain fluorescence originating exclusively from the preparation.

Drugs

Drugs—except for OM—were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). OM was purchased from AdooQ BioScience (Irvine, CA, USA). Stock solutions with final OM concentrations of 1 and 10 mM were prepared in DMSO as solvent and stored at 4 °C. Appropriate volumes of these concentrated stock solutions were dissolved in the bathing medium to obtain final OM concentrations of 1 or 10 μM. Accordingly, DMSO concentration in these solutions was 0.1% uniformly. The control (OM-free) solutions contained the same amount of DMSO.

Statistics

Results are expressed as mean ± SEM values. Statistical significance of differences from control was evaluated using analysis of variance (ANOVA) followed by Dunnett's test. Differences were considered significant when p was less than 0.05.

Results

At the constant pacing frequency of 1 Hz, 1 and 10 μM OM significantly reduced both diastolic and systolic cell lengths (Fig. 1b–d). However, the fractional shortening, an indicator of the amplitude of unloaded contraction, was increased only by 1 μM OM, but not by 10 μM (Fig. 1e). On the other hand, time to peak tension and time of 90% relaxation were progressively increased with increasing concentration of OM (Fig. 1f, g). In contrast to the marked alterations in cell shortening, action potential configuration was little affected by OM (Fig. 1a).

Changes in the cytosolic Ca^{2+} concentration were monitored using the Ca^{2+}-sensitive dye, Fura-2. OM did not alter significantly diastolic and systolic Ca^{2+} concentrations, or the

amplitude of intracellular Ca^{2+} transients, as indicated by the unchanged fluorescent ratios in Fig. 2a–d.

Since the actual heart rate may change—typically increase—during physical activity in both humans and dogs, the effects of OM on cell shortening were also studied in a frequency-dependent manner (Fig. 3). In these experiments, the pacing frequency was increased from 0.5 to 1 Hz, and from 1 to 2 Hz, allowing the stabilization of parameters for 100 cycles at the new stimulation frequency. Moderate frequency dependence of the OM effect on unloaded cell shortening was observed: a stronger tendency of reduction of both systolic and diastolic cell lengths was evident at the frequency of 2 Hz than that at 1 Hz, but systolic shortening was always preserved under these conditions (Fig. 3a–c). A significant increase in fractional shortening was evoked by 1 μM OM at each studied frequency; however, its magnitude was the highest at the lowest stimulation frequency of 0.5 Hz. This effect was fully absent in the presence of 10 μM OM; furthermore, an inverse action (i.e., significant reduction of fractional shortening) was obtained in the presence of 10 μM OM at 2 Hz (Fig. 3d). Little frequency dependence was observed in time to peak and 90% relaxation time values (Fig. 3e, f).

Inspired by this unexpected observation discussed earlier, the effect of OM on cytosolic Ca^{2+} transients was also studied in a frequency-dependent manner, focusing now on the higher frequencies ranging from 1 to 4 Hz. Due to the higher frequency and longer exposure to OM in this arrangement, the effect of 1 μM OM was studied only. As indicated in Fig. 4a–c, OM did not alter the magnitude of either systolic or diastolic $[Ca^{2+}]_i$, even at the highest stimulation frequency of 4 Hz, accordingly, no change was observed in the amplitude of Ca^{2+} signal.

An interesting phenomenon called "overshoot of relaxation" was observed in the presence of 1 and 10 μM of OM. Following the elongated contraction, cell length became transiently longer than the baseline value of its resting length (Fig. 5a). Such behavior has never been observed in the absence of OM, and in contrast to the cell length record, no related hump on the action potential or the Ca^{2+} transient could be detected. The amplitude of this overshoot was independent of the pacing frequency but increased with the concentration of OM (Fig. 5b). These results suggest that the overshoot of relaxation may directly be related to the OM-modified myosin-actin interaction with no connection to electrical or $[Ca^{2+}]_i$ changes.

Discussion

### Effects of OM at cellular level

In this study, we have shown that OM causes a concentration-dependent reduction in both systolic and diastolic cell lengths
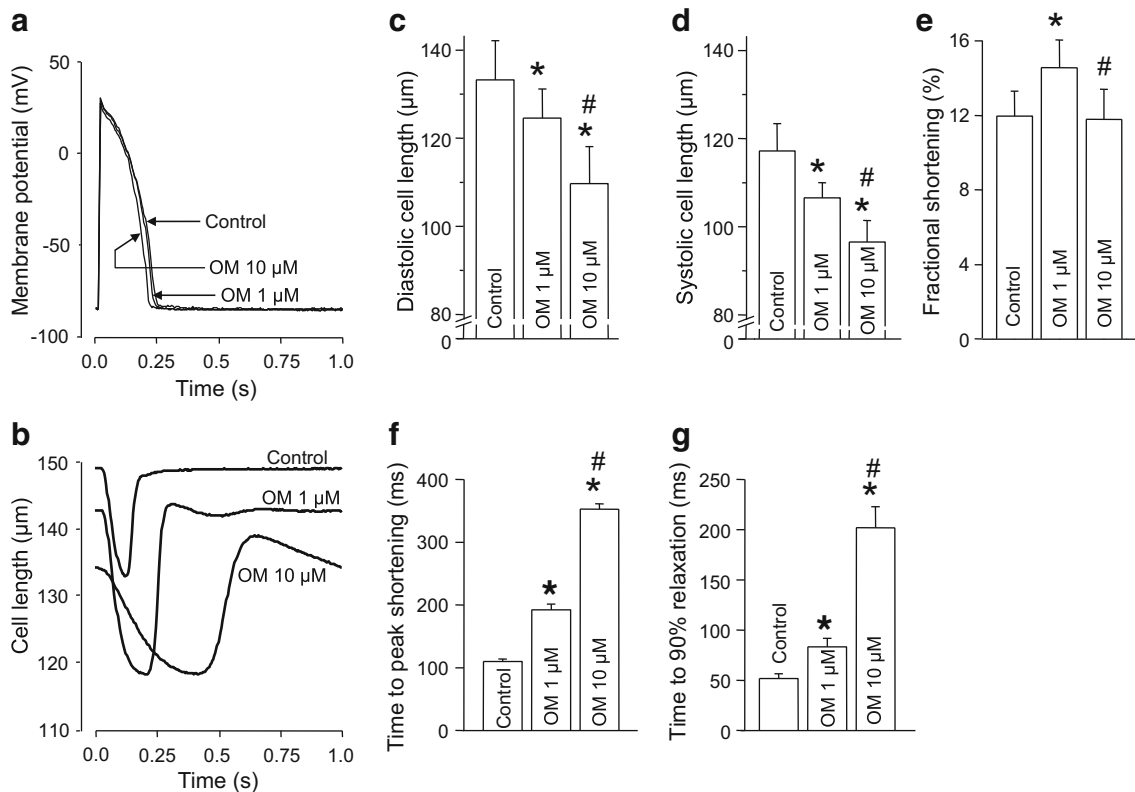
Fig. 1 Concentration-dependent effects of OM on action potential configuration and the parameters of unloaded cell shortening at 1 Hz. **a**, **b** Representative superimposed analogue records of action potentials (**a**) and unloaded cell shortening (**b**) in control and in the presence of 1 and 10 μM OM. **c**, **d** Diastolic and systolic cell length, respectively. **e** Fractional shortening where the amplitude of shortening was normalized to the resting (end-diastolic) cell length. **f**, **g** Time required to reach peak shortening and 90% of relaxation. Columns and bars denote arithmetic mean ± SEM values obtained in five cardiomyocytes. Asterisks indicate significant ($p < 0.05$) differences from control, while double crosses from 1 μM OM data

Fig. 2 Effect of 10 μM OM on intracellular Ca^{2+} transients. **a** Superimposed analogue records taken before, in the presence of, and after washing out of 10 μM OM. Intracellular Ca^{2+} is estimated as a fluorescent ratio (F_{340}/F_{380}) obtained with the Ca^{2+}-sensitive dye Fura-2. **b**–**c** Diastolic and systolic fluorescent ratios, respectively. **d** Fluorescent ratio amplitude. All these changes in fluorescence were normalized to the pre-drug control values. Symbols and bars denote arithmetic mean ± SEM values obtained in five myocytes
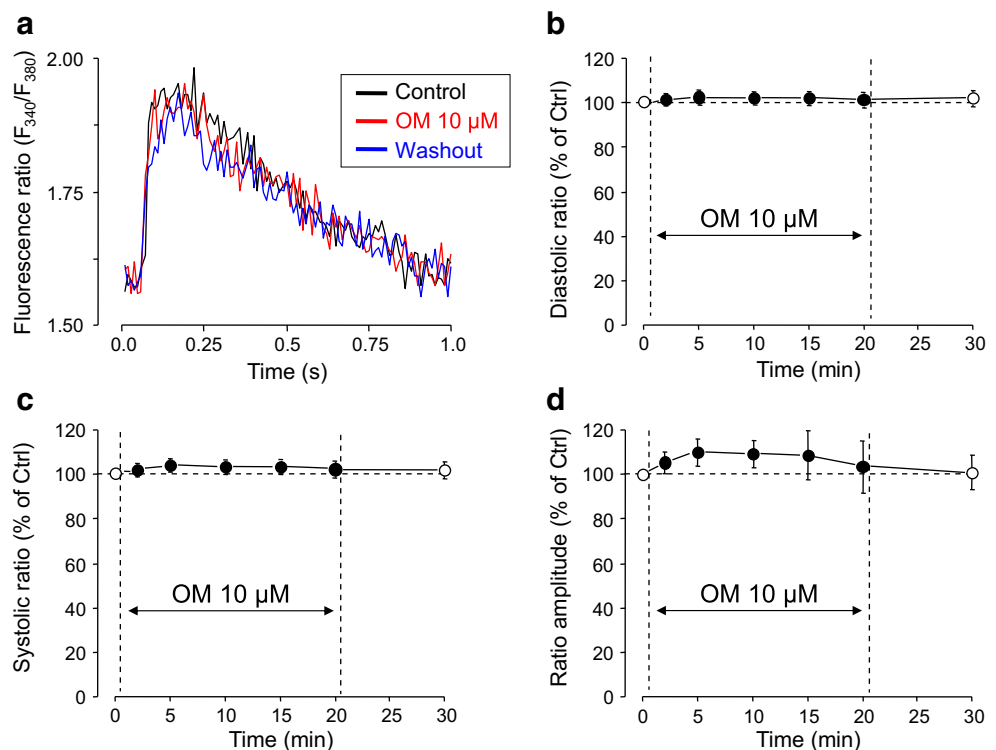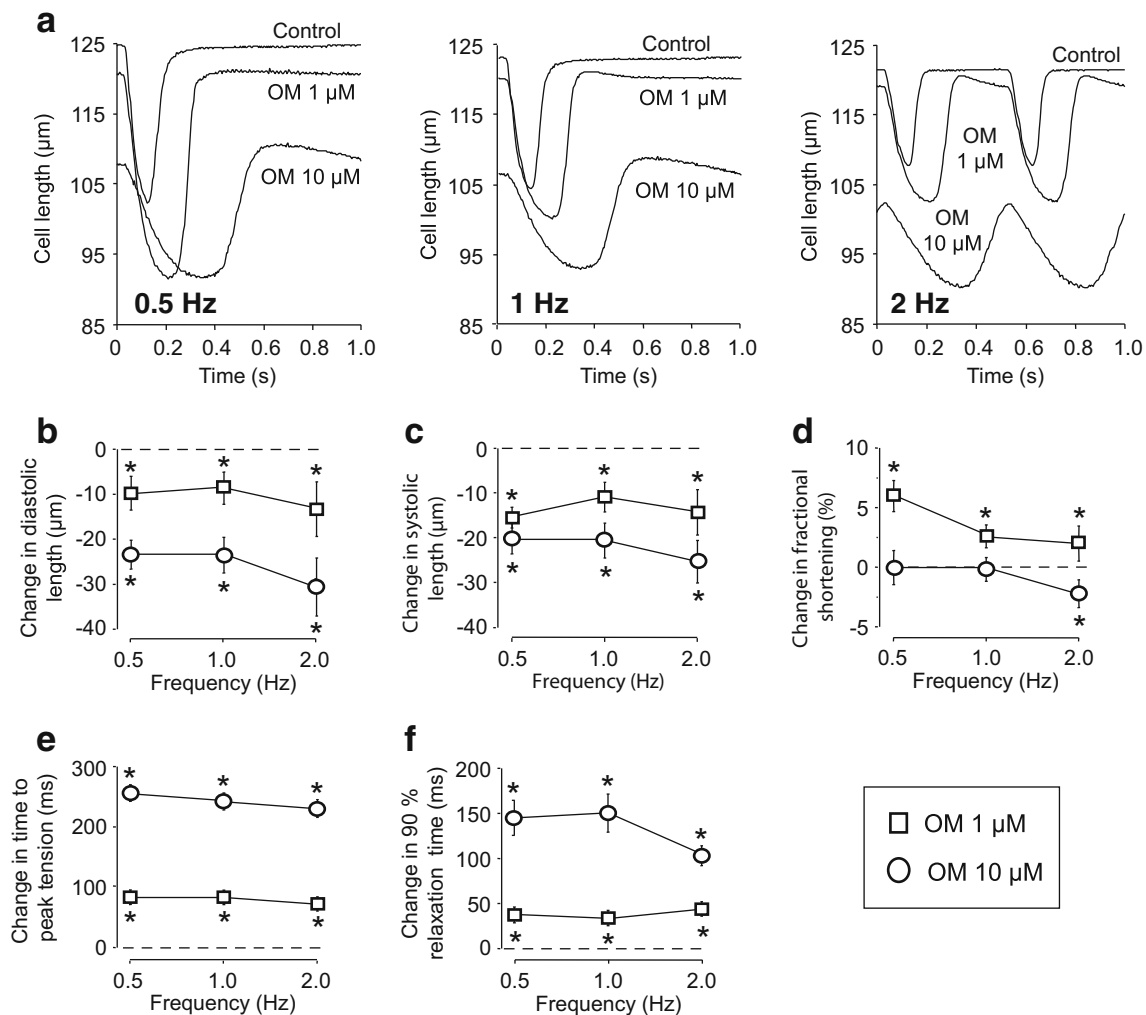
Fig. 3 Frequency-dependent effects of OM on the parameters of unloaded cell shortening. **a** Representative analogue records of unloaded cell shortening obtained at various pacing frequencies (0.5, 1, and 2 Hz) in control and in the presence of 1 and 10 μM OM. **b**–**f** Effects induced by 1 and 10 μM OM are plotted as a function of the pacing frequency. Symbols and bars denote arithmetic mean ± SEM values obtained in five myocytes; asterisks indicate significant changes ($p < 0.05$)

in isolated canine ventricular cardiomyocytes. These effects were combined with prolongation of the time required to reach peak tension and 90% relaxation, but were not accompanied by increasing the duration of action potentials or changes in cytosolic $[Ca^{2+}]_i$ transients (see Figs.1a and 2a). This latter result in our canine cardiomyocyte model confirms the previous report in rat cardiac cells (Malik et al. 2011), which preparation is known to display Ca^{2+} handling properties substantially different from those of larger mammals (Szigligeti et al. 1996). It must be noted, however, that the demonstrated lack of effect of OM on $[Ca^{2+}]_i$ has to be restricted exclusively to our experimental conditions. The effect of 10 μM OM on $[Ca^{2+}]_i$ was studied only at the frequency of 1 Hz, because high-frequency stimulation was not well tolerated by the cells in the presence of 10 μM OM. Consequently, the effect of OM on $[Ca^{2+}]_i$ at high heart rates cannot fully be excluded.

According to the present results, OM increases cell shortening without altering intracellular Ca^{2+} handling. From this

point of view, its effect is similar to that of Ca^{2+} sensitizers (Utter et al. 2015; Nagy et al. 2015; Swenson et al. 2017), although OM does not affect the Ca^{2+} sensitivity of troponin C. From this, it follows that some effects of higher OM concentrations may be similar to those of Ca^{2+} sensitizers, such as diastolic stiffness. Indeed, supratherapeutic OM plasma concentrations (above 500 ng/ml) were associated with comparable reductions of end-diastolic and end-systolic volumes (by 16 and 15 ml, respectively) in humans (Cleland et al. 2011). In line with this, the amplitude of fractional shortening—corresponding to the force of cardiac contraction—was increased only by 1 μM but not by 10 μM OM in the present study (Fig. 1e). More importantly, fractional shortening was significantly *decreased* by 10 μM OM when the pacing frequency was increased to 2 Hz (Fig. 3d), because the OM-induced decrease in diastolic cell length exceeded the reduction of the systolic value under these conditions. This is in a good agreement with the results of King et al. (2011), who demonstrated that

Fig. 4 Frequency-dependent effect of 1 μM OM on intracellular Ca^{2+} transients. **a**, **b** Diastolic and systolic fluorescent ratios, respectively. **c** Fluorescent ratio amplitude. The intracellular Ca^{2+} level is characterized by the fluorescent ratios presented on the ordinate. Symbols and bars denote arithmetic mean ± SEM values obtained in five myocytes
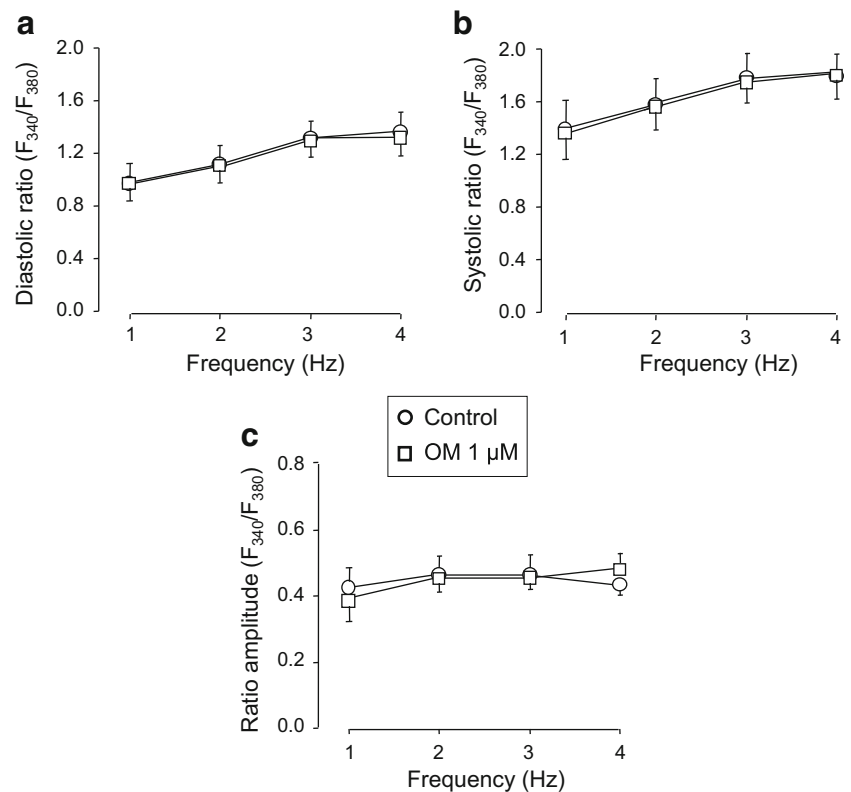

actomyosin interaction is not fully switched off during diastole, since it could be further reduced with actomyosin inhibitors. Consequently, enhancement of coupling between myosin and actin cannot be restricted to systole—it may increase also the diastolic tension resulting in the well-known in vivo consequences (Nagy et al. 2015). This is consistent with the OM-induced reduction of diastolic cell length observed in our study in absence of changes in diastolic $[Ca^{2+}]_i$.

Clinical implications

Frequency dependence of the OM effect on sarcomere shortening was found to be atypical when it was compared to that of several other inotropic agents. Similarly to our results, Butler et al. (Butler et al. 2015) reported reduction of sarcomere shortening at short cycle lengths (0.33 and 0.5 s), while augmentation was seen at longer ones (1 and 2 s) in the

Fig. 5 Overshoot of relaxation induced by 1 and 10 μM OM. As shown in panel **a**, cell length following relaxation was transiently longer than its resting length. The amplitude of overshoot (indicated by arrow) was normalized to the amplitude of cell shortening and plotted against the pacing frequency in panel **b**. Data could not be collected at the highest frequency of 2 Hz in the presence of 10 μM OM due to the extremely elongated contractions. Note that the overshoot of relaxation has never been observed in the absence of OM. Symbols and bars denote arithmetic mean ± SEM values obtained in five myocytes
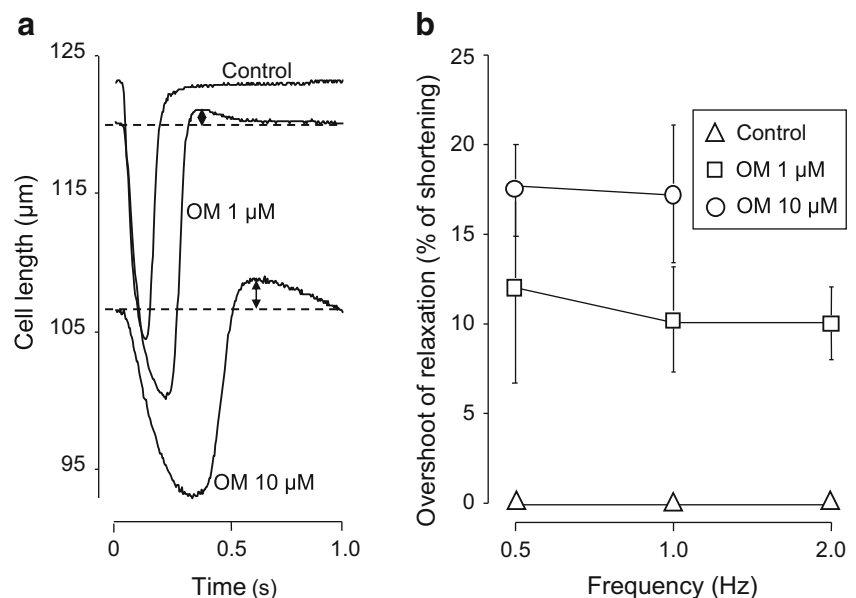
presence of 1–3 μM OM. This unusual frequency profile of the OM effect may carry important clinical implications suggesting that patients with tachycardia may be potentially more exposed to symptoms of diastolic dysfunction in the case of OM overdose. On the other hand, patients with normal heart rates may also develop occasionally tachycardic episodes resulting in a similar outcome, in spite of the fact that OM was shown to decrease the heart rate moderately (Teerlink et al. 2016a).

The clinical relevance of the present data can be estimated when comparing the applied OM concentrations to the OM plasma levels found in human studies. In an early phase II study, OM increased systolic ejection time at concentrations higher than 100 ng/ml, while stroke volume was significantly increased only above 200 ng/ml OM. This effect saturated at concentrations higher than 400 ng/ml (Cleland et al. 2011). In that study, higher OM plasma concentrations have also been reported, however, values above 1200 ng/ml were not tolerated by the patients. In another study, mean OM plasma concentrations of 295 and 550 ng/ml (depending on the dose applied) were achieved in patients with ischemic heart disease, which concentrations were well tolerated in this high-risk population of patients (Greenberg et al. 2015). Finally, in the two more recent phase II studies, performed in patients with chronic (COSMIC-HF) and acute (ATOMIC-AHF) heart failure, peak OM concentrations ranged from 200 to 300 and 100 to 500 ng/ml, respectively (Teerlink et al. 2016a; Teerlink et al. 2016b). Although these values are typically higher than the therapeutic plasma concentrations, OM was well tolerated in these studies. Considering all available results, the typical therapeutic OM plasma concentration may be lower than 1 μM, the smaller OM concentrated tested in our study, corresponding to 400 ng/ml, while the higher OM concentration of 10 μM may be achieved only as a consequence of overdose. It must be born in mind, however, that the plasma protein binding of OM was estimated 82% in humans (Palaparthy et al. 2016) which may make the comparison of present data with clinical results somewhat difficult.

Although phase II clinical studies in patients with acute and chronic heart failure yielded promising results with OM (Cleland et al. 2011; Teerlink et al. 2011; Teerlink et al. 2016a; Teerlink et al. 2016b), the final evaluation of the recently started phase III trial (GALACTIC-HF study n.d.) involving 8000 patients may be decisive regarding the concerns arose. Until this, extra care has to be taken with patients overdosed with OM, especially in tachycardic cases.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and institutional guidelines for the care and use of animals were followed. The experiments were performed according to protocols approved by the local ethical committee (license no. 18/2012/DEMÁB).

References

Butler L, Cros C, Oldman KL, Harmer AR, Pointon A, Pollard CE, Abi-Gerges N (2015) Enhanced characterization of contractility in cardiomyocytes during early drug safety assessment. Toxicol Sci 145:396–406

Cleland JGF, Teerlink JR, Senior R, Nifontov EM, McMurray JJV, Lang CC, Tsyrlin VA, Greenberg BH, Mayet J, Francis DP, Shaburishvili T, Monaghan M, Saltzberg M, Neyses L, Wasserman SM, Lee JH, Saikali KG, Clarke CP, Goldman JH, Wolff AA, Malik FI (2011) The effects of the cardiac myosin activator, omecamtiv mecarbil, on cardiac function in systolic heart failure: a double-blind, placebo-controlled, crossover, dose-ranging phase 2 trial. Lancet 378:676–683

GALACTIC-HF study (n.d.) A double blind, randomized, placebo-controlled, multicenter study to assess the efficacy and safety of omecamtiv mecarbil on mortality and morbidity in subjects with chronic heart failure with reduced ejection fraction. clinicaltrials.gov; NCT02929329

Greenberg BH, Chou W, Saikali KG, Escandón R, Lee JH, Chen MM, Treshkur T, Megreladze I, Wasserman SM, Eisenberg P, Malik FI, Wolff AA, Shaburishvili T (2015) Safety and tolerability of omecamtiv mecarbil during exercise in patients with ischemic cardiomyopathy and angina. JACC Heart Fail 3:22–29

James J, Robbins J (2011) At the source: treating heart failure by altering muscle motor function. Circ Res 109:5–7

King NM, Methawasin M, Nedrud J, Harrell N, Chung CS, Helmes M, Granzier H (2011) Mouse intact cardiac myocyte mechanics: cross-bridge and titin-based stress in unactivated cells. J Gen Physiol 137:81–91

Kistamás K, Szentandrássy N, Hegyi B, Váczi K, Ruzsnavszky F, Horváth B, Bányász T, Nánási PP, Magyar J (2015) Changes in intracellular calcium concentration influence beat-to-beat variability of action potential duration in canine ventricular myocytes. J Physiol Pharmacol 66:73–81

Liu Y, White HD, Belknap B, Winkelmann DA, Forgacs E (2015) Omecamtiv mecarbil modulates the kinetic and motile properties of porcine β-cardiac myosin. Biochemistry 54:1963–1975

Magyar J, Bányász T, Zs B, Pacher P, Szentandrássy N, Fülöp L, Kecskeméti V, Nánási PP (2002) Electrophysiological effects of risperidone in mammalian cardiac cells. Naunyn Schmiedeberg's Arch Pharmacol 366:350–356

Magyar J, Szentandrássy N, Bányász T, Kecskeméti V, Nánási PP (2004) Effects of norfluoxetine on the action potential and transmembrane ion currents in canine ventricular cardiomyocytes. Naunyn Schmiedeberg's Arch Pharmacol 370:203–210

Malik FI, Morgan BP (2011) Cardiac myosin activation part 1: from concept to clinic. J Mol Cell Cardiol 51:454–461

Malik FI, Hartman JJ, Elias KA, Morgan BP, Rodriguez H, Brejc K, Anderson RL, Sueoka SH, Lee KH, Finer JT, Sakowicz R, Baliga R, Cox DR, Garard M, Godinez G, Kawas R, Kraynack E, Lenzi D, Lu PP, Muci A, Niu C, Qian X, Pierce DW, Pokrovskii M, Suehiro I, Sylvester S, Tochimoto T, Valdez C, Wang W, Katori T, Kass DA, Shen YT, Vatner SF, Morgans DJ (2011) Cardiac myosin activation: a potential therapeutic approach for systolic heart failure. Science 331:1439–1443

Nagy L, Kovács Á, Bódi B, Pásztor ET, Fülöp GÁ, Tóth A, Édes I, Papp Z (2015) The novel cardiac myosin activator omecamtiv mecarbil increases the calcium sensitivity of force production in isolated cardiomyocytes and skeletal muscle fibres of the rat. Br J Pharmacol 172:4506–4518

Palaparthy R, Banfield C, Alvarez P, Yan L, Smith B, Johnson J, Monsalvo ML, Malik F (2016) Relative bioavailability, food effect, and safety of the single-dose pharmacokinetics of omecamtiv mecarbil following administration of different modified-release formulations in healthy subjects. Int J Clin Pharmacol Ther 54:217–227

Ruzsnavszky F, Hegyi B, Kistamás K, Váczi K, Horváth B, Szentandrássy N, Bányász T, Nánási PP, Magyar J (2014) Asynchronous activation of calcium and potassium currents by isoproterenol in canine ventricular myocytes. Naunyn Schmiedeberg's Arch Pharmacol 387:457–467

Shen Y-T, Malik FI, Zhao X, Depre C, Dhar SK, Abarzua P, Morgans DJ, Vatner SF (2010) Improvement of cardiac function by a cardiac myosin activator in conscious dogs with systolic heart failure. Circ Heart Fail 3:522–527

Simkó J, Szentandrássy N, Harmati G, Bárándi L, Horváth B, Magyar J, Bányász T, Lőrincz I, Nánási PP (2010) Effects of ropinirole on action potential characteristics and the underlying ion currents in canine ventricular myocytes. Naunyn Schmiedeberg's Arch Pharmacol 382:213–220

Swenson AM, Tang XW, Blair CA, Fetrow CM, Unrath WC, Previs MJ, Campbell KS, Yengo CM (2017) Omecamtiv mecarbil enhances the duty ratio of human cardiac myosin resulting in increased calcium sensitivity and slowed force development in cardiac muscle. J Biol Chem 292:3768–3778

Szabó G, Szentandrássy N, Bíró T, Tóth BI, Czifra G, Magyar J, Bányász T, Varró A, Kovács L, Nánási PP (2005) Asymmetrical distribution of ion channels in canine and human left ventricular wall: epicardium versus midmyocardium. Pflugers Arch 450:307–316

Szabó L, Szentandrássy N, Kistamás K, Hegyi B, Ruzsnavszky F, Váczi K, Horváth B, Magyar J, Bányász T, Pál B, Nánási PP (2013) Effects of tacrolimus on action potential configuration and transmembrane ion currents in canine ventricular cells. Naunyn Schmiedeberg's Arch Pharmacol 386:239–246

Szentandrássy N, Bányász T, Bíró T, Szabó G, Tóth BI, Magyar J, Lázár J, Varró A, Kovács L, Nánási PP (2005) Apico-basal inhomogeneity in distribution of ion channels in canine and human ventricular myocardium. Cardiovasc Res 65:851–860

Szentandrássy N, Horváth B, Váczi K, Kistamás K, Masuda L, Magyar J, Bányász T, Papp Z, Nánási PP (2016) Dose-dependent electrophysiological effects of the myosin activator omecamtiv mecarbil in canine ventricular cardiomyocytes. J Physiol Pharmacol 67:483–489

Szigligeti P, Pankucsi C, Bányász T, Varró A, Nánási PP (1996) Action potential duration and force-frequency relationship in isolated rabbit, guinea pig and rat cardiac muscle. J Comp Physiol B 166:150–155

Teerlink JR (2009) A novel approach to improve cardiac performance: cardiac myosin activators. Heart Fail Rev 14:289–298

Teerlink JR, Metra M, Zaca V, Sabbah HN, Cotter G, Gheorghiade M, Cas LD (2009) Agents with inotropic properties for the management of acute heart failure syndromes. Traditional agents and beyond. Heart Fail Rev 14:243–253

Teerlink JR, Clarke CP, Saikali KG, Lee JH, Chen MM, Escandon RD, Elliott L, Bee R, Habibzadeh MR, Goldman JH, Schiller NB, Malik FI, Wolff AA (2011) Dose-dependent augmentation of cardiac systolic function with the selective cardiac myosin activator, omecamtiv mecarbil: a first-in-man study. Lancet 378:667–675

Teerlink JR, Felker GM, McMurray JJ, Solomon SD, Adams KF Jr, Cleland JG, Ezekowitz JA, Goudev A, Macdonald P, Metra M, Mitrovic V, Ponikowski P, Serpytis P, Spinar J, Tomcsányi J, Vandekerckhove HJ, Voors AA, Monsalvo ML, Johnston J, Malik FI, Honarpour N, COSMIC-HF Investigators (2016b) Chronic Oral Study of Myosin Activation to Increase Contractility in Heart Failure (COSMIC-HF): a phase 2, pharmacokinetic, randomised, placebo-controlled trial. Lancet 388:2895–2903

Teerlink JR, Felker GM, McMurray JJV, Ponikowski P, Metra M, Filippatos GS, Ezekowitz JA, Dickstein K, Cleland JGF, Kim JB, Lei L, Knusel B, Wolff AA, Malik FI, Wasserman SM, on behalf of the ATOMIC-AHF Investigators (2016a) Acute treatment with omecamtiv mecarbil to increase contractility in acute heart failure: the ATOMIC-AHF study. J Am Coll Cardiol 67:1444–1455

Utter MS, Ryba DM, Li BH, Wolska BM, Solaro RJ (2015) Omecamtiv mecarbil, a cardiac myosin activator, increases Ca^{2+} sensitivity in myofilaments with a dilated cardiomyopathy mutant tropomyosin E54K. J Cardiovasc Pharmacol 66:347–353

Váczi K, Hegyi B, Ruzsnavszky F, Kistamás K, Horváth B, Bányász T, Nánási PP, Szentandrássy N, Magyar J (2015) 9-Anthracene carboxylic acid is more suitable than DIDS for characterization of calcium-activated chloride current during canine ventricular action potential. Naunyn Schmiedeberg's Arch Pharmacol 388:87–100