The myosin activator omecamtiv mecarbil: a promising new inotropic agent

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Abstract

Heart failure became a leading cause of mortality in the past decades with a progressively increasing prevalence. Its current therapy is restricted largely to the suppression of the sympathetic activity and the renin-angiotensin system in combination with diuretics. This restrictive strategy is due to the potential long term adverse effects of inotropic agents despite their effective influence on cardiac function when employed for short durations. Positive inotropes include inhibitors of the Na^+/K^+ pump, β-receptor agonists and phosphodiesterase inhibitors. Theoretically, Ca^{2+} sensitizers may also increase cardiac contractility without resulting in Ca^{2+} overload nevertheless their mechanism of action is frequently complicated by other pleiotropic effects. Recently a new positive inotropic agent, the myosin activator omecamtiv mecarbil has been developed. Omecamtiv mecarbil binds directly to β-myosin heavy chain and enhances cardiac contractility by increasing the number of the active force-generating cross-bridges, presumably without major off-target effects. This review focuses on recent *in vivo* and *in vitro* results obtained with omecamtiv mecarbil, and discusses its mechanism of action at a molecular level. Based on clinical data, omecamtiv mecarbil is a promising new tool in the treatment of systolic heart failure.

Keywords: inotropic agents, calcium sensitizer, cardiac myosin activator, omecamtiv mecarbil, CK-1827452, cardiac contractility, heart failure.

Current therapeutic strategies against heart failure

Heart failure is diagnosed when the heart is not able to provide the required amount of cardiac output. Heart failure can be categorized from several viewpoints, *e.g.*: systolic *vs*. diastolic, left *vs.* right heart, compensated *vs*. decompensated, and by well-defined criteria for its progression (Felker et al. 2003). Regarding the therapeutic implications, the sharpest difference is between the acute and the chronic forms of heart failure, since in the former case the pump function of the heart has to be effectively facilitated using inotropic agents, regardless of the potential long-term shortcomings, while in the latter case the strategy may be the opposite, i.e. suppression of the neurohormonal compensation may increase the life span and improve the quality of life (Bristow 2000; Middlekauff and Mark 1998).

In spite of the relative therapeutic success in the field of chronic heart failure in the past decades, little progress is seen in the treatment of acute heart failure. The potential positive inotropic strategies are displayed in Fig. 1. The first agents being applied to improve cardiac performance were digitalis glycosides. These agents suppress the activity of the Na^+/K^+ pump in the surface membrane resulting in a reduced transmembrane Na^+ gradient, which in turn leads to cellular Ca^{2+} overload *via* the Na^+/Ca^{2+} exchange (Altamirano et al. 2006; Lee 1985). In spite of the beneficial acute effects of these glycosides, long term improvement of contractility has been questioned (The Digitalis Investigation Group 1997), and more importantly, they displayed a significant proarrhythmic activity due to the possibility of Ca^{2+} overload. Indeed, development of delayed afterdepolarizations has frequently been reported in digitalis-intoxicated patients (January and Fozzard 1988; Kass et al. 1978).

Positive inotropic action can be evoked by activation of the cardiac β-adrenergic receptors, since this is the natural way to increase the cardiac output through an increase in heart rate, positive inotropy and lusitropy. Ca^{2+} entry into the cardiomyocytes is strongly enhanced by the prtotein kinase A-dependent phosphorylation of L-type Ca^{2+} channes (Kamp and Hell 2000; Van der Heyden et al. 2005). The increased rate of Ca^{2+} sequestration into the SR, due to phosphorylation of phospholamban by protein kinase A is also crucial, since it results in reduction of diastolic but elevation of systolic Ca^{2+} concentration due to the increased Ca^{2+} content of the SR in addition to the positive effect of synchronization of SR Ca^{2+} release by β-adrenergic stimulation (Frank and Kranias 2000; Song et al. 2001).

Unfortunately, sympathomimetic agents are also strongly proarrhythmic due to several reasons. First of all, intracellular Ca^{2+} concentration is increased, which may generate delayed afterdepolarizations (Fozzard 1992; Tweedie et al. 2000). Furthermore, early afterdepolarizations manifested in torsade de pointes type serious cardiac arrhythmias are also generated by β-adrenergic activators under *in vitro* as well as *in vivo* conditions (January and Riddle 1990; Priori and Corr 1990). This is why β_1-receptors are better to be suppressed than activated at longer time scale. Secondly, β-adrenergic activation increases myocardial oxygen consumption, which is unfavorable for the failing heart (Neubauer et al. 1994). However, selective activation of β_3-adrenergic receptors, acting through the NO/cGMP pathway, may diminish the unfavorable consequences of cardiac remodeling, and thus may be useful in treatment of heart failure (Belge et al. 2014).

Similarly to β-adrenergic activation, inhibition of the phosphodiesterase enzyme is also a suitable tool to increase the contractile force, since the cAMP level can be increased either by increased production or decreased demolition of cAMP. Amrinone and milrinone were the first molecules applied as positive inotropes acting by PDE inhibition (Honerjäger 1989). However, there are two major problems with this strategy. The first is largely identical with the application of β-adrenergic agonists, i.e. the resultant Ca^{2+} overload increases arrhythmia propensity (Fozzard 1992; Priori and Corr 1990). Secondly, since cardiomyocytes may contain several phosphodiesterase isoenzymes, each having its characteristic signal transduction pathway and compartmentalization profile, selective inhibition of one single isoenzyme is possibly required (Steinberg and Brunton 2001; Takimoto et al. 2007; Vandecasteele et al. 2006). Suppression of the PDE-III isoform was widely used in heart failure with variable success.

In addition to the increased arrhythmia propensity due the resultant Ca^{2+} overload, common problem with β-adrenergic agonists and PDE inhibitors is that they increase the ATP utilization of working myocardium. This is because of the increased rate of Ca^{2+} cycling, *i.e.* more Ca^{2+} has to be actively removed from the cytoplasm under these conditions within a certain period of time. This may be unfavorable in chronic heart failure, since the failing heart is in poor metabolic conditions (Ardehali et al. 2012). Recently a new strategy of "RyR_2 stabilization" has emerged. This is based on the fact that in some forms of heart failure the RyR_2 is leaky allowing Ca^{2+} to leak out of the SR during diastole (Shannon and Lew 2009; Wehrens and Marks 2003). This strongly deteriorates the pump function because of the increased diastolic Ca^{2+} level (diastolic

failure) combined with the reduced SR Ca^{2+} content (systolic failure). Ryanodine receptor stabilizers - by suppressing this diastolic leak - are believed to improve the contractile performance and reduce the energy demand of the heart (Ezekowitz 2013; Kaneko 1994).

An alternative approach of inotropic strategy is to increase the "efficacy" of the Ca^{2+} signal, i.e. more force to be generated by a given rise of cytosolic Ca^{2+}, or less Ca^{2+} should be enough to generate a certain level of force (Endoh 2001). Increasing the affinity of troponin C to Ca^{2+}, Ca^{2+} sensitizers improve the mechanical performance of the myocardium without increasing markedly its oxygen demand (Follath 2009; Lehmann et al. 2003). Since - at least theoretically - Ca^{2+} cycling is not enhanced, intracellular Ca^{2+} level and the concomitant arrhythmia incidence should not be elevated either (Endoh 2008). Pimobendan and levosimendan, the best known representatives of conventional Ca^{2+} sensitizers, display also PDE inhibitory actions (Endoh 2002). Theoretically, the combination of Ca^{2+} sensitizer and PDE inhibitory effects may be clinically beneficial due to a PDE dependent counteraction of a predictable diastolic dysfunction caused by pure Ca^{2+} sensitization (Endoh 2001). The hemodynamic effects of levosimendan are further complicated as this drug opens ATP-sensitive K^+ channels in vascular smooth muscle cells resulting in vasodilation (Papp et al. 2012). Furthermore, activation of these channels located in the mitochondrial membrane has been shown to display cardioprotective effects (Papp et al. 2012). Collectively, all the above mechanisms are likely to contribute to the therapeutic effects of levosimendan. The term positive inotropy with a "downstream mechanism" of action refers to a special way of Ca^{2+} sensitization, *i.e.* when myocardial systolic force is enhanced the modifications of the molecular events of the actin-myosin cycle directly (Endoh 2008).

Myosin activators - new strategy for increasing contractility

As it was shown in the previous chapter, each of the known inotropic strategies suffers from more or less drawbacks. The ideal inotropic agent is expected (1) to enhance cardiac contractility selectively (i.e. having positive inotropic and lusitropic actions without chronotropic, dromotropic or bathmotropic responses), (2) not to increase the energy demand of the heart, and most importantly (3) not to increase arrhythmia propensity (Campia et al. 2010). It is also useful if its positive inotropic action is augmented under pathological conditions, e.g. in a failing heart. Since these goals could not be fully achieved by interventions acting upstream to the Ca^{2+}-troponin binding, the research began to focus on the downstream steps. Ca^{2+} binding to troponin C engages a set of protein-protein interactions resulting finally in force generation. Indeed, interactions with these steps may provide several options to regulate contractility (Solaro 2009; Solaro 2010; Sun et al. 2008). Probably the best of these strategies may be the application of myosin activators which increase the force of contraction by interacting directly with the myosin heavy chain, as omecamtiv mecarbil, the first selective cardiac myosin activator, does (Morgan et al. 2010).

The first molecule screened out for increasing the ATPase activity of myosin was CK-0156636. Substitution of the nitrate group for fluorine in CK-1032100 improved the water solubility of the molecule diminishing this way the binding to plasma proteins. CK-1122534 was the fist agent to increase the fractional shortening in rat ventricular myocardium, however, the ATP-sensitive K^+ channels were also activated by the compound. CK-1213296 was free of this effect, but inhibited the CYP 1A2 enzyme. All

side-effects were successfully eliminated from CK-1317138, and the optimized structure was achieved by CK-1827452, omecamtiv mecarbil (Morgan et al. 2010)**.** This latter molecule was more potent by one order of magnitude than its ancestor, CK-1317138 (Malik and Morgan 2011).

In vitro **and** ***in vivo*** **effects of omecamtiv mecarbil**

Omecamtiv mecarbil displays its positive inotropic effect by selective binding to the S1 domain of the cardiac β-myosin heavy chain where the relay helix and converter domain converge at the base of the force-producing lever arm. Binding of omecamtiv mecarbil to this site results in a conformational change in the nucleotide-binding domain of the myosin head contributing to the allosteric activation of its enzymatic and mechanical properties (Malik and Morgan 2011). As a consequence of allosteric modulation of the nucleotide-binding domain of myosin, omecamtiv mecarbil was shown to accelerate the release of inorganic phosphate, which is the rate-limiting step of the actomyosin cycle, due to the increased ATPase activity of the myosin heavy chain, thereby accelerating the transition rate from the weakly bound to the strongly bound configuration of actin associated myosin heads by decreasing the energy barrier between them (Malik et al. 2011). This effect results in an increased number of force-generating cross-bridges within the sarcomere and associated with the enhancement of force generation (Malik et al. 2011; Teerlink 2009). Experiments performed in heterologously reconstituted combinations of actin-myosin systems revealed that the stimulation of ATPase by omecamtiv mecarbil occurs exclusively in the presence of the cardiac and slow skeletal muscle myosin isoforms (α, β) independently of the origin of the thin filament. Healthy

human hearts contain dominantly the β and to a lesser extent the α isoform, while α isoform practically disappears from the failing heart. Myosins from fast skeletal or smooth muscles are not activated by omecamtiv mecarbil suggesting a "quasi" cardiospecific binding of the drug (Leinwand and Moss 2011; Malik et al. 2011; Teerlink 2009). In fact, omecamtiv mecarbil was shown to increase the contractility in slow skeletal muscle fibers (e.g. diaphragm) which may widen the potential therapeutic spectrum of the agent (Nagy et al. 2015). Omecamtiv mecarbil, in line with stabilization of the "strongly bound" configuration of myosin heads, reduced unloaded shortening velocity of porcine and human ventricular myosins when monitored by an *in vitro* mobility assay (Aksel et al. 2015; Liu et al. 2015; Wang et al. 2014). Since this effect was equally observed with α and β isoforms (Aksel et al. 2015), both atrial and ventricular contractility can readily be improved by omecamtiv mecarbil.

Increasing the ATPase activity of the myosin heads is expected to increase cardiac oxygen consumption. Since omecamtiv mecarbil fails to increase (actually decreases) the actin-independent release of inorganic phosphate, the overall oxygen consumption of the heart is not expected to increase by the drug (Teerlink 2009). In absence of the extra energy demand related to an enhanced Ca^{2+} cycling, cardiac energy utilization can be more effective in the presence of omecamtiv mecarbil (Shen et al. 2010). Moreover, the mammalian heart is apparently robust enough to tolerate myosin motor tuning during long term administrations (James and Robbins 2011). Transgenic rabbits containing α and β isoforms of myosin at a ratio of 1:1 failed to show symptoms of cardiomyopathy, they were rather cardioprotected in an overdrive-induced heart failure model (James et al.

2005). In addition, the lifespan of these transgenic animals was similar to their normal littermates (James and Robbins 2011).

Experiments studying the effects of omecamtiv mecarbil at a cellular level revealed the enhancement the fractional shortening in isolated rat cardiomyocytes without any alterations in the Ca^{2+} handling monitored by fluorescent Ca^{2+} indicators. Importantly, myosin activation resulted in an increase in both the amplitude and the duration of contractions (Malik et al. 2011). Omecamtiv mecarbil was also effective under *in vivo* conditions when the fractional shortening was monitored by echocardiography in anesthetized dogs and rats. Interestingly, canine hearts were more sensitive to omecamtiv mecarbil than rat hearts (Malik et al. 2011).

In an *in vivo* canine model of pacing-induced systolic heart failure after myocardial infarction or chronic pressure overload, omecamtiv mecarbil infusion was shown to enhance left ventricular stroke volume, cardiac output and systolic ejection time in addition to reduction of heart rate, total peripheral resistance and loading pressures (Shen et al. 2010; Teerlink 2009). On the other hand, myocardial O_2 consumption and the rate of pressure development were not affected by the drug when compared with traditional inotropes (Shen et al. 2010). Furthermore, omecamtiv mecarbil produced larger augmentation of the systolic performance in failing canine hearts than in healthy animals (Shen et al. 2010). In contrast to other inotropic agents, including catecholamines, the inotropic action of omecamtiv mecarbil was enhanced at longer while reduced at shorter pacing cycle lengths in unloaded canine cardiomyocytes (Butler et al. 2015).

It is important to bear in mind that any increase in the systolic ejection time may be displayed only at the expense of diastole, impeding thereby the ventricular filling as well as the coronary flow. However, since intravenous administration of omecamtiv mecarbil was reported to decrease heart rate, moderate improvements of systolic emptying should not dramatically compromise diastolic function or coronary flow (Dickstein 2011; Nagy et al. 2014).

Surprisingly, no cellular electrophysiological results have been reported with omecamtiv mecarbil so far. However, accordingly to our preliminary unpublished results, relatively high (10 μM) concentration of omecamtiv mecarbil caused small but statistically significant changes in canine ventricular action potential configuration as demonstrated in Fig. 2. These changes included the reduction of phase-1 repolarization, suppression of the plateau amplitude and shortening of action potential duration. Although regarding the involvement of the underlying ion currents we can only speculate in absence of relevant voltage clamp data, the smaller phase-1 amplitude indicates a reduction of the transient outward current (I_{to}), while depression of the plateau potential is congruent with a decreased L-type Ca^{2+} current. Clearly, further electrophysiological studies are required to map all possible effects of omecamtiv mecarbil on cardiac ion currents, since even relatively small shifts in these currents may be proarrhythmic in certain groups of sensitive patients.

Clinical trials

Based on promising preclinical results obtained with the myosin activator omecamtiv mecarbil (Meijs 2012), the drug was tested in a phase I study (Teerlink et al. 2011). The

primary goal was to determine the maximum-tolerated dose and plasma concentrations of the drug applied intravenously for a period of 6 hours, once a week for 4 weeks in 34 healthy volunteers. The secondary goal was the evaluation of pharmacodynamic properties of the effects on left ventricular systolic function assessed by transthoracic echocardiography, safety and tolerability (Teerlink et al. 2011). In this first-in-men dose-escalating study, omecamtiv mecarbil was shown to effectively enhance left ventricular systolic function in a dose-dependent manner applying doses of 0.005–1 mg/kg/h. Within this range a linear dose-related correlation was found between the plasma concentrations and systolic ejection time. Improvement of the systolic performance of the heart was not associated with impairment of diastolic function. The maximum-tolerated dose was 0.5 mg/kg/h; no adverse effect was observed at this concentration. The dose-limiting toxic effect was myocardial ischemia due to the prolongation of the systolic ejection time at the expense of diastole (Teerlink 2009; Teerlink et al. 2009; Teerlink et al. 2011).

A double-blind, randomized, placebo-controlled, dose-ranging phase II trial was performed in 45 patients with stable heart failure and left ventricular systolic dysfunction treated with ACE inhibitor (Cleland et al. 2011). The primary aim was to study the safety and tolerability of the drug in this group of patients, who received omecamtiv mecarbil infusion for 2, 24, or 72 hours. Omecamtiv mecarbil increased left ventricular ejection time by up to 80 ms from the baseline value, while stroke volume was increased by up to 9.7 ml. These changes were accompanied by reduction of heart rate by up to 2.7 beats/min. Higher plasma concentrations resulted a decrease in end-systolic and end-diastolic volumes by 15 and 16 ml, respectively (Cleland et al. 2011). Comparing these results to those obtained from phase I trial, it can be concluded that beneficial effects of

omecamtiv mecarbil are comparable in healthy individuals and heart failure patients. The well-tolerated plasma concentration was within the range of 0.1-1.2 μg/ml. At higher plasma levels (1.35 and 1.75 μg/ml), however, two patients complained for consequences of myocardial ischemia due to the excessive prolongation of systolic ejection time (Cleland et al. 2011).

A randomized, controlled phase IIb trial was performed to evaluate the safety and efficacy of omecamtiv mecarbil in patients suffering from acute heart failure (ATOMIC-AHF, 2014). This study revealed that the omecamtiv mecarbil-induced myosin activation failed to meet the primary end point since no significant effect on dyspnea could be demonstrated. It was finally concluded that the administration of omecamtiv mecarbil is clinically safe, and the results showed a tendency of slowing the progression of heart failure (ATOMIC-AHF, 2014; Garg and Frishman 2013; Valentova and von Haehling 2014).

Recently, omecamtiv mecarbil was studied in a set of patients hospitalized with cardiomyopathy combined with ischemic heart disease (angina pectoris) in order to monitor the safety and tolerability of the agent in ischemic cardiomyopathies (Greenberg et al. 2015). This combination was critically important, since myocardial ischemia due to the excessive prolongation of systolic ejection time has already been observed in case of overdose. In this double-blind, placebo-controlled study the effect of 20 hours infusion with omecamtiv mecarbil was evaluated on the cardiac performance during a treadmill test. The results indicate that omecamtiv mecarbil at doses producing plasma concentrations sufficient to effectively increase systolic function is well tolerated during physical exercise even in patients with ischemic cardiomyopathy and angina (Greenberg

et al. 2015). Since at higher concentrations (i.e. in case of overdose intoxication) these ischemic consequences may really be anticipated, extra caution has to be applied with the dosage of omecamtiv mecarbil due to the unknown magnitude of interpersonal differences in drug-sensitivity and pharmacokinetics.

Data from 3 clinical trials with omecamtiv mecarbil were analyzed using a nonlinear mixed-effects model to investigate pharmacokinetic properties of the drug and the relationship between plasma concentration and left ventricular outflow tract stroke volume and systolic ejection time (Vu et al. 2015). Oral absorption half-life of the drug was 0.62 hour and absolute bioavailability was estimated as 90%, while the elimination half-life was 18.5 hour. Plasma concentrations were directly correlated with increases in stroke volume and ejection time in healthy volunteers as well as in patients with heart failure. Model-based simulations for several immediate-release oral dose regimens (37.5, 50 and 62.5 mg doses every 8, 12 and 24 hours) showed that a pharmacodynamic effect could be maintained in the absence of excessive omecamtiv mecarbil plasma concentrations (Vu et al. 2015).

Taken all data together, omecamtiv mecarbil seems to be a very promising approach for the treatment of systolic heart failure indicating that the strategy of myosin activation may be effectively translated into the clinical practice (Nagy et al. 2014).

Perspectives

In light of the results discussed above the group of Ca^{2+} sensitizer agents provides the most suitable therapy for both acute and chronic heart failure - at least according to our present knowledge. The conventional approach of increasing the Ca^{2+} cycling in

myocytes was not supported by positive therapeutic experience. The reasons are clearly the increased energy consumption and arrhythmia propensity. Ca^{2+} sensitizers - theoretically - should be free of these side-effects. In case of acute heart failure Ca^{2+} sensitizers alone may probably be the main-stream therapy in the future. In chronic heart failure application of these drugs, in combination with the conventional β-blocker + ACE inhibitor + diuretic therapy, gives a chance to the "pharmacologically suppressed" heart to enhance its mechanical performance, which may significantly improve the quality of life of the patients. Since omecamtiv mecarbil can be considered as a representative of a novel group of Ca^{2+} sensitizers, the question whether conventional Ca^{2+} sensitizers or myosin activators will provide better therapeutic results has to be answered with no doubt (Spinarova and Spinar 2015). Further clinical and preclinical studies are required to reveal and to discriminate between their potential effects on cardiac oxygen consumption and cardiac rhythm during various cardiac pathologies (Givertz et al. 2013; Hasenfuss and Teerlink 2011; Kehat 2012; Rajapreyar et al. 2014; Selby and Teerlink 2013; Tarone et al. 2014). Properly designed clinical trials will hopefully also help to reach final conclusions. The most dangerous potential pitfall with omecamtiv mecarbil may be the compromised diastolic filling (Bers and Harris 2011) which is not compensated by suppressed PDE activity as in the case of other Ca^{2+} sensitizers.

As previously mentioned, no change in intracellular Ca^{2+} transients was observed with omecamtiv mecarbil in ventricular myocytes of the rat (Malik et al. 2011). This was considered as a strong argument supporting the pure "downstream" mechanism of action of the drug (i.e. the lack of action on Ca^{2+} release and Ca^{2+} reuptake). Unfortunately, the rat is not an ideal experimental model from this point of view, because neither

electrophysiological properties, nor the Ca^{2+} handling in rat is similar to that found in larger mammals including humans. This doubt can easily be eliminated by testing omecamtiv mecarbil on Ca^{2+} handling in larger mammals.

The cardioselective nature of the action of omecamtiv mecarbil is usually mentioned as an advantage, since myosins from fast skeletal and smooth muscles are not affected. However, the potential therapeutic benefits of omecamtiv mecarbil, related to facilitation of slow skeletal muscle contractility, cannot be overemphasized (Bers and Harris 2011; Leinwand and Moss 2011; Nagy et al. 2015). Slow-twitch skeletal muscles, such as the diaphragm, are equipped with a set of myosins similar to that found in the heart. Therefore omecamtiv mecarbil is likely to improve the mechanical performance of the diaphragm (and other slow-twitch muscles in the body), which may be important for patients on ventilators or healthy individuals in elderly (Bers and Harris 2011). In support of this idea omecamtiv mecarbil was shown to facilitate contractility in rat diaphragm similarly to cardiac myocytes. These experiments clearly indicated the Ca^{2+} sensitizer properties of omecamtiv mecarbil based on slowing the activation-relaxation kinetics (Nagy et al. 2015).

Since omecamtiv mecarbil is selectively bound to cardiac myosins, it can be used for labeling in order to visualize the myosin heads in the heart. Indeed, ^{18}F-labeled analogues of omecamtiv mecarbil were applied for cardiac myosin imaging under conditions of positron emission tomography (Zhang et al. 2013). This technique - when combined with sarcomere length nanometry (Shintani et al. 2014) - may give a new insight into the mechanics of the contractile machinery.

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Figure legends

Fig. 1. Ca^{2+} handling and positive inotropic straregies in the heart. Red arrows indicate transmembrane and intracellular Ca^{2+} movements, including the L-type Ca^{2+} channel, sarcolemmal Ca^{2+} pump, Na^{+}/Ca^{2+} exchanger, RyR_2 receptor and SERCA pump. Blue arrows show sites of possible inotropic interventions (see details in the text).

Fig. 2. Effects of omecamtiv mecarbil in enzymatically isolated canine ventricular myocytes. **A**: Representative superimposed action potentials recorded at 37 ^{o}C using a sharp 3M KCl-containing microelectrode in Tyrode solution (Control), after superfusion with 10 μM omecamtiv mecarbil (OM) for 10 min, and following the washout of the drug. **B**: Average results obtained with 10 μM omecamtiv mecarbil in 14 canine ventricular cells. Phase-1 amplitude was defined as a difference between overshoot potential and voltage of the notch. Plateau amplitude vas measured as the amplitude of the plateau (the actual membrane potential *minus* resting potential) obtained at the time of 50% repolarization. Action potential duration was determined at 90% level of repolarization. Columns and bars indicate mean ± SEM values, respectively, asterisks denote significant ($P<0.05$) changes from control. **C**: Time course of development of drug-effects and their partial elimination upon washout.

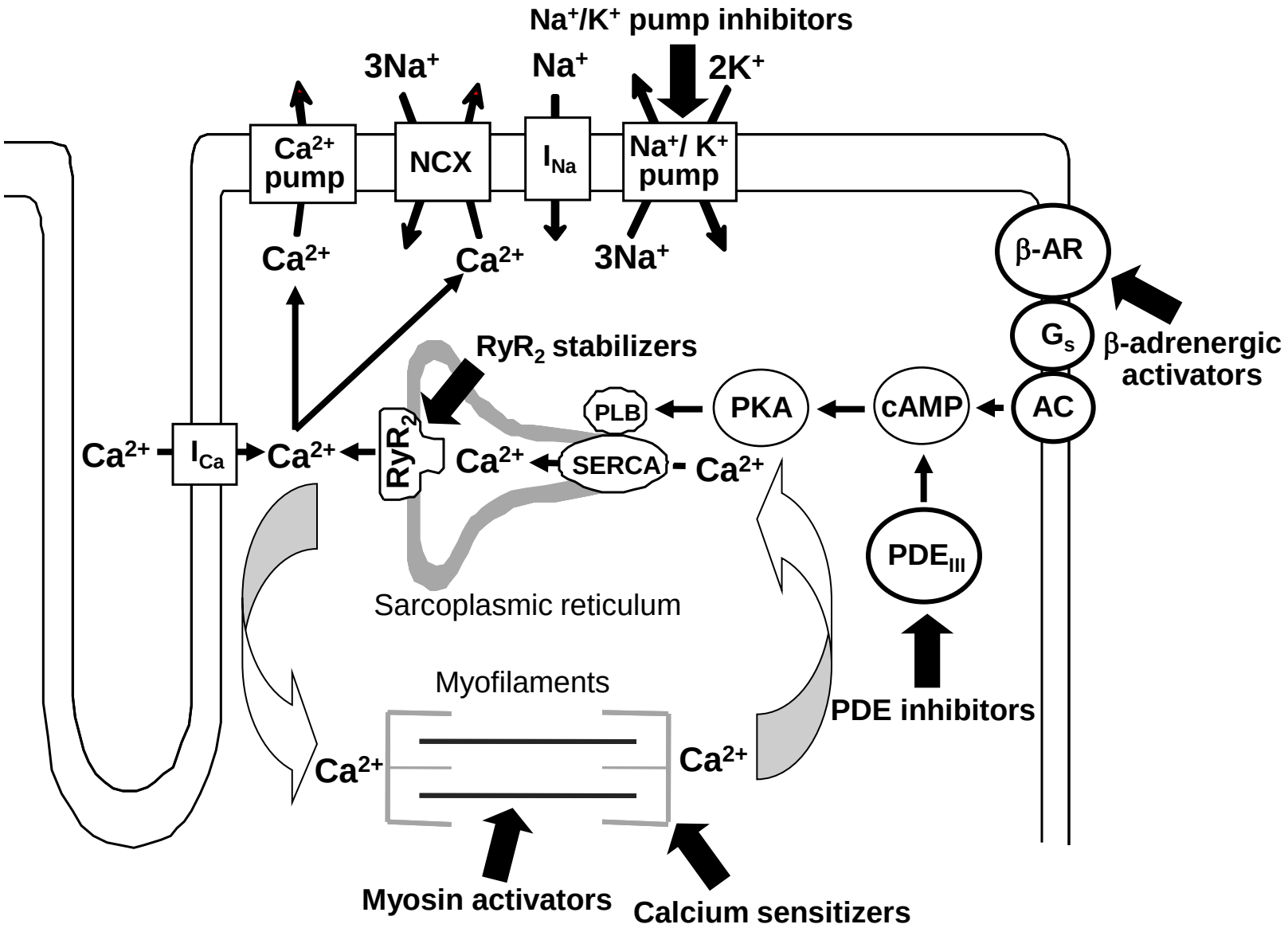

Fig. 1.

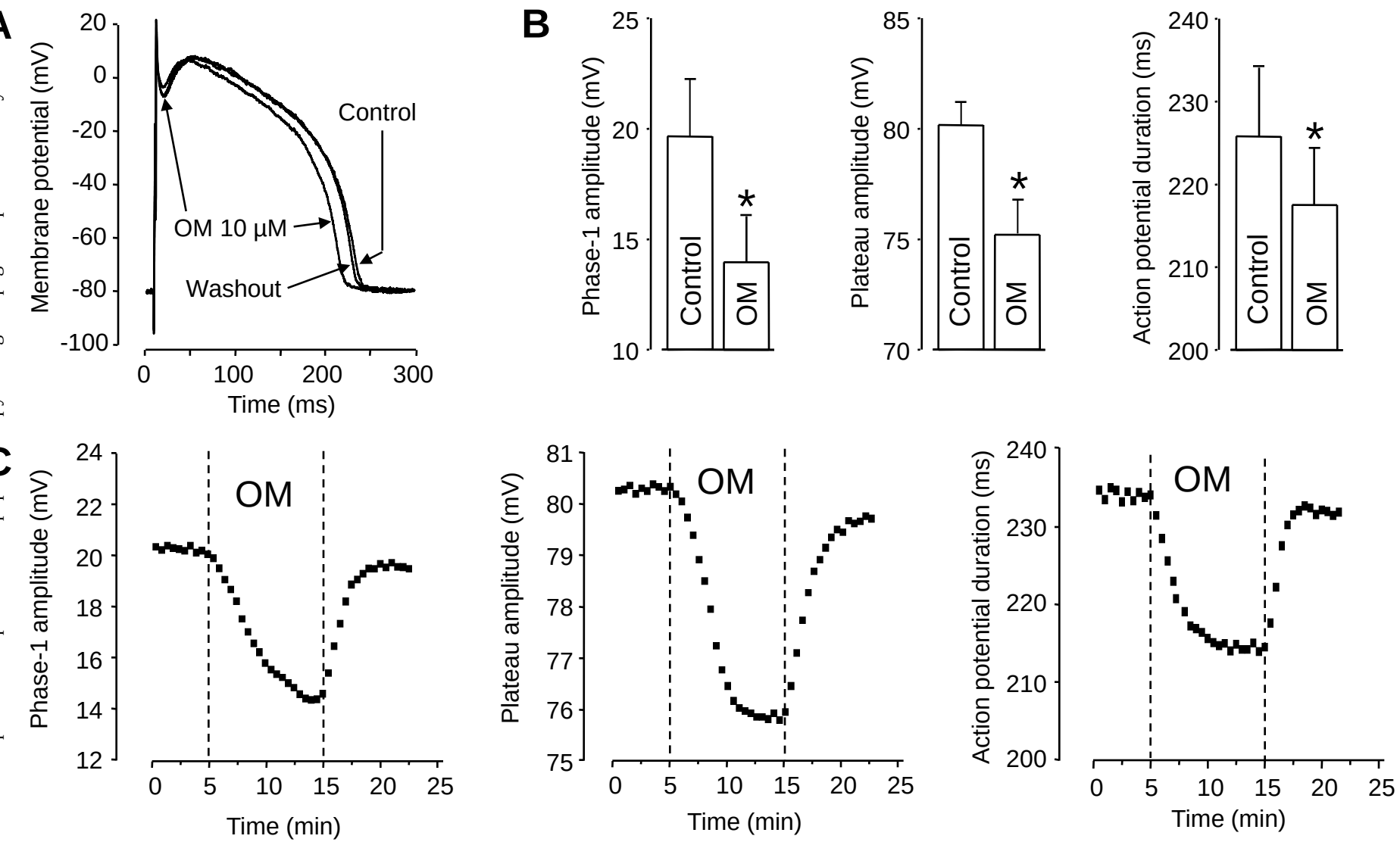

Fig. 2.