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DOSE-DEPENDENT ELECTROPHYSIOLOGICAL EFFECTS OF THE MYOSIN ACTIVATOR OMECAMTIV MECARBIL IN CANINE VENTRICULAR CARDIOMYOCYTES

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Omecamtiv mecarbil (OM) is a myosin activator agent recently developed for treatment of heart failure. Although its action on extending systolic ejection time and increasing left ventricular ejection fraction is well documented, no data is available regarding its possible side-effects on cardiac ion channels. Therefore, the present study was designed to investigate the effects of OM on action potential morphology and the underlying ion currents in isolated canine ventricular myocytes using sharp microelectrodes, conventional patch clamp, and action potential voltage clamp techniques. OM displayed a concentration-dependent action on action potential configuration: 1 μM OM had no effect, while action potential duration and phase-1 repolarization were reduced and the plateau potential was depressed progressively at higher concentrations (10 – 100 μM; $P < 0.05$ compared to control). Accordingly, OM (10 μM) decreased the density of the transient outward K^+ current (I_{to}), the L-type Ca^{2+} current (I_{Ca}) and the rapid delayed rectifier K^+ current (I_{Kr}), but failed to modify the inward rectifier K^+ current (I_{K1}). It is concluded, that although the therapeutic concentrations of OM are not likely to influence cardiac ion currents significantly, alterations of the major cardiac ion currents can be anticipated at concentrations above those clinically tolerated.

Key words: *omecamtiv mecarbil, myosin activators, calcium sensitizers, cardiac action potential, cardiac ion currents, canine myocytes*

INTRODUCTION

Heart failure represents a progressively increasing cause of mortality in the European population. Its therapy is based on the application of positive inotropic agents, including inhibitors of phosphodiesterase and the Na^+/K^+ pump, β-adrenoceptor agonists and Ca^{2+} sensitizers. Recently, a novel strategy of enhancing the systolic performance of the heart has been developed. This is based on increasing the number of the active force-generating cross-bridges without any effect on the troponin-tropomyosin system. The lead molecule of this myosin motor activator group, OM acts directly on the β-myosin heavy chain without increasing the intracellular Ca^{2+} concentration in cardiac cells (1-7). More importantly, early clinical studies revealed that the improvement of the systolic performance was not accompanied with impairment of the diastolic function (5).

In contrast to the well documented actions of OM on extending systolic ejection time and increasing left ventricular ejection fraction, no systematic cellular electrophysiological research has been performed to reveal the possible effects of OM on cardiac ion channels. According to the single preliminary report available in this regard, 10 μM OM caused depression of the plateau, reduction of early repolarization and shortening of action potentials (8). Present study was designed to investigate the effects of OM on the major cardiac ion currents underlying the action potential, including I_{Ca}, I_{to}, I_{Kr} and I_{K1}, in isolated canine ventricular myocytes. This preparation was chosen since it is believed to resemble most the human ventricular cells regarding their electrophysiological properties (9, 10) and because dog hearts were shown to be more sensitive to OM than the hearts of small rodents (3).

MATERIALS AND METHODS

Isolation of single canine ventricular myocytes

Adult mongrel dogs of either sex (15 animals) were anaesthetized with intramuscular injections of 10 mg/kg ketamine hydrochloride (Calypsol, Richter Gedeon, Hungary) + 1 mg/kg xylazine hydrochloride (Sedaxylan, Eurovet Animal Health BV, The Netherlands) according to protocols approved by the local ethical committee (license No: 18/2012/DEMAB) in line with the ethical standards laid down in the Declaration of Helsinki in 1964 and its later amendments. The hearts were quickly removed and placed in Tyrode solution containing (in mM) NaCl, 144; KCl, 5.6;

$CaCl_2$, 2.5; $MgCl_2$, 1.2; HEPES, 5; and dextrose, 11; at pH = 7.4. Single myocytes were obtained by enzymatic dispersion using the segment perfusion technique, as described previously (11, 12). 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) for 5 min. This was followed by 35 min perfusion with Joklik solution supplemented with 1 mg/ml collagenase (Type II. Worthington, Chemical Co.) 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, however, contained dominantly midmyocardial myocytes. After gradually restoring the normal external Ca^{2+} concentration, the cells were stored in Minimum Essential Medium Eagle until use.

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 a final OM concentration of 10 mM were prepared in dimethyl sulfoxide (DMSO) as solvent and stored at 4°C. Appropriate volumes of the concentrated stock solutions were dissolved in the bathing medium to obtain final OM concentrations of 1, 10 or 100 µM. Accordingly, DMSO concentrations in these solutions were 0.01, 0.1 and 1 %, respectively. The control (OM-free) solutions contained the same amounts of DMSO. Each cell was exposed to OM for 10 min.

Recording of action potentials

All electrophysiological measurements were performed at 37°C as previously described (11, 12). The rod-shaped viable cells showing clear striation were sedimented in a plexiglass chamber of 1 ml volume allowing continuous superfusion (at a rate of 2 ml/min) with oxygenized Tyrode solution. The cells were paced through the recording electrode at steady cycle length of 1 s using 1 – 2 ms wide rectangular current pulses having amplitudes of twice the diastolic threshold. Myocytes were allowed to equilibrate in the DMSO-containing control solution for 10 min before the control records were taken in order to develop a stable action potential configuration. Then the cells were exposed to OM (1, 10 or 100 µM) for 10 min, which was followed by a 15 min period of washout in OM-free solution. Transmembrane potentials were recorded using 3 M KCl filled sharp glass microelectrodes having tip resistance between 20 and 40 MΩ. These electrodes were connected to the input of Multiclamp 700A or 700B amplifiers (Molecular Devices, Sunnyvale, CA, USA). Since the cytosol was not dialyzed, time dependent changes in action potential morphology were negligible for the period of our experimental protocol lasting typically not longer than 30 min. Action potentials were digitized at 200 kHz using Digidata 1322A or 1440A cards (Molecular Devices, Sunnyvale, CA, USA) and stored for later analysis.

Conventional voltage clamp

The cells were superfused with oxygenized Tyrode solution at 37°C. This superfusate was supplemented with 3 mM 4-aminopyridine when measuring I_{Ca}, while 1 µM nisoldipine was added extracellularly in case of K^+ current recordings. When I_{Kr} was measured the superfusate contained also 1 µM HMR 1556 to prevent the contamination with I_{Ks}. Suction pipettes, made of borosilicate glass, had tip resistances of 2 MΩ after filling with pipette solution containing (in mM) K-aspartate, 100; KCl, 45; $MgCl_2$, 1; HEPES, 5; EGTA, 10; K-ATP, 3; or alternatively, KCl, 110; KOH, 40; HEPES, 10; EGTA, 10; TEACl, 20; K-ATP, 3; when measuring K^+ or Ca^{2+} currents, respectively (pH = 7.2 in both cases). Membrane currents were recorded with Multiclamp-700A or 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) using the whole cell configuration of the patch clamp technique. After establishing high (1 – 10 GΩ) resistance seal by gentle suction, the cell membrane beneath the tip of the electrode was disrupted by further suction or by applying 1.5 V electrical pulses for 1 ms (13). After stabilization of current amplitudes (usually 5 – 10 min) in the presence of 0.1% DMSO, the cells were superfused with 10 µM OM for 10 min. Outputs from the amplifier were digitized at 100 kHz under software control (pClamp 10.0, Molecular Devices, Sunnyvale, CA, USA). Ion currents were normalized to cell capacitance, determined in each cell using hyperpolarizations from 0 to –10 mV for 15 ms. Series resistance was typically 4 – 8 MΩ before compensation (usually 50 – 80%). Experiments were discarded when the series resistance was high or substantially increased during the measurement.

Action potential voltage clamp

After establishment of whole-cell configuration, action potentials were recorded in current clamp mode from the myocytes superfused with Tyrode solution. The pipette solution was identical to that used for potassium current measurement under conventional voltage clamp conditions, except for EGTA, which was reduced to 10 µM. As a consequence, the experimental conditions applied in action potential voltage clamp experiments were close to physiological. The cells were continuously paced through the recording electrode at steady stimulation frequency of 1 Hz so as a 1 – 2 ms gap between the stimulus artifact and the upstroke of the action potential could occur. Ten subsequent action potentials were recorded from each cell, which were on-line analyzed. One of these action potentials, having duration closest to the average of the 10 recorded action potentials, was delivered to the same cell at the identical frequency as command voltage after switching the amplifier to voltage clamp mode. The current trace obtained under these conditions was a horizontal line positioned at the zero level except for the very short segment corresponding to the action potential upstroke (14, 15). The profile of the OM-sensitive composite current was obtained by subtracting the post-drug curve (measured in the presence of OM) from the pre-drug one (recorded in control). This procedure resulted in a brief early upward deflection representing an outward current identified as I_{to}, which was followed by a longer downward deflection (inward current) associated with Ca^{2+} current. Finally, a small outward deflection corresponding to terminal repolarization was considered as an approximate estimate for I_{Kr} - according to the known fingerprints of these currents (16, 17).

Statistical analysis

Results are expressed as mean ± S.E.M. values. Statistical significance of differences from control was evaluated using one-way ANOVA followed by Student's t-test. Differences were considered significant when P was less than 0.05 .

RESULTS

Effects of omecamtiv mecarbil on action potential configuration

Parameters of the cardiac action potential configuration were not influenced significantly by 1 µM OM - in spite of the vigorous contractions seen always after exposure to the drug.

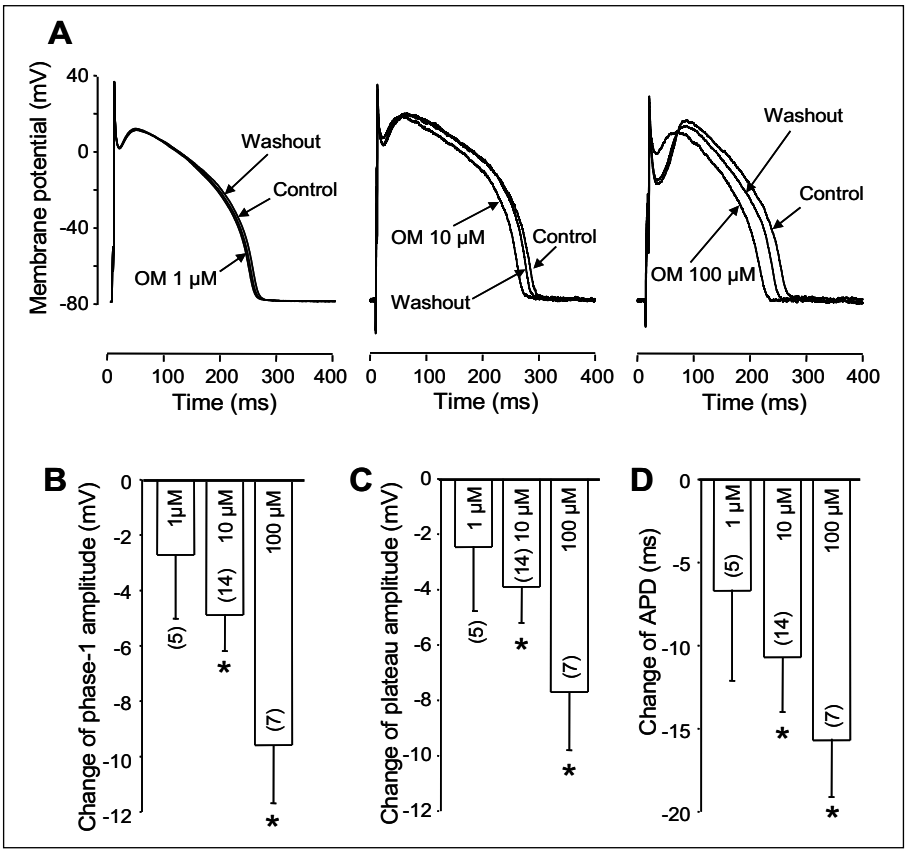

Fig. 1. Concentration-dependent effects of OM on action potential morphology. (A): Superimposed action potentials recorded using sharp microelectrodes before, in the presence of, and after washing out of 1, 10 and 100 µM OM. The average effects on phase-1 amplitude, plateau amplitude and action potential duration are displayed in panels (B), (C) and (D), respectively. Columns and bars represent mean ± S.E.M. data, asterisks denote statistically significant ($P < 0.05$) changes from control, numbers in parentheses indicate the number of myocytes studied.

Superfusion with higher concentrations (10 and 100 µM) of OM caused concentration-dependent complex changes in action potential morphology in canine ventricular myocytes paced at a constant frequency of 1 Hz (*Fig. 1A*). These changes included reduction of early repolarization (phase-1 amplitude was defined as a difference between overshoot potential and voltage of the notch, *Fig. 1B*), the plateau amplitude (plateau potential measured at half-duration of action potential *minus* resting potential, *Fig. 1C*) and action potential duration (measured at 90% level of repolarization, *Fig. 1D*). In other words, the *incisura* became shallower, the plateau depressed and the action potential became shorter in the presence of 10 or 100 µM OM. These changes were partially reversible upon washout in OM-free Tyrode solution, as shown in *Fig. 1A*. On the other hand, OM failed to alter the magnitude of the resting potential, overshoot potential and action potential amplitude even at the highest concentration studied.

Effect of omecamtiv mecarbil on cardiac ion currents measured by conventional voltage clamp

Since 10 µM OM exerted significant changes in the parameters of the action potential, its effects the major ion currents were studied using conventional voltage clamp arrangement.

I_{to} was activated by depolarizations to +60 mV, arising from the holding potential of –80 mV, at a rate of 0.2 Hz. Before each test pulse a short (5 ms) depolarization to –40 mV was applied in order to inactivate the fast Na^+ current, while I_{Ca} was blocked by 1 µM nisoldipine. Here and later on current amplitudes were determined as differences between peak and pedestal values, which were normalized to cell capacitance and expressed as current densities. The peak amplitude of I_{to} was decreased significantly by 10 µM OM without changes in current kinetics in the 11 myocytes exposed to the drug (*Fig. 2*). In addition to changes in current amplitude, the decay time constant estimated for I_{to} was moderately increased by 10 µM OM (from 7.1 ± 0.5 to 8.2 ± 0.8 ms, $P < 0.05$).

I_{Ca} was recorded during depolarizations to +5 mV, arising from the holding potential of –80 mV, at a rate of 0.2 Hz. Similarly to the I_{to} measurements, a depolarizing pre-pulse to -40 mV was applied (here for 20 ms) before the test pulse. In addition, this superfusate was supplemented with 3 mM 4-aminopyridine in order to eliminate K^+ currents.

As demonstrated in *Fig. 3*, the amplitude of I_{Ca} was significantly reduced by 10 µM OM. Inactivation of I_{Ca} followed biexponential kinetics. Neither the faster, nor the slower time constant of inactivation was significantly altered by 10 µM OM (16.8 ± 0.7 versus 17.0 ± 1.1 ms and 130 ± 10 versus 121 ± 13 ms, respectively).

Abnormalities of the rapid component of the delayed rectifier K^+ current (I_{Kr}) are known to be responsible for the generation of cardiac arrhythmias (18). The current was activated by 300 ms long depolarizing pulses to +50 mV, arising from the holding potential of –40 mV. The cycle length here was 20 s. I_{Kr} was assessed as tail current amplitudes recorded following repolarization to the holding potential. I_{Ca} and I_{Ks} were suppressed by 1 µM nisoldipine and 1 µM HMR-1556, respectively. As shown in *Fig. 4*, the amplitude of the I_{Kr} tail was reduced significantly by OM in the 6 myocytes challenged, but no change was observed in the deactivation time constant of I_{Kr}.

I_{K1} was assessed as a steady-state current recorded at the end of a hyperpolarizing pulse clamped to –135 mV for 200 ms from the holding potential of –80 mV. These pulses were delivered at a cycle length of 5 s. OM displayed no significant effect on I_{K1} in the 11 myocytes exposed to the compound (*Fig. 5*).

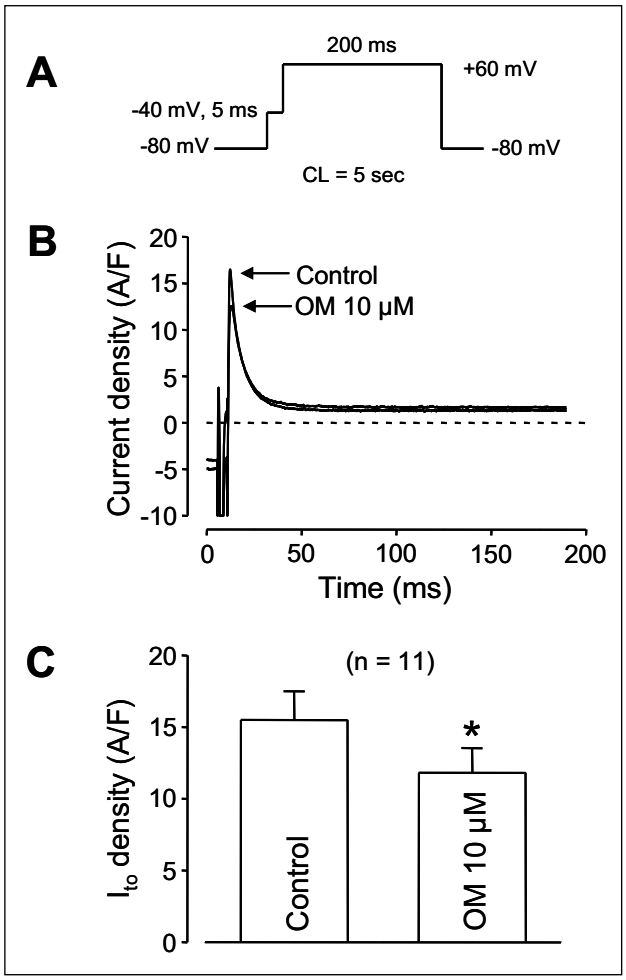

Fig. 2. Effect of 10 µM OM on transient outward K^+ current (I_{to}). (A): Pulse-protocol applied. (B): Representative experiment showing superimposed I_{to} records. The arrows indicate peak currents. (C): Average of peak I_{to} densities recorded from 11 myocytes. Columns and bars are mean ± S.E.M. data, the asterisk denotes statistically significant ($P < 0.05$) difference between the pre-drug and post-drug values.

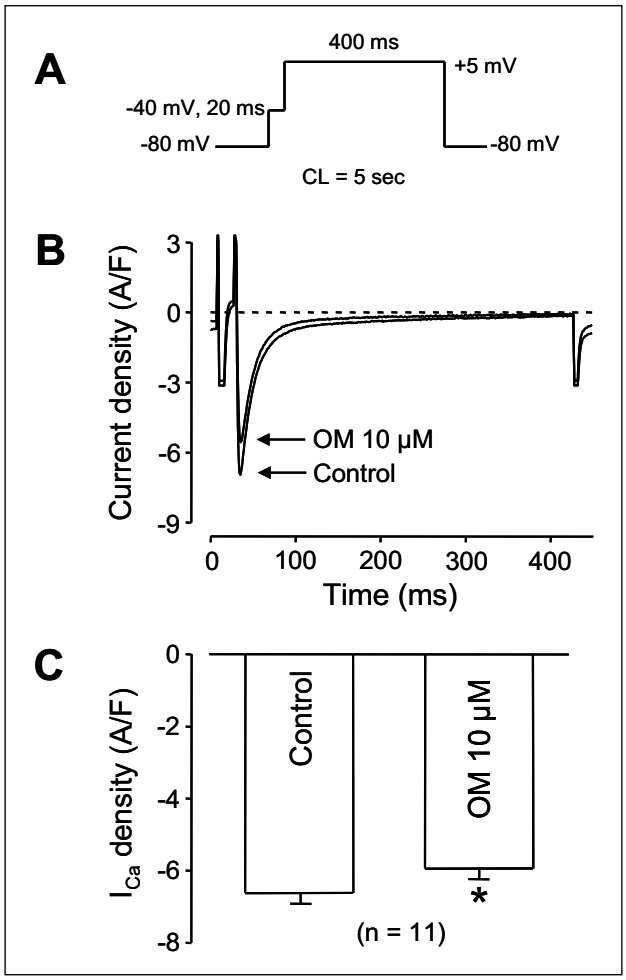

Fig. 3. Effect of 10 µM OM on L-type Ca^{2+} current (I_{Ca}). (A): Pulse-protocol applied. (B): Representative experiment showing superimposed I_{Ca} current records. The arrows indicate peak currents. (C): Average of peak I_{Ca} densities recorded from 11 cells. Columns and bars are mean ± S.E.M. data, the asterisk denotes statistically significant ($P < 0.05$) difference between the pre-drug and post-drug values.

Effect of omecamtiv mecarbil on ion currents under action potential clamp conditions

The profile of an ion current may be markedly different when comparing under conventional voltage clamp and action potential clamp conditions. An advantage of the action potential clamp technique is that the effect of any drug on the net membrane current can be recorded allowing thus to monitor drug-effects simultaneously on more than one ion current. Furthermore, this technique enables us to record true current profiles flowing during an actual cardiac action potential. Of course, in the case of a drug acting on more than one ion current, such as OM, a series of peaks can be detected on the current trace, each of them corresponding to the fingerprint of an individual ion current. Accordingly, the early outward current peak, shown in *Fig. 6A* and *6B*, arises when I_{to} is suppressed, while the inward deflection indicates a blockade of I_{Ca} (probably containing also some late I_{Na}). The late outward current peak, coincident with terminal repolarization of the canine action potential, can be considered as a crude estimate of I_{Kr} (contaminated somewhat by I_{K1}). As demonstrated in *Fig. 6C-6E*, 10 and 100 µM concentrations of OM progressively suppressed I_{to}, $I_{Ca,}$ I_{Kr} indeed - in line with results of conventional voltage clamp experiments.

DISCUSSION

In this study we are first to present the effects of OM on cardiac ion currents. 1 µM OM failed to alter the parameters of the action potentials suggesting that the underlying ion channels are not much influenced by this low concentration of OM. At higher concentrations (10 – 100 µM), however, three major ion currents (I_{to}, I_{Ca} and I_{Kr}) were suppressed by OM, as demonstrated using conventional as well as the action potential voltage clamp experiments. These changes in current densities are in line with the observed alterations in action potential morphology. The most prominent change was found in I_{to}, in accordance with the marked suppression of the phase-1 amplitude and the concomitant reduction of the *incisura.* As a consequence, the more positive membrane potential during the early plateau is likely to decrease the driving force for Ca^{2+} entry

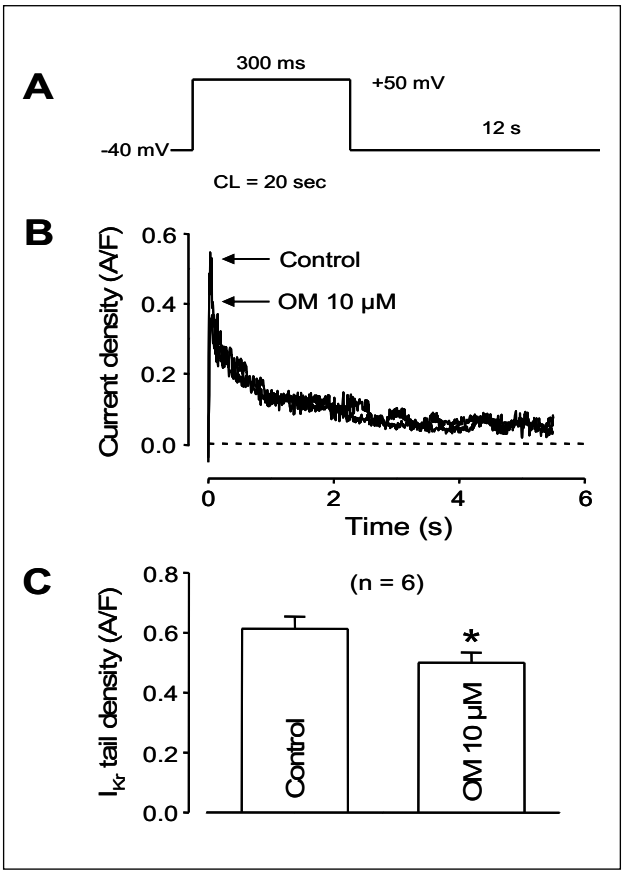

Fig. 4. Effect of 10 µM OM on the rapid delayed rectifier K^+ current (I_{Kr}). (A): Pulse-protocol applied. (B): Representative experiment showing superimposed I_{Kr} tail current records. Arrows indicate the initial peak values of the tails. (C): Average of peak I_{Kr} tail current densities recorded from 6 myocytes. Columns and bars are mean ± S.E.M. data, the asterisk denotes statistically significant ($P < 0.05$) difference between the pre-drug and post-drug values.

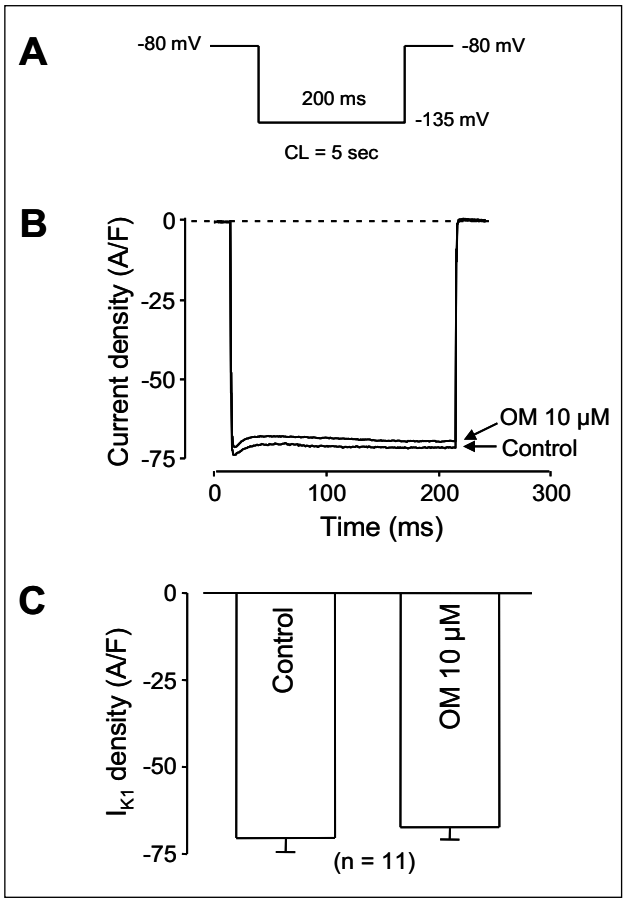

Fig. 5. Lack of effect of 10 µM OM on the inward rectifier K^+ current (I_{K1}). (A): Pulse-protocol applied. (B): Representative experiment showing superimposed I_{K1} records. The arrows indicate steady-state current levels recorded at the end of the 200 ms long hyperpolarizing pulse. (C): Average of steady-state I_{K1} densities obtained in 11 myocytes. Columns and bars are mean ± S.E.M. data.

(19). This effect, combined with the reduced Ca^{2+} conductance, may result in a compromised Ca^{2+} entry, which may be beneficial for the myopathic heart (20) due to the smaller energy demand of relaxation associated with the concomitantly decreased Ca^{2+} removal (21). Although the depressed plateau amplitude can well be explained by the reduced density of I_{Ca}, the inhibition of the late Na^+ current cannot be ruled out either. Its contribution is supported by the prominent late component of the inward current excised by OM under action potential voltage clamp conditions, and also by the observed shortening of action potentials in spite of the moderately decreased I_{Kr} (22).

Our study cannot answer the question, that the alterations of ion currents observed in the presence of high concentrations of OM are direct drug effects on the underlying ion channels, or alternatively, are consequences of the modified myosin-actin interaction. A similar mechanism has been suggested in gelsolin deficient mice resulting in increased amplitude of I_{Ca} with unchanged current kintetics (23).

Clinical relevance of the present results can be estimated only when comparing to human OM plasma levels. Therapeutic plasma concentrations were reported to vary from 300 to 600 ng/ml (24). In another study the well-tolerated plasma concentration varied between 100 – 1200 ng/ml. High values of 1350 and 1750 ng/ml have also been observed, however, plasma levels above 1200 ng/ml - corresponding to 3 µM - were not tolerated (25). Considering other results, the typical OM plasma concentration was around 400 ng/ml in average (1, 5), which corresponds to 1 µM. Accordingly, the 1 µM concentration of OM applied in this study well corresponds to the usual therapeutic plasma level, while the higher concentrations (10 – 100 µM) are relevant clearly for cases of overdose. It must be noted, however, that the plasma protein binding of OM was estimated 82% in humans (26) which may distort the comparison of the present data with clinical results.

In this study we have focused mainly on the electrophysiological effects of 10 µM OM. This is reflected by the variable sample size, which was always higher in the case of 10 µM OM than the respective numbers referring to the lower or higher concentrations, as evident from the data presented in *Figs. 1* and *6*. Regarding the conventional voltage clamp experiments, where the effects of 10 µM OM were studied on the individual ion currents, the sample size was uniformly 11 - except for the case of I_{Kr}, when only those experiments were evaluated where the change of tail current amplitude was less than 5% before the application of OM. This variability of the sample size can be considered as a limitation of the study, however, it is not likely affecting our conclusions.

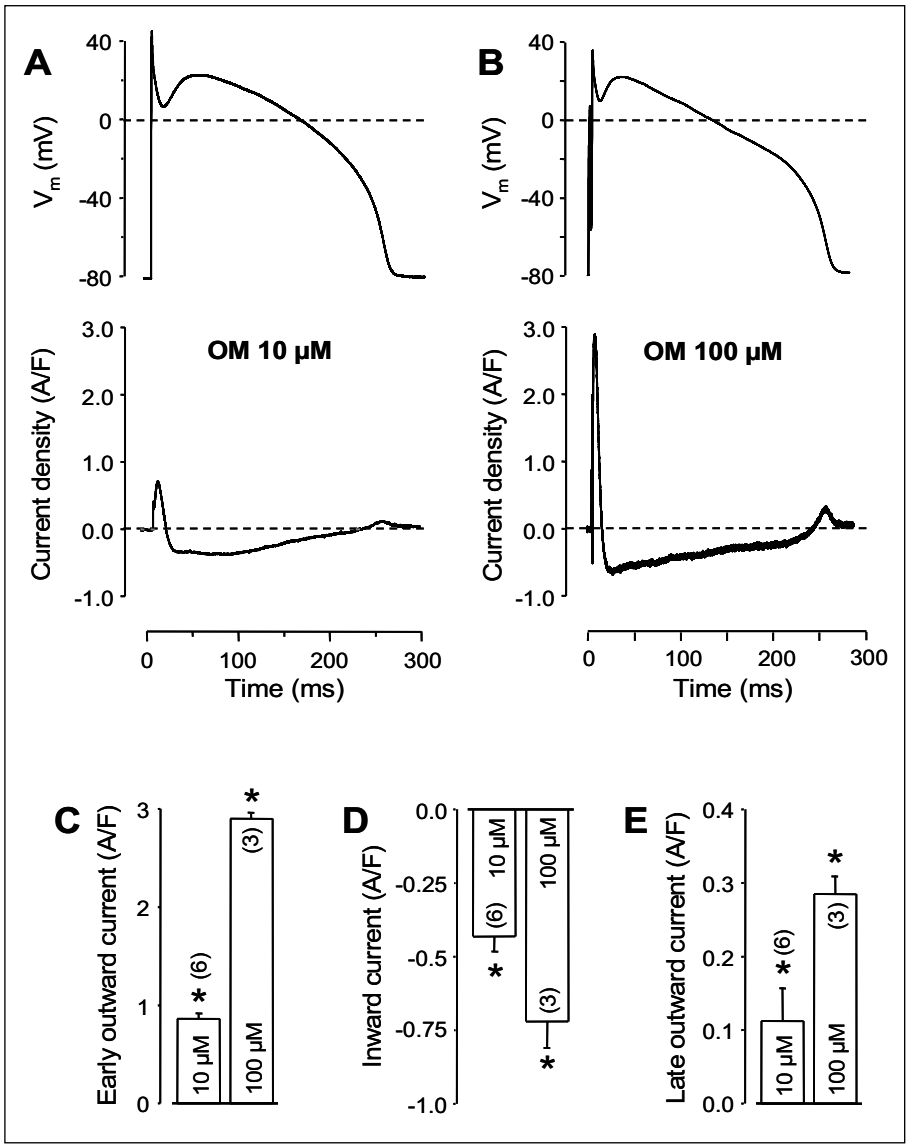

Fig. 6. Effects of OM (10 and 100 µM in panels (A) and (B), respectively) on cardiac ion currents under action potential voltage clamp conditions. Command action potentials are displayed as upper traces, while the OM-sensitive current records are shown below. The average densities of the early outward peak (I_{to}), the inward peak (I_{Ca}) and the late outward peak (I_{Kr}) are displayed in panels (C), (D) and (E), respectively. Columns and bars represent mean ± S.E.M. data, asterisks denote statistically significant ($P < 0.05$) changes from control, numbers in parentheses indicate the number of experiments.

In summary, OM appears to be a relatively safe drug regarding its cardiac electrophysiological properties, however, an increased prevalence of cardiac arrhythmias cannot be ruled out under conditions of overdose intoxication due to the resultant modification of cardiac ion currents.

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Conflict of interest: None declared.

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