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ORIGINAL ARTICLE

The Investigation of Combined Na^+/Ca^{2+} Exchanger and the L-type Ca^{2+}- Channel Inhibition in Langendorff-Perfused Isolated Guinea Pig Hearts

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ABSTRACT

Objective: The sodium/calcium exchanger (NCX) and the L-type Ca^{2+}-channel (LTCC) are nowadays considered the major transmembrane transport mechanisms that control Ca^{2+} homeostasis. In pathophysiological conditions the altered function of these currents may influence the Ca^{2+} homeostasis and cardiac contractility and thereby, may enhance the development of severe tachyarrhythmias. The blockade of NCX current has been proposed as possible approach in the prevention and/or suppression of arrhythmias; however, this mechanism is not always favourable because the inhibition of both modes of NCX may induce Ca^{2+}-overload. The decrease of the Ca^{2+} level by partial LTCC inhibition may be beneficial in increasing the antiarrhythmic efficacy. Therefore, the aim of our study was to investigate the antiarrhythmic effects of combined NCX and LTCC blockade in the *ex vivo* guinea pig arrhythmia model.

Methods: We have performed Langendorff experiments in isolated guinea pig hearts. We have recorded electrocardiograms (ECG) and left ventricle pressure. We have applied 1 μM ORM-10962 (ORM), a compound that block NCX current and 30 nM nisoldipine for the inhibition of LTCC. Arrhythmias have been provoked by decreasing the activity of the sodium/potassium pump with 5 μM ouabain.

Results: We found that neither LTCC nor NCX blockade alone increased, while the combined inhibition of the two currents significantly delayed ($p<0.05$) the mean time of appearance of ouabain-induced ventricular fibrillation. The heart frequency was affected by none of the drugs, only the left ventricular pressure (end-systolic and diastolic difference) was significantly elevated by ORM ($p<0.001$).

Conclusion: In the Langendorff-perfused guinea pig heart, specific, combined NCX and LTCC blockade may be favourable than the inhibition of NCX or LTCC alone. However, further investigations are necessary to identify the pathological settings in which this combined cardiac drug therapy may be a potential new approach.

Keywords: Langendorff-perfused hearts, guinea pig, NCX current, L-type calcium current, antiarrhythmic effect.

REZUMAT

Obiective. Transportorul de schimb sodiu/calciu (NCX) și curentul de calciu tip L (LTCC) sunt considerate actualmente drept principalele mecanisme de transport transmembranar ce controlează homeostazia calciului. În condiții patologice, alterarea funcțională a acestor curenți ionici poate interfera cu homeostazia calciului și contractilitatea cardiacă, cu posibilitatea apariției tahiaritmiilor severe. Blocarea schimbătorului NCX a fost propusă drept posibilă intervenție de prevenire sau supresie a acestor aritmii; cu toate acestea, acest mecanism poate fi nefavorabil deoarece blocarea ambelor moduri de funcționare ale curentului NCX poate induce supraîncărcarea de Ca^{2+}. Reducerea nivelului de Ca^{2+} prin inhibiția parțială a curentului LTCC este considerată și ea drept un tratament antiaritmic eficace. Ca atare, scopul studiului nostru a fost investigarea efectului de blocare combinată a canalului LTCC și a schimbătorului NCX pe un model de aritmii induse *ex vivo* la cobai.

Metode: Inimile izolate de cobai au fost perfuzate retrograd tip Landendorff cu înregistrarea electrocardiogramei (ECG) și a presiunii în ventriculul stâng. În vederea inhibiției schimbătorului NCX a fost aplicat 1 μM ORM-10962 (ORM) și, respectiv, pentru blocarea curentului LTCC a fost utilizată nisoldipina (30 nM). Aritmiile au fost induse prin reducerea activității pompei sodiu/potasiu (INa/K) cu ajutorul ouabainei (5 μM).

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Rezultate: Inhibarea combinată a LTCC și a NCX (dar nu și blocarea lor separată) a condus la o creștere semnificativă ($p<0.05$) a duratei medii de apariție a fibrilației ventriculare indusă prin blocarea pompei Na/K cu ouabainã. Compușii farmacologici nu au influențat frecvența cardiacă, dar presiunea ventriculară stângă (calculată ca diferența dintre cea telesistolică și diastolică) a crescut semnificativ dupa aplicarea compusului ORM ($p<0.001$).
Concluzii: Pe modelul de inimă izolată de cobai, blocarea combinată a NCX și LTCC exercită un efect superior inhibiției separate a celor doi curenți ionici. Investigații suplimentare sunt necesare pentru a identifica condițiile patologice în care această terapie farmacologică combinată își va dovedi eficiența terapeutică.
Cuvinte cheie: inimi perfuzate Langendorff, cobai, transportor de schimb NCX, curent de calciu tip L, effect antiaritmic.

INTRODUCTION

The cardiovascular diseases are life threatening pathological conditions, which are best illustrated by EU statistics indicating that the ischaemic heart diseases were the main cause of death in Europe[13,14]. It is well-known that prevalence of arrhythmias is extremely high in patients diagnosed with different heart diseases as acute myocardial infarction, congestive heart failure, etc, and we may state that typically in these patients the cardiac arrhythmias are the main cause of death. Unfortunately, the nowadays applied antiarrhythmic drugs, tools or other forms of treatment do not meet the expected therapeutical demand, thereby there is a high expectation in developing new and efficient drugs for preventing or suppressing cardiac arrhythmias.

The cardiac action potential is generated by the highly organized and regulated function of a number of different transmembrane ion channels and electrogenic ion transport mechanisms. Among several other ion currents and transporters, the sodium/calcium exchanger (NCX) is considered as a crucial contributor to Ca^{2+} homeostasis in the myocardium[4,5,24,25]. The NCX is tightly regulated by the transmembrane Na^+ and Ca^{2+} gradients and by the actual level of the membrane potential. NCX can operate in a bidirectional fashion. In the forward mode NCX extrudes one Ca^{2+} from the cell which is coupled with three Na^+ ions entering the cell. Thus, the forward mode generates inward current, which eventually depolarizes the cell membrane. The reverse mode of the NCX is activated when the cytoplasmic Na^+ level is increased and the Ca^{2+} concentration is low and/or the membrane potential is depolarized. In this mode the NCX extrudes three Na^+ from the cell and moving one Ca^{2+} into the cell, and it can generate repolarizing net current. Under physiologic conditions, NCX operates mainly in forward mode and removes the same amount of Ca^{2+} that entered the cell through ICaL maintaining the beat-to-beat Ca^{2+} balance. At the same time, Ca^{2+} elimination leads to Na^+ influx[8]. In the excitation-contraction coupling (ECC), Ca^{2+} ions enter the cell from the extracellular space (Ca^{2+}-influx) mainly via inward L-type calcium current (ICaL, LTCC). These channels are primarily present at sarcolemmal-sarcoplasmatic reticulum (SR) junctions, in the vicinity of ryanodine receptors (RyRs). The Ca^{2+} influx increases the Ca^{2+} concentrations near the RyRs, which triggers Ca^{2+} release from the sarcoplasmic reticulum (SR). This mechanism is called "Ca^{2+}-induced Ca^{2+} release" (CICR), which leads more Ca^{2+} to be released into the cytosol, resulting in a Ca^{2+} transient (CaT). In cardiac muscle CaTs can be observed as a summary of spatio-temporally restricted Ca^{2+} sparks, which can be monitored by optical (fluorometric) techniques. The free intracellular Ca^{2+} binds to the myofilaments and triggers contraction. After the contraction the released $[Ca^{2+}]i$ is eliminated by both Ca^{2+} reuptake to the SRvia the activity of the SR Ca^{2+} pump (SERCA2a), and Ca^{2+} extrusion (efflux) from the cell (primarily by the forward mode activity of the NCX)[5-7]. Sequestration of the released Ca^{2+} and stabilization of the diastolic $[Ca^{2+}]i$ between contractions is crucial in the regulation of the beat-to-beat Ca^{2+} balance under varying conditions. The majority of the released Ca^{2+} is reuptake by SERCA2a. A much smaller amount of $[Ca^{2+}]$ i is extruded from the cell, primarily via the forward mode activity of the NCX. An ATP-dependent Ca^{2+} transporter, the Ca^{2+} pump of the plasma membrane (PMCA) may also extrude Ca^{2+}, however, its contribution to maintaining Ca^{2+} balance is much less important, and its role is suggested to be primarily restricted to fine tuning of the diastolic Ca^{2+} level[11]. In steady state, during each cycle, the released and reuptake Ca^{2+}, as well as the entered and extruded Ca^{2+} must be equal.

Since the extrusion of one Ca^{2+} is coupled with 3 Na+ entering the cell, during the forward mode of the NCX net inward current is carried which, when intracellular Ca^{2+} is elevated, can cause substantial depolarization leading to early (EAD) and delayed (DAD) after

depolarizations. EAD and DAD is generally thought to play an important role in arrhythmogenesis[16,26], especially under conditions where potassium conductance is decreased, such as heart failure[21]. In the past two decades, several NCX inhibitors were developed having different potency and selectivity. The KB-R7943 markedly suppressed the NCX current however exerted poor selectivity[9]. The SEA-0400 (SEA) is a widely used, potent NCX inhibitor which considerably improved our knowledge about the NCX function however a ~ 20% inhibition of ICaL made the data interpretation difficult[9]. The ORM-1010317,23 and ORM-1096218 and GYKB-663515 are recently synthesised, novel NCX inhibitors having an appropriate selectivity profile to investigate the NCX functionon the cardiac action potential waveform and repolarization.

The blockade of NCX current has been proposed as possible approach in the prevention and/or suppression of arrhythmias[21], however this mechanism is not always favourable because the inhibition of both modes of NCX may induce Ca^{2+}-overload. The decrease of the Ca^{2+} level by partial LTCC inhibition may be beneficial in the antiarrhythmic efficacy.

The combined NCX+LTCC blockade was first time studied by Bourgonje et al. (2013) by investigating the combined effect of SEA-0400 + verapamil in a chronic AV block dog model. They concluded that the combination of the two drugs may be beneficial by counterbalancing the negative side effects of the monotherapy of the LTCC blockade, ie, unlike specific inhibition of LTCC, combined NCX and LTCC inhibition has no negative effects on cardiac hemodynamics. This study, however, has some weaknesses because either the selectivity of SEA-0400 (blocks not only but also ICaL at the same concentration range), or verapamil (blocks repolarising K+ currents) is questionable. These observation makes questionable the results of the study of Bourgonje et al. (2013), because it is difficile to identify which compound (SEA or verapamil) was the main antiarrhythmic factor of the combined therapy.

Therefore, the aim of our study was to investigate the antiarrhythmic effects of combined ORM-10962+nisoldipine blockade in *ex vivo* guinea pig arrhythmia model. The selectivity of ORM-10962 on NCX current18 and of nisoldipine on ICaL current[19] are well documented and confirmed. We focused on two directions:

1. How the heart frequency and pump function is affected by partial perse NCX or by combined NCX + LTCC blockade, respectively?
2. Is or not a beneficial therapeutic intervention for preventing/suppressing cardiac arrhythmias the partial per se NCX or combined NCX + LTCC blockade, respectively, in isolated (Langendorff perfused) guinea pig hearts?

METHODS

Animals

Langendorff perfused guinea hearts were used for ouabain induced arrhythmia studies. Adult guinea pigs of either sex weighing 300-500g obtained from a recognised supplier were used for the study. All experiments were conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication No. 85-23, revised 1996) and conformed to Directive 2010/63/EU of the European Parliament. The protocols were approved by the Review Board of the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development, Hungary (XIII./1211/2012).

Isolated heart experiments

Guinea pigs of either sex weighing 300-500 g were used for these experiments, that we described earlier[15]. Animals were anaesthetised with sodium-pentobarbital (500 mg/kg, i.p.) and injected with heparin sodium (300 IU i.v.). Hearts were rapidly excised, mounted via the aorta on a Langendorff apparatus and perfused retrograde with warm (37°C), modified Krebs–Henseleit bicarbonate (KHB) buffer at a constant pressure (60 mmHg). The KHB solution contained (in mmol/l): $NaHCO_3$ 25; KCl 4.3; NaCl 118.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; glucose 10; $CaCl_2$ 1.8, having a pH of 7.4±0.05 when gassed with carbogene (95% O_2 + 5% CO_2). The Langendorff perfused apparatus is a suitable technique for investigating drug effects and for modelling ischaemia-reperfusion injury diseased models. The main advantage of the model is that in isolated hearts ceases the regulation of the neurohumoral enervation, which makes enable the better investigation of the direct drug effects[22].

Perfusion flow and the electrocardiogram (ECG) were simultaneously recorded using the WinWCP software (V4.9.1. Whole Cell Electrophysiology Analysis Program, John Dempster, University of Strathclyde, UK).

Arrhythmia diagnosis: For the induction of arrhythmia, we used ouabain (Sigma) and the drugs (ORM-10962, Nisoldipine) or solvent were administered 10 minutes before starting ouabain perfusion.

Ventricular extra beat (VEB), bigeminia, salvo, ventricular tachycardia (VT) and ventricular fibrillation (VF) were defined according to the Lambeth Conventions I and II, respectively[27].

Statistics: All data from independent samples. Following data were expressed as mean ± standard error of the mean (SEM) and the groups were compared in pairs by means of the "*t*-test". Statistically significant differences are marked in figures by * $p<0.05$.

Chemicals

Except for the nisoldipine (gift from Bayer AG, Leverkusen, Germany) and ORM-10962 (gift from Orion

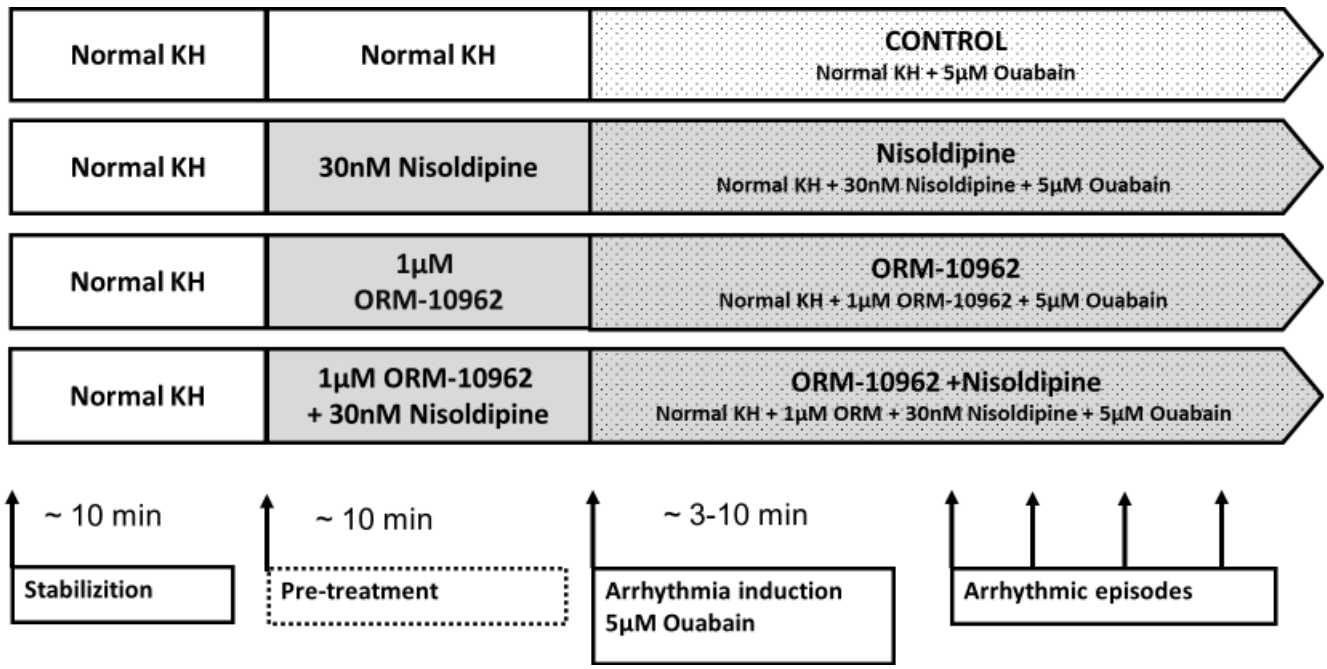

Figure 1. The investigational protocol of the ouabain induced arrhythmia model in isolated guinea pig hearts. The bands represent the respective experimental groups: 1st row: control; 2nd row: test nisoldipine; 3rd row: test ORM-10962; 4th row: test ORM+nisoldipine. The demarcation lines represent the 3 distinct periods of the experiments: stabilization period, pre-treatment period and arrhythmia provocation period.

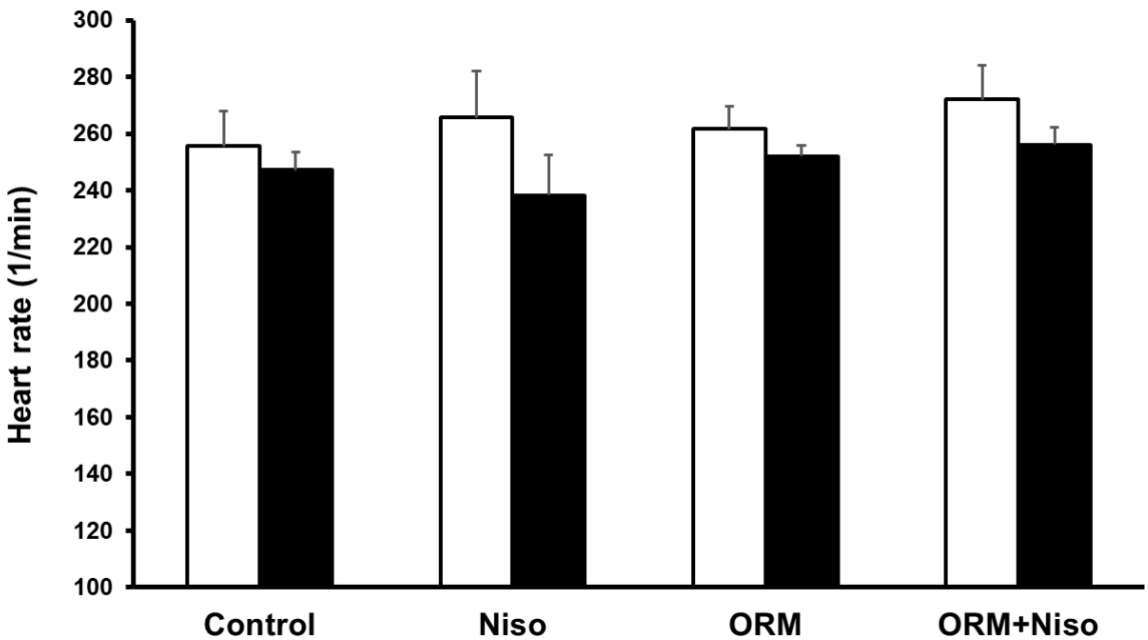

Figure 2. Heart rate values from isolated guinea pig hearts for control and tested drugs. The bars represent mean values before (white) and after (black) treatment ± SEM.

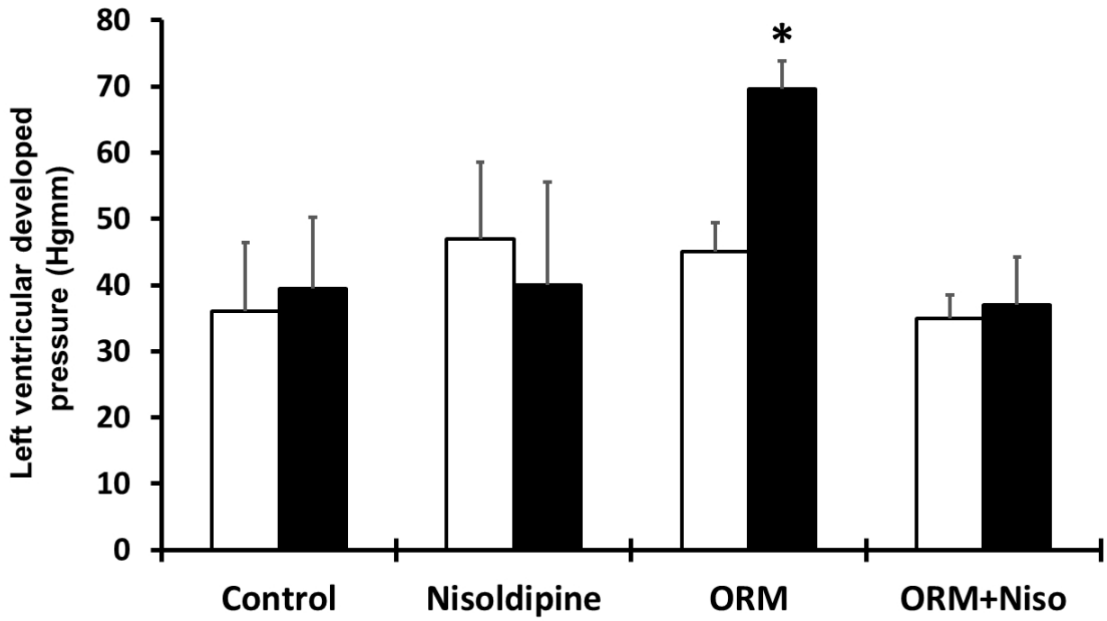

Figure 3. Investigation of changes in left ventricular pressures from isolated guinea pig hearts in the control state and after drug administration. Ventricular pressure was calculated from the difference between end-systolic and end-diastolic pressures. ORM treatment showed a significant difference compared to the control ($p < 0.001$). The bars represent mean values before (white) and after (black) treatment ± SEM.

Pharma, Espoo, Finland) all chemicals were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO, USA). ORM-10962, nisoldipine and ouabain were dissolved in DMSO to yield a 1mM stock solution, respectively. These stock solutions were diluted to reach the desired final concentration in the Langendorff apparatus.

Investigational protocols

On order to test the blocking effects of NCX and L-type Ca^{2+}-currents in mono and combined therapy by applying a ouabain induced arrhythmia model in isolated guinea pig hearts, we applied the following experimental protocol (Figure 1). Our experiments were divided in four groups.

The first group was the time control. The second and third groups contained the animal which were tested with the effect of nisoldipine (30nM Niso, 2nd group) and ORM-10962 (1 μM ORM, 3. group) monotherapy, respectively, while the last group of guinea pigs where, which we analysed the combined effects of the NCX and LTCC blockers (same amount of Niso+ORM).

Each set of experiments consisted of three distinct periods: i) stabilization period of 10 minutes of the isolated hearts with superfusion with KH solution; ii) preincubation period, then for another 10 minutes with a KH solution which contained the drug solvent (1st group) or test drug (2nd-4th groups); iii) arrhythmia provocation period, we have superfused the hearts with 5 μM ouabain in the continuous presence of the compound form the second period (solvent or test drugs). The numbers of experiments in all groups were as follow: control – 5 hearts; nisoldipine - 5 hearts, ORM -5 hearts; Niso+ORM – 6 hearts.

Statistics

All data are expressed as means ± SEM. Statistical analysis was performed with Student's *t*-test for paired data. The results were considered statistically significant when P was < 0.05.

RESULTS

In each experimentwe examined changes in the parameters of left ventricular pressure and the arrhythmias before and after ouabain (5 μmol/L) administration in isolated guinea-pig hearts. The heart rate was not significantly affected by either agent, however, the change in left ventricular developed pressure (systole minus end-diastole pressure) was significantly increased by ORM ($p<0.001$). Averaging the time to onset of ventricular fibrillation, we found that neither LTCC inhibition nor NCX inhibition had a significant protective effect on hearts, whereas combined inhibition

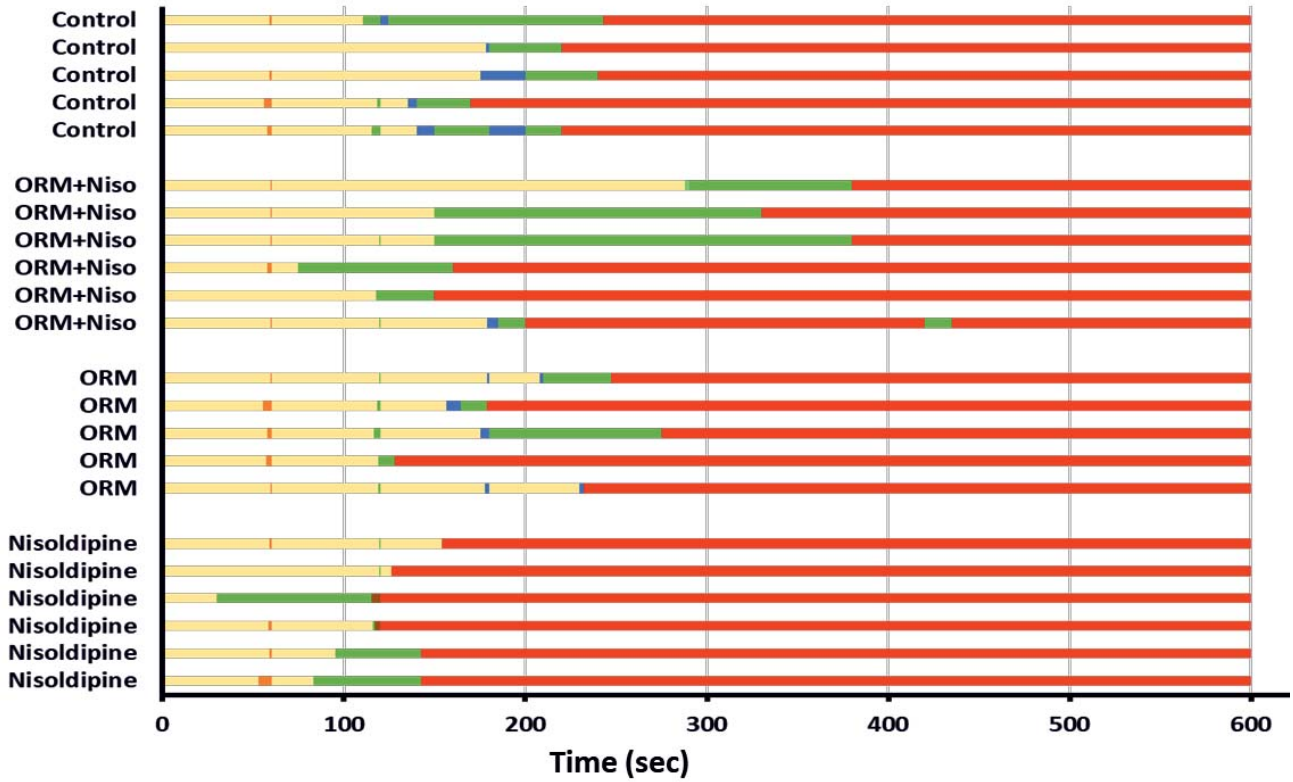

Figure 4. The total arrhythmia map analysed from all hearts. The X axis shows the time after ouabain perfusion; Y axis: arrhythmia diagrams of 20 individual hearts. The hearts were divided into 4 groups: Control; Nisoldipine 30nM, ORM-10962 1 μM and 1 μM ORM+ 30nM Nisoldipine. Each of the horizontal lines represent a single heart. Time periods with the various arrhythmia stages are shown in colour: regular sinus rhythm (yellow); multiple extrasystoles (orange); bigeminy (blue): ventricular tachycardia (green): ventricular fibrillation (red).

significantly delayed the onset of ventricular fibrillation compared to control ($p<0.05$).

Heart rate values were calculated from the minutes 9 and 19 before and after drug administration (Figure 2). The first periods of our experiment (Normal) are the white bars, the treatments are the black bars. The ORM, nisoldipine and combination treatments did not cause a significant difference compared to normal heart rate (control 256±19.5 vs. 240±24.5 1/min; Nisoldipine 265.7±17.4 vs. 238.3±15.4 1/min; ORM 261.6±4.8 vs. 252±3.8 1/min; ORM+Niso 272±10.7 vs. 256±6.4 1/min)

Developed left ventricular pressure values were measured using a latex balloon placed in the left ventricle and the recorded data were analysed offline. The mean differences in systolic and diastolic end pressure values were calculated at 9 and 19 minutes before and after 10 minutes drug administration. Figure 3 shows that for ORM we found a significant difference between the stabilization period (before white bar) and the treatment period (after black bar) ($p<0.001$) (control 36.0 ± 10.4 vs. 39.4 ± 10.8 Hgmm, Nisoldipine 47.5 ± 11.5 vs. 40.0 ± 15.6 Hgmm, ORM 45.6 ± 4.0 vs. 69.6 ± 4.3 Hgmm, ORM + Niso 35.2 ± 3.6 vs. 37.0 ± 7.2 Hgmm).

Registered ECGs were analysed from the 20th minute of our experiments, and the arrhythmia map was made after the addition of 5μM Ouabain. We examined which types of arrhythmias develop / are present at a given minute (Figure 4). Each category was represented by different colours: the arrhythmia-free time interval was marked in yellow, followed by arrhythmias in different colours: ventricular extrasystoles (brown), bigeminias, salvos (blue), ventricular tachycardia (green), and ventricular fibrillation (red). Values were plotted for 7 minutes (420 seconds) because thereafter, cardiac function no longer changed, and fibrillation stabilized in all cases. The ORM-1962 alone (ORM) and combined with Nisoldipine (ORM+Niso) significantly delayed the number of arrhythmias and fibrillation episodes compared to control (Figure 4).

In the control group, the first arrhythmias, developed around the second minute. Several minutes later, ventricular tachycardia appeared, that followed

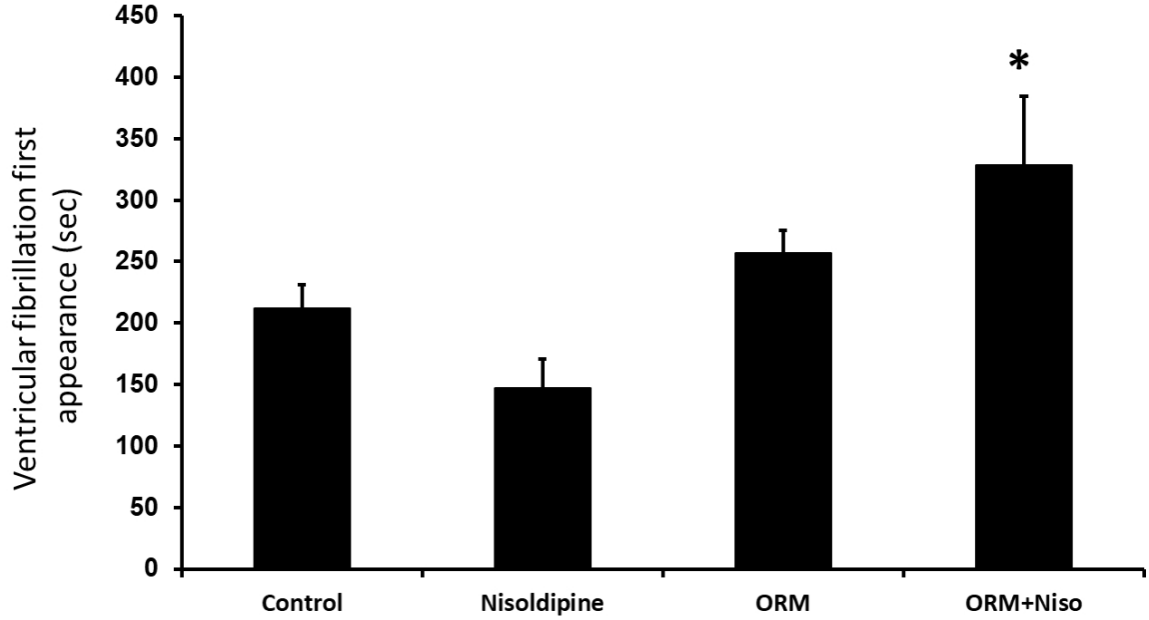

Figure 5. The first appearance of VF in all groups after ouabain administration. The efficacy of individual inhibitors and inhibitor combinations has been evaluated against the control hearts. The bars represent mean values ± SEM, after drug or vehiclepre-treatment and ouabain administration.

by fibrillation around the fourth minute after starting ouabain administration. The time of the first appearance of ventricular fibrillation (Figure 5) was calculated from the beginning of ouabain administration and the elapsed time was expressed in seconds. A significant difference was observed only for ORM + nisoldipine compared to the control (*$p<0.05$), which is marked with an asterisk. Nisoldipine and ORM-10962 pre-treatment were no significantly effect, but ORM showed a tendency on First VF time.

DISCUSSION

Main findings

The main finding of this study was: i) the sodium-calcium exchanger (NCX) blocker ORM-10162 -according with literature data- during preincubation period significantly increased the left ventricular pressure, while the L-type calcium channel (LTCC) inhibitor nisoldipine neither alone nor in combination with ORM compound altered substantially the cardiac pump function; ii) ORM and Niso monotherapy (preincubation with ORM or Niso) did not prevent the ouabain induced cardiac arrhythmias, ie, did not delayed the time of appearance of VT/VF, while the combination of the drugs significantly delayed it, ie. the combined NCX+LTCC blockade possess antiarrhythmic potency against triggered arrhythmias.

The ORM-induced pressure increasing effect is in accord with the pharmacological profile of the compound, however this effect disappeared when the drug was tested in combination with the LTCC inhibitor nisoldipine (Figure 3). Our data demonstrates that the additional application of a selective LTCC inhibitor may prevent and/or avoid the Ca^{2+}-overload induced by the NCX blockade. One may speculate that this combination may be efficacious to prevent Ca^{2+}-overload induced arrhythmias, for example in ischaemia-reperfusion injuries. This speculation is strengthened by a previous observation which reported that NCX blockade with ORM-10962 monotherapy could not prevent the I/R induced arrhythmias neither in coronary artery occlusion-reperfusion induced arrhythmias anesthetized rat model nor in zero flow ischaemia-reperfusion induced arrhythmias in guinea-pig Langendorff hearts[23,18]. Neither ORM and Niso nor ORM+Niso combination affected the heart frequency.

Our results indicate that 5 μM ouabain induced life-threatening arrhythmias in all investigated hearts. The preincubation with the combined ORM and Niso (NCX+LTCC) blockade significantly decreased the incidence rate of arrhythmias (Figure 4), and first appearance of ventricular fibrillation (Figure 5). This information is in good agreement with the data reported by Kohajda et al. (2016), where ORM-10962 significantly supressed the VT/VF in anaesthetized guinea pig mo-

del. Nisoldipine preincubation alone did not possess any protective effect against ouabain induced arrhythmias. We may argue that the applied concentration (30 nM nisoldipine) is extremely low, but obviously we must state that we have opted for application of nisoldipine because it well-known as selective and potent ICaL blockers, and not as typical antiarrhythmic agent. Finally, the results of the combined application of NCX+LTCC blockers support our hypothesis.

The combined NCX+LTCC blockade was first time studied by Bourgonje et al (2013) by investigating the combined effect of SEA0400+verapamil in a chronic AV-block dog model of arrhythmia. They reported that 1 μM SEA-0400 blocked by more than 50% either forward or reverse modes of NCX and at least 33% the L-type calcium current. SEA compound did not affect left ventricular pressure and effectively decreased in a concentration dependent manner the development of the dofetilide induced Torsades de Pointes (TdP) arrhythmias. Verapamil exerted a negative inotropic effect, but also prevented the prevalence of TdPs. They concluded that the combination of the two drugs may be beneficial by counterbalancing the negative side effects of the monotherapy of the LTCC blockade, ie, unlike specific inhibition of LTCC, combined NCX and LTCC inhibition has no negative effects on cardiac hemodynamics. This study, however, has several weaknesses: i) the selectivity of SEA-0400 is questionable, because at the concentration range where the compound effectively inhibits both NCX modes (»1 μM) it block also least 30-50% the amplitude of the ICaL current also (1); ii) the selectivity of verapamil is also questionable, several studies reported that verapamil may also block repolarizing potassium currents for example IKr/HERG current[20]; 2. These observation makes questionable the results of the study of Bourgonje et al. (2013), because it is difficile to identify which compound (SEA or verapamil) was the main antiarrhythmic factor of the combined therapy.

Limitation of the study

We must emphasize two limitations:

First, nisoldipine was not the best option in this investigation because unfortunately possess strong coronary dilating and smooth muscle relaxing effects, which may diminish the potential antiarrhythmic effect of the combined drug effects. Second, the other limitation of our investigation was the relative low number of experimenst in each study groups, however, the satistics were clear and conlusive, and we could draw the main conclusion that the combined blockade is efficacious in preventing (by delay) or suppresing of ouabain induced (delayed afterdepolarization based) arrhythmias.

CONCLUSION

Our results indicated that the decrease of the Ca^{2+}overload by combined NCX+LTCC blockade is more favourable than specific separate NCX or LTC inhibitions alone.

Our results indicate that it would be worthwile to continue this direction and investigate the possible effect of combined NCX+LTCC blockers using better compounds as nisoldipine. Finally, we may also conclude that this combination may be useful in heart failure or any other Ca^{2+}-overload related cardiac diseases as ischaemia since the drug combination did not affect the pump function or the heart frequency, but may prevent any arrhythmias induced by altered Ca^{2+}-homeostasis.

Compliance with ethics requirements:

The authors declare no conflict of interest regarding this article. The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study.

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List of abbreviations

$[Ca^{2+}]i$	intracellular Ca^{2+}concentration
CaT	Ca^{2+} transient
CIRC	Ca^{2+}induced Ca^{2+}release
DAD	delayed afterdepolarization
DMSO	dimethyl-sulphoxide
EAD	early afterdepolarization
ICaL	L-type Ca^{2+}-current
IKr	rapid component of the delayed rectifier K^+-current
KH-solution	Krebs-Henseleit solution

LTCC	L-type Ca^{2+}-channel
NCX	Na^+/Ca^{2+}exchanger
Niso	nisoldipine
ORM	ORM-10962 substance
PMCA	plasma membrane Ca^{2+}-ATPase
RyR	Ryanodin receptor
SEA	SEA-0400 substance
SERCA	sarcoplasmic reticulum membrane Ca^{2+}-ATPase
SR	sarcoplasmic reticulum
TdP	Torsades de Pointes arrhythmia
VT/VF	ventricular tachycardia/fibrillation

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