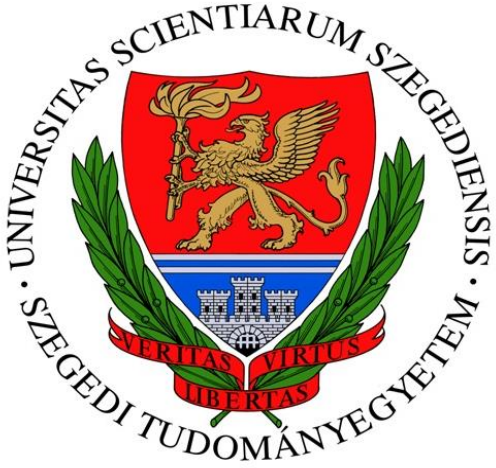
EXPERIMENTAL INVESTIGATION OF THE ACTION POTENTIAL DURATION INFLUENCING ANTIARRHYTHMIC DRUGS IN VIVO

Nikolett Farkas-Morvay, MSc

Summary of Ph.D. Thesis



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Department of Pharmacology and Pharmacotherapy

University of Szeged

Doctoral School of Multidisciplinary Medicine

Supervisor: Prof. Dr. István Leprán PhD, D.Sc.

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PUBLICATIONS RELATED TO THE THESIS

I. Long-term pretreatment with desethylamiodarone (DEA) or amiodarone (AMIO) protects against coronary artery occlusion induced ventricular arrhythmias in conscious rats.

Morvay N, Baczkó I, Sztojkov-Ivanov A, Falkay G, Papp JG, Varró A, Leprán I.
Canadian Journal of Physiology and Pharmacology 93 (9):773-7 (2015)

II. The effect of a novel highly selective inhibitor of the sodium/calcium exchanger (NCX) on cardiac arrhythmias in *in vitro* and *in vivo* experiments.

Kohajda Z, **Farkas-Morvay N**, Jost N, Nagy N, Geramipour A, Horváth A, Varga RS, Hornyik T, Corici C, Acsai K, Horváth B, Prorok J, Ördög B, Déri S, Tóth D, Levijoki J, Pollesello P, Koskelainen T, Otsomaa L, Tóth A, Baczkó I, Leprán I, Nánási PP, Papp JG, Varró A, Virág L.
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V. Investigation of cardiovascular effeccts of long-term endurance exercise training in rabbits and dogs

Polyák A, Kui P, **Morvay N**, Leprán I, Ágoston G, Varga A, Baczkó I, Farkas A, Papp JGy, Varró A, Farkas A

CARDIOLOGIA HUNGARICA 47: Suppl.G pp. G40-46. , 7 p. (2017)

VI. Long-term endurance training-induced cardiac adaptation in new rabbit and dog animal models of the human athlete's heart

Polyák A, Kui P, **Morvay N**, Leprán I, Ágoston G, Varga A, Nagy N, Baczkó I, Farkas A, Papp JGy, Varró A, Farkas AS

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1. INTRODUCTION

1.1. Epidemiology of cardiovascular diseases

Cardiovascular diseases (CVD) are the leading cause of death both in developed and in developing countries. According to the Global Burden of Disease Study 30% of all death worldwide were caused by CVD. This incidence is even higher in Europe and CVD are responsible for over 4 million deaths per year in Europe, close to half of all death. A common precipitating factor of CVD are arrhythmias. This high prevalence and mortality rate promoted the development of a large number of antiarrhythmic drugs in order to prevent life-threatening arrhythmias and associated subsequent deaths

1.2. Arrhythmias

Pathological changes of the cellular electrical activity were found in the background of the development of arrhythmias. The electrical activity of cardiac tissue is determined by the highly regulated flow of ions across the cell membrane during the cardiac action potential, mediated by several ion channels, currents as well as transport proteins. The relatively long cardiac action potential, resulting in a relatively long refractory period is very important in the maintenance of normal cardiac rhythm.

If the depolarizating currents re-activate earlier – ventricular premature beats can occur. These abnormal depolarizations of cardiac myocytes are called afterdepolarizations, differentiated according to their occurrence during the action potential: early or delayed afterdepolarization (EAD or DAD). Both are the results of the elevated intracellular Ca^{2+} concentration and play a significant role in arrhythmogenesis.

The narrowing or occlusion of a coronary artery (ischaemia), thereby the reduced blood supply of the heart, can contribute to the development of severe arrhythmias.

The restoration of the blood flow, i.e. reperfusion, is a generally accepted method in case of myocardial ischaemia to reduce the irreversible damage of cells. However, reperfusion itself can be harmful due to the Ca^{2+} overload, reactive oxygen species (ROS) formation and accumulation, the rapid change of the intracellular pH and the increase of mitochondrial permeability. All of these changes together can cause endothelial dysfunction, reversible loss of myocardial contractility (myocardial stunning), arrhythmias, necrosis and apoptosis, which are known as reperfusion injury. The above-mentioned electrophysiological changes can induce different type of arrhythmias; reentry due to the slowed conduction, unidirectional block or inhomogeneity in the refractory period and/or triggered activity due to Ca^{2+} overload.

1.3. Antiarrhythmic therapies

During the last decades, the still high rate of cardiac arrhythmia-related death has been promoted the innovations of both interventional (catheter ablation, implantable cardioverter defibrillator) and pharmacological therapies.

The targets of pharmacological intervention are 1) the suppression of the automaticity (decreasing the ectopic activity) by the inhibition of the Na^+ and Ca^{2+} channels, 2) the lengthening of the refractory period (terminating the reentry), 3) the reduction of triggered activity and 4) the decrease of the activity of the sympathetic autonomous nervous system.

1.3.1. Amiodarone-Desethylamiodarone

According to the Vaughan Williams classification, amiodarone (AMIO, iodine-containing benzofuran derivative) is a representative of the class III antiarrhythmic drugs, with complex antiarrhythmic effects. The main effects of AMIO are the prolongation of the action potential duration and the inhibition of the K^+ channels. Moreover, it can also inhibit other ion channels, e.g. cardiac voltage dependent sodium-, and calcium-channels, as well as produce a β-adrenergic blocking effect.

The therapeutic use is, however, limited by serious extracardiac adverse effects, e.g. pulmonary fibrosis, hepatotoxicity, photodermatosis, cornea deposits. Its unique pharmacokinetic properties (extremely long elimination half-life and its metabolic interactions with several other drugs) also produce difficulties during the therapeutic use. During chronic AMIO treatment an electrophysiologically active metabolite, desethylamiodarone (DEA) is produced which accumulates in the blood plasma and tissues, including the myocardium. DEA produces very similar pharmacologic effects to AMIO.

Acute administration of both the AMIO and DEA significantly prolonged the QRS, QT and QTc intervals of the electrocardiogram in rats. DEA showed a significantly greater potency in this respect. Acute treatments with either AMIO or DEA suppressed ventricular arrhythmias after a two-stage coronary artery ligation in dogs. Long-term administration of AMIO or DEA in rabbits prolonged the APD and the effective refractory period.

However, there are no data available comparing the effects of a long-term treatment with AMIO and DEA on arrhythmias under *in vivo* conditions.

1.4. Sodium/calcium exchanger

The Na^+/Ca^{2+} exchanger (NCX) is considered the main transmembrane intracellular Ca^{2+} remover in the cardiac cell. During systole, the elevated cytosolic Ca^{2+} concentration induces NCX to extrude 1 Ca^{2+} from the cytosol into the extracellular space, transporting 3 Na^+ from the extracellular space to the cytosol. This ionic exchange generates an inward current (I_{NCX}) leading to an increase in net I_{Na} which delays repolarization. Therefore, increased I_{NCX} – by prolonging repolarization – can lead to the formation of DAD, triggering tachyarrhythmias. On the other hand, reduced I_{NCX} would decrease I_{Na} thus APD shortens, leading to decreased formation of triggered arrhythmias.

NCX plays an important role in ischaemia/reperfusion (I/R) injuries, as well. Under pathological conditions, the intracellular Na^+ level raises due to the inhibition of the Na^+/K^+ - ATPase. During high intracellular Na^+ concentration, the NCX is working in a reverse mode, resulting in the elevation of intracellular Ca^{2+} concentration. Under ischaemia the intracellular acidosis activates the Na^+/H^+ exchanger (NHE), increasing further the intracellular Na^+ level. This process further activates the reverse mode of NCX, resulting in a Ca^{2+} overload, causing cell injury and myocardial stunning.

In the clinical practice, class I/B antiarrhythmic drugs (lidocaine, phenytoin), or intravenously administered magnesium can be applied for the elimination of DAD-induced arrhythmias. The specific inhibition of NCX is thought to be an effective therapeutic tool against Ca^{2+} overload related arrhythmias (EAD, DAD).

KB-R7943 and SEA 0400 are two widely investigated NCX blockers. SEA 0400 seems to be about 100 times more selective than KB-R7943, without the inhibition of Na^+ and K^+ ion channels, while inhibiting Ca^{2+} channels. Although, neither of them is completely selective, their antiarrhythmic effects have been elucidated in different *in vitro* as well as *in vivo* animal models. Both KB-R7943 and SEA 0400 exert protective effects due to the limitation of the infarct size after coronary artery occlusion/reperfusion as well as decreasing the incidence of ventricular arrhythmias. However, the antiarrhythmic effect is controversial and negative results are also found.

More recently an even more selective NCX inhibitor, ORM 10962 became in focus of researches. Some in vitro experimental results proved the selectivity and effectiveness of ORM 10962, however no in vivo results on arrhythmias are available.

1.5. Cardiac glycosides

Cardiac glycosides (e.g. digoxin, ouabain) have positive inotropic effect so they are widely used to improve the force of the cardiac contraction in failing heart. However, they often induce arrhythmias due to the excess inhibition of the Na^+/K^+-ATPase, leading to the excess accumulation of intracellular Ca^{2+}. It has been found that NCX blockers such as KB-R7943 and SEA 0400 have protective effects against ouabain-induced arrhythmias in guinea-pigs. However, due to the questionable selectivity of these inhibitors, it is very speculative to assume that this effect is mediated by the selective inhibition of NCX. A highly selective NCX inhibitor (i.e. ORM 10962) could help to elucidate this question.

1.6. Aims of the study

The aims of the present investigations were to elucidate:

1. the effectiveness of long-term pretreatment with AMIO or with its active metabolite, DEA, on ventricular arrhythmias, induced by acute coronary occlusion in conscious rats.
2. the effects of highly selective and effective NCX inhibitor ORM 10962, and to compare it to the effects of SEA 0400, on ouabain- or ischaemia/reperfusion-induced cardiac arrhythmias *in vivo*.

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague-Dawley CFY rats and Hartley guinea-pigs were used. The animals were fed with standard chow for rats or guinea-pig, respectively, and were allowed to drink tap water *ad libitum*. 12 h dark/light cycles were maintained.

2.2. Coronary artery ligation-induced arrhythmias in conscious rats

In a preliminary surgery under ether anaesthesia, we opened the chest in the 4th intercostal space. A loose silk loop was led around the left main coronary artery, and then the chest was closed. Small needle-electrodes were implanted subcutaneously in order to register the ECG. After the complete recovery and healing of the animals, the loose silk loop was tightened to occlude the coronary artery in conscious, freely moving animals. During the first 15 min of myocardial infarction (MI) a bipolar ECG was recorded continuously.

The survival rate during the acute phase (first 15 min) and during the following 16 hours after coronary artery occlusion was followed. The incidence and duration of arrhythmias in the acute phase were evaluated according to the Lambeth Conventions. An arrhythmia score was given, including the type and duration of different arrhythmias by giving a grade to each animal.

The size of the myocardial infarction was quantified in the animals surviving for 16 hours after coronary artery occlusion. AMIO and DEA concentration of the plasma and the myocardium was determined.

2.3. Myocardial ischaemia/reperfusion-induced arrhythmias in anesthetized rats

During pentobarbitone anaesthesia in rats the chest was opened between the fourth and fifth ribs and the heart was exposed. A loose loop of atraumatic silk was placed around the left main coronary artery. After the surgery artificial respiration was started. After 10 minutes normalization we occluded the coronary artery by tightening the loop for 6 minutes, followed by a reperfusion period for 5 minutes. Arterial blood pressure was measured via the right arteria carotis communis. The left vena femoralis was prepared and cannulated for drug administration.

The four limb lead ECG was recorded continuously. The survival rate and incidence of arrhythmias was determinated. No attempt was made artificially to revert VF during ischemia or reperfusion.

2.4. Ouabain-induced arrhythmias in guinea-pigs

2.4.1. Prevention of ouabain-induced arrhythmias

Male guinea-pigs were anaesthetized with pentobarbitone. The trachea was prepared, cannulated and the animals were ventilated using a respirator with room air. The right arteria carotis communis was prepared and cannulated in order to measure the arterial blood pressure. The left vena jugularis was cannulated for the drug administration and later for the ouabain infusion. Ouabain was continuously infused intravenously via a polyethylene cannula.

2.4.2. Suspension of ouabain-induced arrhythmias

The above-described *(2.4.1)* preparation was performed with the modification that the investigated compound was administered intravenously after the termination of ouabain infusion at the 16^{th} min.

3. RESULTS

3.1. Comparing the antiarrhythmic effects of amiodarone and desethylamiodarone in vivo

Neither AMIO nor DEA administration produced any visible behavioral changes of the animals during the long-term treatment, or any difference in the gaining of body weight.

Coronary artery occlusion in conscious rats within 4-6 minutes resulted in various arrhythmias (ventricular extrasystole, bigeminia, ventricular tachycardia), leading frequently to irreversible ventricular fibrillation. Both AMIO (100 mg/kg/day) and DEA (50 mg/kg/day) pretreatments (one month long) significantly improved the survival rate during the acute phase of experimental myocardial infarction (82% and 64% vs. 31%) and delayed the onset time of the first arrhythmias. However, the mean duration of the arrhythmic attacks in the surviving animals did not change significantly (32 sec and 46 sec vs. 41 sec). Interestingly, some animals did not develop arrhythmias at all during the first 15 min after coronary artery occlusion, in spite of the characteristic ECG changes (e.g. the QRS-complex distortion and T-wave elevation) and developing myocardial damage 16 hours later.

The mean values of the baseline heart rate did not differ among the control and pretreated groups before the coronary artery occlusion (321 and 330 beat/min vs. 327 beat/min). A modest elevation in the heart rate was detected in each experimental group during the coronary artery occlusion.

As a result of AMIO treatment the plasma concentration of its active metabolite (i.e. DEA) was only 1/4 of the parent molecule (AMIO) (0.15 μg/ml vs. 0.68 μg/ml). The tissue concentration of AMIO in the myocardium was about 10-times higher than in the plasma, and was equivalent to the tissue concentration of DEA. Long-term oral DEA treatment resulted in similar myocardial tissue DEA concentration to that measured after AMIO pretreatment (7.35 μg/ml vs. 8.95 μg/ml).

3.2. Investigation of the antiarrhythmic effects of sodium/calcium exchange inhibition

3.2.1. Effects of SEA 0400 and ORM 10962 pretreatments on coronary artery occlusion/reperfusion-induced arrhythmias in rats

Coronary artery occlusion in anesthetized rats resulted in sporadic arrhythmias starting around the 5th minutes of ischaemia. The 6-minutes-long occlusion rarely produced life-threatening arrhythmias, however, reperfusion after this ischaemia rapidly induced severe arrhythmias, leading to death in some of the rats. One-third of the control animals died due to irreversible ventricular fibrillation. Other serious arrhythmias, e.g. tachycardia, frequently occurred. Interestingly, ventricular fibrillation, that was spontaneously reversible within 1 minute, also developed in 3 out of the 14 surviving control animals. One animal survived the reperfusion period without developing any arrhythmias.

Neither SEA 0400, nor ORM 10962 pretreatments could significantly influence the development, or severity of these arrhythmias, the survival rate, as well as the heart rate or blood pressure response, compared to the control.

3.2.2. Effects of SEA 0400 and ORM 10962 pretreatments on ouabain-induced arrhythmias in guinea-pigs

Intravenous ouabain infusion induced various arrhythmias in guinea-pigs. The first arrhythmias, such as extrasystole and bigeminia, developed around the 24th minute in the control group. Later, ventricular tachycardia appeared, which followed by ventricular fibrillation or AV block around the 37th minute after starting ouabain infusion.

Both the SEA 0400 and ORM 10962 pretreatments significantly delayed the onset time of ventricular arrhythmias as compared to the control group (VEB: 36.4±2.12 min and 36.6±2.64 min vs. 24±1.73 min). The two NCX inhibitor did not produce significant differences in the heart rate or blood pressure parameters before the drug administration.

Ouabain increased the heart rate and the blood pressure during its infusion. All of the pretreatments shifted the blood pressure increasing effect of ouabain to a later time point, in parallel with the delaying of the appearance of arrhythmias.

3.2.3. Effects of ORM 10962 on the suspension of ouabain-induced arrhythmias

Intravenous administration of ouabain did not produce severe arrhythmias during the 16 min infusion. Termination of the infusion of ouabain at this time point resulted in the progression of arrhythmias. All of the control animals developed some kind of arrhythmia; e.g. ventricular tachycardia developed in three animals, and even reversible ventricular fibrillation occurred only in one animal. Heart rate and blood pressure could not be determined during this period due to frequent arrhythmias. After ORM 10962 treatment, the progression of ouabain-induced arrhythmias was less expressed. The duration of the arrhythmic period was significantly shorter compared to the control group and the arrhythmias during this time were not continuous.

4. DISCUSSION

4.1. Antiarrhythmic effect of amiodarone and its stable metabolite, desethylamiodarone

Because of the influencing effect of the anesthetic agent, artificial ventilation and acute surgery on the development of arrhythmias, in the present experiments a conscious animal model is used. This means that the acute phase of myocardial infarction develops in intact, freely moving animals.

We demonstrated that chronic oral DEA pretreatment (using half of the dose of AMIO) produced similar DEA levels in the plasma and myocardium as with double dose of AMIO. We also demonstrated that DEA – the main metabolite of AMIO – exerted similar antiarrhythmic effects to its parent compound, after long-term oral pretreatment in an acute coronary artery ligation-induced ventricular arrhythmia model in conscious rats.

AMIO treatment is still the most frequently used and effective therapy to prevent arrhythmias in the clinical practice. The advantages are its negligible negative inotropic effect and the very low incidence of proarrhythmia. On the other hand, the long-term routine use may result in several problems, due to the unique pharmacokinetic properties and its serious extracardiac adverse effects.

During the first pass metabolism, AMIO is extensively and variably metabolized to DEA. Both AMIO and DEA are further metabolized by dealkylation, hydroxylation and deamination. Enzyme polymorphism, the co-administration of enzyme inhibitors or inducers greatly influence the rate of conversion of amiodarone and it is responsible for the great individual variations in response and for its interaction with other drugs or food.

Previous investigations showed, that DEA – given as a single dose of 50 mg/kg intraperitoneally, 30 min before the coronary artery ligation in a similar arrhythmia model in conscious rats – significantly improved the survival. Our present investigations, using long-term oral pretreatment with DEA, supports and extends these earlier experiments. In the present experiments DEA offered significant protection against the early phase of arrhythmias, as well as produced similar plasma concentrations as it is measured after the double dose of AMIO. Therefore, DEA may produce less adverse effects and less problem of drug interactions during its long-term use.

4.2. Antiarrhythmic effects of sodium/calcium exchange inhibition

According to our investigations, selective NCX blockade by SEA 0400 and ORM 10962 proved to be effective against DAD related arrhythmogenesis in *in vivo* ouabain-induced cardiac arrhythmias in guinea-pigs, however, it failed to exert cardioprotection against ischaemia/reperfusion-induced arrhythmias in rats.

The ouabain-induced arrhythmia model is widely used to investigate antiarrhythmic effects in animals e.g. guinea-pigs and dogs. Ouabain increases the arterial systolic- and diastolic blood pressure and has a positive inotropic effect via the inhibition of the Na^+/K^+-ATPase, resulting in the accumulation of intracellular Na^+ and thereby activating the reverse mode of NCX, therefore it can be arrhythmogenic due to the Ca^{2+} overload. The mechanism of the antiarrhythmic effect may be based on the inhibition this increase in intracellular Ca^{2+} level via the inhibition of the reverse mode activity of NCX.

The selectivity and potency of the recently investigated NCX blockers are in the following order: KB-R7943<SEA 0400<ORM 10103. Although, SEA 0400 is more potent and selective, than KB-R7943, its effectiveness to prevent arrhythmias is controversial.

ORM 10962 shows higher activity and even better selectivity to inhibit NCX current, and can be administered in *in vivo* conditions, as well. According to our results, ORM 10962 delayed the development of ouabain-induced ventricular arrhythmias in anesthetized guinea-pigs, presumably due to the inhibition of the reverse mode of NCX. No significant difference was found between SEA 0400 and ORM 10962, although ORM 10962 was administered in lower dose, than SEA 0400. In addition, ORM 10962 was effective also when it is applied after intracellular Ca^{2+} overload, when arrhythmias are already established. The antiarrhythmic effect in this case may be explained by the inhibition of the NCX forward mode, resulting in less depolarization and thereby eliminating DAD's in Purkinje fibres and suppressing arrhythmias.

During myocardial ischaemia, anaerobic metabolism results in intracellular acidosis and ATP depletion, thus inhibiting the Na^+/K^+-ATPase and activating the Na^+/H^+ exchanger (NHE). Hence, the intracellular Na^+ increases, which activates the reverse-mode of NCX resulting in a Ca^{2+} overload, cell injury and myocardial stunning. Selective inhibition of the reverse mode of NCX theoretically may offer a potential therapeutic tool for cardioprotection against ischaemia/reperfusion injury.

According to our results, neither SEA 0400, nor ORM 10962 pretreatments resulted in any significant protection on the incidence or severity of ischaemia/reperfusion-induced ventricular arrhythmias in rats *in vivo.* The explanation of the failure of NCX inhibitors may be the complexity of the development of arrhythmias in this case, involving several different mechanisms, e.g. reentry, spontaneous automacy, DAD's, and NCX may not play important role in each mechanism. In addition, it is suggested, that the initiating step to develop ischaemic damage is the activation of NHE. NHE inhibition offered a protective effect against ischaemia/reperfusion injury. According to the literature, the NHE blocker cariporide provided more powerful antiarrhythmic effect when applied simultaneously with an NCX inhibitor. The inhibition of NCX alone to limit Ca^{2+} overload probably does not play important role in ischemia/reperfusion-induced arrhythmias in anaesthetized rats. These results suggest that the prevention of the initial step, i.e. Na^{+} overload may be more important for cardioprotection than the prevention of Ca^{2+} overload.

We may conclude that the protective effect of SEA 0400 or KB-R7943 in the previous investigations was mainly due to their lower selectivity, i.e. inhibiting Ca^{2+} current, and not to their NCX inhibitory effect.

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