CHARACTERIZATION OF THE EFFECTS OF SARCK$_{ATP}$ AND MITOK$_{ATP}$ MODULATORS ON REPERFUSION-INDUCED ARRHYTHMIAS IN ISOLATED RAT HEARTS

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ABSTRACT

Malignant tachyarrhythmias, ventricular fibrillation (VF) and tachycardia (VT), associated with ischemia/reperfusion (I/R) injury represent a major cause of sudden cardiac death worldwide. Pharmacological modulation of the cardiac ATP-sensitive potassium channels, either sarcolemmal (sarcK$_{ATP}$) or mitochondrial (mitoK$_{ATP}$), has been reported by many (but not all) studies to be cardioprotective against the deleterious effects of postischemic reperfusion. The aim of the present study was to assess the effects of HMR-1098 (10 μM), a cardioselective sarcK$_{ATP}$ inhibitor and of diazoxide (DZX, 50 μM), a mitoKATP opener, given either independently or together, on I/R-related arrhythmias. A brief episode of regional ischemia was elicited by ligation of the left anterior descending coronary artery (LAD) in Langendorff-perfused rat hearts (constant flow mode) and the compounds were given prior to the induction of ischemia. In separate administration, HMR-1098 and DZX significantly reduced the duration of reperfusion-related VF (but not of VT) as compared to the control group. Also, the incidence of both tachyarrhythmias was diminished (albeit not significantly) by the individual administration of these compounds. Intriguingly, neither isolated nor combined administration of HMR-1098 and DZX were able to significantly decrease the duration of ventricular tachycardia (VT). Moreover, in the presence of both compounds the beneficial effect on VF duration, recorded with each of the drug when given solely, was lost. In isolated rat hearts, the independent administration of a sarcK$_{ATP}$ inhibitor and a mitoKATP opener mitigated the duration of reperfusion-induced ventricular fibrillation, whereas the association of these compounds did not influence the incidence and duration of malignant tachyarrhythmias.

Keywords: isolated rat heart, sarcolemmal and mitochondrial ATP-sensitive potassium channels, ischemia-related malignant arrhythmias

INTRODUCTION

Ischemia-related ventricular tachyarrhythmias are currently the major cause of sudden cardiac death worldwide [1]. Preventing malignant arrhythmias in the setting of myocardial ischemia represents an unmet therapeutic target, ultimately aimed at decreasing mortality due to coronary artery disease [2]. The efficiency of current antiarrhythmic therapy is still under debate and a couple of studies have shown that several antiarrhythmic drugs can rather increase the risk of lethal arrhythmias in patients recovering after myocardial infarction [3]. The major mechanism underlying ventricular arrhythmias is represented by reentry, classically promoted by the heterogeneity in action potential duration (APD) and tissue repolarization, respectively. The duration of action potential can be influenced, among others, by the activity of the ATP-sensitive potassium channels (K$_{ATP}$) that couple membrane excitability with cellular energetics [4]. These channels were firstly described by Noma [5] in the sarcolemmal membrane of cardiomyocytes (sarcK$_{ATP}$). A decade later, Inoue *et al.* [6] described a second type of K$_{ATP}$ channels located in the inner mitochondrial membrane (mitoK$_{ATP}$). The ATP-dependent potassium channels are closed during normoxic conditions, when the cellular level of ATP is high, and open when ATP concentration drops during myocardial ischemia, as a result of the accumulation of ischemic metabolites ADP, lactate and H^+ [2].

Paradoxically, both activation and inhibition of sarcK$_{ATP}$ channels have been associated with antiarrhythmic effects [7]. In particular, opening the sarcK$_{ATP}$ channels has been considered responsible for a significant potassium efflux

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with the subsequent shortening of the APD and the increase in the incidence of re-entrant arrhythmias, respectively [3,7-9]. Accordingly, several studies suggested that inhibition of sarcK_{ATP} channels can protect the heart against ischemia/reperfusion-induced arrhythmias, and also improve the survival rates [10-12].

In the past 2 decades, studies have shifted to the investigation of mitoK_{ATP} channels as effectors of cardioprotection [13]. In this regard, opening the mitoK_{ATP} channels was found to preserve mitochondrial structure [14], increase ATP preservation during ischemia and the functional recovery at reperfusion [15], and also to decrease infarct size [16].

In the present study we thought to assess the effect of the combined administration of a cardioselective sarcK_{ATP} inhibitor and a mitoK_{ATP} opener on ischemia-induced arrhythmias in the isolated rat heart model.

MATERIAL AND METHODS

All the experiments were performed using a Langendorff retrograde perfusion system in the constant flow mode. The perfusion buffer Krebs-Henseleit (NaCl 118.0 mmol/L; KCl 3.2 mmol/L; MgSO4 1.2 mmol/L; NaHCO3 25.0 mmol/L; NaH2PO4 1.18 mmol/L; CaCl2 2.5 mmol/L; glucoză 11.1 mmol/L) was oxygenated with carbogen (95% O_2, 5% CO_2), at least 15 minutes prior to the experiment and kept at 37^{0}C and pH=7.4. All experimental procedures were conducted in accordance with the Directive 2010/63/EU and the Romanian Law nr. 43/May 2014 concerning the protection of animals used for scientific purposes. The experimental protocol was approved by the Committee for Research Ethics of "Victor Babes" University for Medicine and Pharmacy of Timişoara, Romania.

Most reagents were purchased from Sigma Aldrich.

Experimental protocol

Male Sprague-Dawley rats (n= 32) were anesthetized using a mixture of xylazine (5 mg/kg) and ketamine (30 mg/kg). After the abdominal laparotomy, 500 IU of heparin was administered via the portal vein to prevent thrombosis. The heart was rapidly excised, rinsed with cold heparinized Krebs-Henseleit solution and mounted on the cannula of the Langendorff apparatus for the retrograde perfusion. After cannulation, hearts were allowed to stabilize for 3 minutes prior being subjected to 8 minutes of regional ischemia followed by 10 minutes of reperfusion. Regional ischemia was elicited by the ligation of left anterior descending (LAD) coronary artery. A brief episode of non-necrotic ischemia was purportedly chosen in order to investigate the 'pure' antiarrhythmic effect, i.e unrelated to the death of the cardiomyocytes. The compounds, HMR-1098 (10 μM) and DZX (50 μM) were dissolved in dimethyl sulfoxide (DMSO, final concentration less than 0.05%) and added to the perfusate before the stabilization period. The experimental protocol is presented in Figure 1.

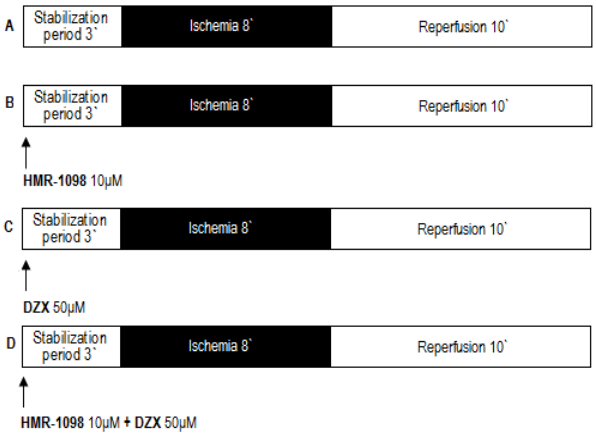

Fig. 1. Experimental protocol.
(A) Control group, (B) HMR-1098, (C) DZX,
(D) HMR-1098 + DZX treated groups.

Statistics

Ventricular arrhythmic events were analyzed as incidence and duration expressed as $\log_{10}$ from the total reperfusion time (600s). Statistical analysis was performed using one-way ANOVA and Tukey's test as Post-hoc comparison among the groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

The present study was purported to assess the 'pure' antiarrhythmic properties of two K_{ATP} modulators, a sarcK_{ATP} inhibitor and a mitoKATP opener, and whether their combined administration might provide an additive effect.

The Effects of HMR-1098 and DZX on Tachyarrhythmias Incidence

The incidence of VT and VF in the study groups is shown in Table 1. The brief 10 min period of postischemic reperfusion was associated with VF in all animals and VT in 5 out of the 8 rats of the control group, respectively. A decreasing tendency in the incidence of both types of malignant arrhythmias in the presence of either K_{ATP} modulator when given independently was recorded. However, when given together, the incidence of both arrhythmias was comparable to the one of the control group (5 out of 8 animals for VT, and 7 out of 8 animals for VF) - Table I.

Table I. The incidence of VF and VT.

	Control	*HMR-1098*	*DZX*	*DZX+ HMR-1098*
VT	5/8	3/8	4/8	5/8
VF	8/8	5/8	6/8	7/8
Normal heart rhythm	2/8	8/8	7/8	6/8

The Effects of HMR-1098 and DZX on Tachyarrhythmia Duration

Surprisingly, neither the individual nor the combined administration of HMR-1098 and DZX were able to reduce the duration of VT as compared to controls (Figure 2).

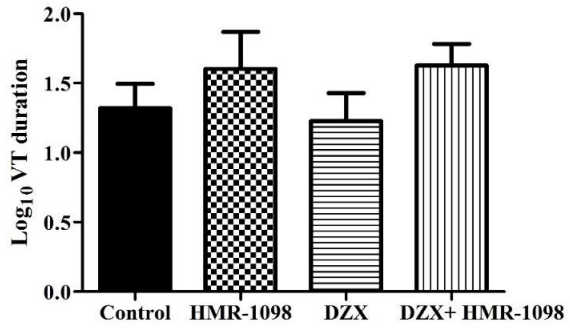

Fig. 2. The isolated and combined effects of HMR-1098 and DZX on VT duration.
(n = 8/group, p = NS)

In contrast, inhibition of sarcK_{ATP} channels with the selective compound HMR-1098 and opening of mitoK_{ATP} channels with DZX was each followed by a significant decrease in the mean duration of VF (Figure 3). However, the association of both K_{ATP} modulators abolished the beneficial effect of the individual administration on VF duration (Figure 3).

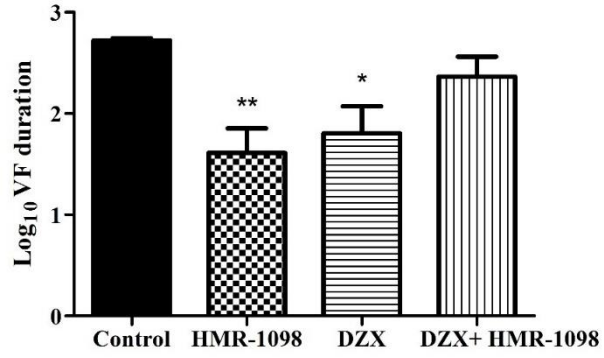

Fig.3. The isolated and combined effects of HMR-1098 and DZX on VF duration.
(n=8/group, **p<0.01 for HMR-1098 *vs*. Control, *p<0.05 for DZX *vs*. Control)

DISCUSSIONS

The major finding of this paper is that the sarcK_{ATP} inhibitor and the mitoKATP opener, albeit beneficial in individual administration against VF, were not able to diminish the incidence and duration of reperfusion-related ventricular arrhythmias when jointly given prior to the ischemic episode.

The cardioprotective effects, including the antiarrhythmic ones, of K_{ATP} modulators were extensively [for recent comprehensive reviews see refs. 17, 18]. In particular, glibenclamide, a non-selective sarcK_{ATP} inhibitor belonging to the second generation of sulphonylureas, was systematically reported to prevent/reduce the incidence of arrhythmias in several *in vitro* and *in vivo* experimental models and also in the clinical arena [17, 22-23]. HMR-1098 is a sulfonylthiourea, reported as cardioselective blocker of cardiac sarcK_{ATP} channels by some (but not all) authors [24] that, at variance from glibenclamide, did not influence the blood glucose levels [25] or the coronary flow under normoxic and hypoxic conditions [26]. Conflicting results are available in the literature with respect to the antiarrhythmic action of HMR-1098. Accordingly, Fischbach *et al.* demonstrated that pre-treatment with 3 μM HMR-1098 was able to prevent the apparition of VF in isolated rabbit hearts, and also to block (at 1μM) the VF induced by pinacidil, a non-selective K_{ATP} agonist [27]. In the canine old myocardial infarction model, HMR-1098 (but not glibenclamide) improved the scores of programmed electrical stimulation-induced ventricular arrhythmias, as reported by Zhu *et al.* [28]. At variance, Gok *et al.* reported that HMR-1098 (3 and 30 μmol/l) was not able to prevent VT and VF in isolated rat hearts subjected to 30 min of coronary occlusion followed by 30 min of reperfusion [29]. In our model of short I/R injury, we observed decrease in the incidence (but not the duration) of reperfusion-associated VT in the presence of HMR-1098. Conversely, both incidence and duration of VF were dimished, albeit statistical significance ($P < 0.01$, *vs*. control) was reached only in case of the latter.

DZX is a benzothiadiazine with important cardioprotective properties that has been reported to exert antiarrhythmic effects in the rat model of I/R injury, both *in vivo* and *in vitro* [reviewed in ref. 17]. In our study, pre-treatment with DZX effects comparable to the ones of HMR-1098 with respect to the incidence and duration of VT and VF. Thus, in independent administration, DZX mitigated (albeit non-significantly) the incidence of both VT and VF, had no effect on VT duration, and significantly diminished the VF duration ($P < 0.05$, *vs*. control). Surprisingly, in combined administration, the K_{ATP} modulators had no beneficial effect on either incidence or duration of VT and VF.

In the setting of I/R injury, cardioprotection associated with mitoK_{ATP} opening has been ascribed to the following

mechanisms: mitochondrial swelling, inhibition of ATP synthesis during ischemia, decreased mitochondrial Ca^{2+} overload, modulation of ROS production during ischemia and reperfusion, respiratory inhibition, and mild uncoupling - mechanisms that underlie the infarct size reduction and the increase in functional recovery at reperfusion [30]. In the literature there are only a few studies that examined the antiarrhythmic effect of mitoK_{ATP} opening. Thus, in anesthetized rabbits, two selective mitoK_{ATP} openers, nicorandil and minoxidil, when administered prior to regional ischemia of LAD, were able to increase the survival rate and decrease arrhythmogenesis, respectively [2]. In the canine model of in vivo I/R injury, DZX protected the myocardium against ischemia/reperfusion- induced arrhythmias, effect blocked by the administration of 5-HD, the classic mitoK_{ATP} blocker [31].

Interestingly, the loss of mitochondrial selectivity has been reported during ischemia for both DZX and nicorandil, when they can activate sarcK_{ATP} as result of increased ADP concentration [32]. In line with this observation we can speculate that the loss of antiarrhythmic effects when associating DZX to HMR-1098 was the consequence of counteracting the effect of the latter on sarcK_{ATP}; however, we did not measure the ADP concentration to confirm that it was decreased. However, other mechanisms can be also incriminated since DZX has been reported to elicit K_{ATP}-independent effects in cardiomyocytes [33]. Further experiments aimed at assessing the particular effect of each drug on the sarcK_{ATP} channel activity are required.

CONCLUSIONS

In isolated rat hearts, the independent administration of a sarcK_{ATP} inhibitor and a mitoKATP opener mitigated the duration of reperfusion-induced ventricular fibrillation, whereas the association of these compounds did not influence the incidence and duration of malignant tachyarrhythmias.

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CARACTERIZAREA EFECTELOR MODULATORILOR SARCK_{ATP} ȘI MITOK_{ATP} ASUPRA ARITMIILOR INDUSE DE REPERFUZIE PE INIMI IZOLATE DE ȘOBOLAN

REZUMAT

Tahiaritmiile maligne, fibrilația și tahicardia ventriculară (FV și TV) asociate ischemiei/reperfuziei (I/R) miocardice reprezintă o cauză majoră de moarte cardiacă subită la nivel global. Modularea farmacologică a canalelor cardiace de potasiu dependente de ATP localizate atât la nivel sarcolemal (sarcK_{ATP}) cât și la nivel mitocondrial (mitoK_{ATP}) a fost asociată cu cardioprotecție în condițiile ischemiei/reprefuziei miocardice în numeroase studii, fără însă ca această observație să fie fără echivoc. Scopul prezentului studiu a fost de a evalua efectele administrării izolate și în asociere a HMR-1098 (10 μM), inhibitorului selectiv al canalelor sarcK_{ATP} și respectiv, a diazoxidului (DZX, 50 μM), deschizătorului canalelor mitoKATP, asupra tahiaritmiilor induse de reperfuzia postischemică. Ischemia regională a fost indusă prin ligatura arterei coronare anterioare descendente stângi la nivelul inimilor izolate de șobolan perfuzate retrograd tip Langendorff (în modul de lucru cu flux constant), iar compușii au fost administrați anterior inducerii ischemiei regionale. În administrare independentă, atât HMR-1098 cât și DZX au redus semnificativ durata FV (dar nu și a TV) comparativ cu lotul martor. De asemenea, aplicarea lor individuală a redus (deși nu semnificativ) incidența ambelor tahiaritmii maligne. Surprinzător, nici administrarea independentă nici cea în asociere a compușilor nu au condus la o reducere semnificativă a duratei TV. Mai mult, administrarea combinată a condus la pierderea efectului benefic obținut în cazul aplicării individuale asupra duratei FV. În concluzie, în cazul inimilor izolate de șobolan, administrarea independentă a unui inhibitor al sarcK_{ATP} și respectiv, a unui deschizător al mitoKATP, a redus durata fibrilației ventriculare la reperfuzia postischemică, în timp ce asocierea acestor compuși nu a influențat incidența și durata tahiaritmiilor maligne.

Cuvinte cheie: inimă izolată de șobolan**,** canale de poatasiu sarcolemale si mitocondriale dependente de ATP, aritmii maligne induse de ischemie