SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

The role and pharmacological treatment options of hyperinsulinemia and metabolic syndrome in cardiac arrhythmogenesis

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The Examination takes place at the Lecture Hall of "Building A", Department of Internal Medicine, Medical and Health Science Center, University of Debrecen
13.00 p.m., November 7, 2013

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The Ph.D. Defense takes place at the Lecture Hall of "Building A", Department of Internal Medicine, Medical and Health Science Center, University of Debrecen
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1. INTRODUCTION

Metabolic syndrome comprising the unique constellation of insulin resistance, obesity, dyslipidemia and hypertension, can be considered as the most relevant public healthcare problem in the 21st century. According to the recent surveys, approximately 20-25% of the global population is suffering from metabolic syndrome, which provide a leading position to the disorder in the ranking of *non-communicable diseases* (*NCD*). The prevalence of metabolic syndrome is considerably high in the highly-industrialized countries but the developing ones also, moreover the incidence of the disease is increasing permanently and rapidly. The explosive incidence of insulin resistance has contributed significantly to become metabolic syndrome as an „epidemic", currently. On the basis of some estimations, 371 millions of people (8,3% of the global population) are affected by *diabetes mellitus* (*DM*). Since *type 2 diabetes mellitus* (*T2DM*) is responsible for 90% of all the diabetic cases, the rapidly growing incidence of insulin resistance contributes particularly to the significant burst of the number of patients suffering from metabolic syndrome.

Patients with metabolic syndrome exhibit elevated cardiovascular risk, since the incidence of cardiovascular diseases (*CVD*) in those is more than doubled as compared to the healthy population. Similar tendency was observed at diabetics also, because approximately 80% of the mortality of *DM* is caused by the direct or indirect consequences of *CVD*. The studies investigating the possible link between the abovementioned metabolic abnormalities and *CVD* has taken particularly into account the cases of ischemic heart disease (*IHD*) and peripheral artery disease (*PAD*). Limited number of research is available examining the potential influence of diseases based on insulin resistance on cardiac arrhythmogenesis. Consequently, it has not been entirely clarified yet, in what extent the arrhythmias are responsible for the elevated cardiac morbidity and mortality associated with the metabolic disorders resulted from insulin resistance. Obviously, this scientific gap is even the main cause of the total

lack of antiarrhythmic drugs in the abundant pharmacological treatment options of the metabolic diseases caused by insulin resistance.

2. PURPOSES

Taking rigorously into account the principal preclinical and clinical manifestations of the main phases of insulin resistance (compensatory hiperinsulinemia, obvious insulin resistance), the main goals of the present research were as follows:

To investigate the

- arrhythmia inducibility,
- and the underlying mechanisms of arrhythmia development

associated with hiperinsulinemia and metabolic syndome on preclinical animal models exhibiting reliably the essential signs and symptoms of the abovementioned metabolic disorders.

Moreover to test

- drugs that can mitigate effectively the arrhythmia inducibility,
- and to determine those potential cardioprotective mechanisms of action.

3. METHODS

3.1. General aspects

Experiments of the present research were divided according to the progression of insulin resistance as a preclinical and clinical entity (ie. „*Hyperinsulinemia protocol*" and „*Metabolic syndrome protocol*"). The applied experimental protocol has been approved by the local ethical boards of the University of Debrecen (licence numbers: *6/2007 DE MÁB, 13/2007 DE MÁB*).

3.1.1. Experimental animals

The experiments were carried out on male *New Zealand White* (*NZW*) rabbits (*Charles-Rivers Laboratories, Isaszeg, Hungary*) weighing from 3 to 3,5 kilogramms (kg).

3.1.2. Surgical procedure

Implantation of pacemaker electrode catheter (*Eledyn 2/F4 S®, B. Braun Melsungen AG, Melsungen, Germany*) into the animals was prerequisite for the electrophysiological measurements and *programmed electrical stimulation* (*PES*) performed in the subsequent part of the experiments.

3.1.3. Electrophysiological and hemodynamic measurements

Detection, recording, evaluation

A digitally-recorded, 12-lead body surface ECG (*EXP-ECG-P, Experimetria Kft., Budapest, Hungary*) was obtained by using bipolar limb- and unipolar chest leads in each rabbits. The electrodes (ie. precordial and standard leads) were placed analoguous as those applied in humans. The electrophysiological and hemodynamic variables were detected and recorded continuously during the set of experiments. The subsequent analysis of the recordings was performed by employing *Haemosys software* (*Experimetria Kft., Budapest, Hungary*). To determine the electrophysiological parameters, a signal-averaged ECG for 30-cycle periods devoid of extrasystoles was computed. Recordings obtained from the lead V_2 and V_3 were considered with the highest priority in the process of evaluation.

The examined elecrophysiological parameters

QT interval is a surrogate marker to indicate the increased myocardial susceptibility for threatening arrhythmias and its determination is paramount regarding the assessment of the individual risk for cardiac rhythm disturbances. *QT* interval was

considered as time interval from the initial point of the Q-wave to the end point of the T-wave. The end of the T-wave was established by using the tangential method. Respecting the strict frequency dependence of the *QT* interval, the heart rate-corrected value (QT_c) of that was calculated according to the *Bazett-formula.*

Recent evidence suggest that the principal substrate for the development of ventricular arrhythmias is better reflected on ECG signs of *transmural dispersion of repolarization* (*TDR*) than those of *QT* prolongation (ie. *QT*, QT_c). Amplification of *TDR* is considerably associated with numerous familiar and acquired proarrhythmic states, whereas the incidence of cardiac rhythm disturbances is significantly elevated. Since *TDR* correlates closely with the time interval measured between the peak and the end of the T-wave (T_{peak}-T_{end} /T_p-T_e/), therefore we determined also that. The maximal amplitude of the T-wave (T_{peak} /T_p/) was indicated by the peak of that. The end of the T-wave (T_{end} /T_e/) was even determined by the tangential method. ECG recordings obtained from the precordial leads (V_2, V_3, V_4) were used for assessing T_{peak}-T_{end} interval, since these electrodes provide look to the heart in coronal section.

QT_{peak} interval (QT_p) was considered the time interval starting from the initial point of the Q-wave and lasting to the peak of the T-wave.

At the determiantion of the *Ventricular Effective Refractory Period* (*VERP*) we applied *PES*. Briefly, electrical square impulses (S_1) of 1,5 ms duration at twice diastolic threshold were delivered via the previously-implanted pacemaker electrode by means of a programmable stimulator (*ST-02*, *Experimetria Kft.*, *Budapest, Hungary*). A single programmed extrastimulus (S_2) was introduced late in the diastole following 12 basic driven beats (S_1) of 200-ms cycle length. The coupling interval of S_2 was then gradually shortened in 2-ms steps until the disappearence of signs of ventricular activation on the surface ECG. The longest interval that failed to produce activation of the ventricules was referred to as VERP.

RR interval was measured by using the time interval between two consecutive R-wave. *Heart rate* (*HR*) was calculated from the *RR interval*.

Mean Arterial Blood Pressure (*MABP*) was continuously measured via a percutaneous cannula (*Vasofix Braunüle® G22, B. Braun Melsungen AG, Germany*) inserted into the the ear artery of the experimental animal and attached to an electromanometer device (*EXP-D2, Experimetria Kft., Budapest, Hungary*).

Arrhythmia induction

In order to evoke cardiac arrhythmias in the preclinical experimental model, we carried out *PES*. The setup of the stimulation was nearly analoguous as empolyed at the determinetion of the *VERP*. In brief, two consecutive programmed extrastimuli (S_2 & S_3) were applied late in the diastole subsequently to 12 basic driven beats (S_1) at 200-ms cycle length. The coupling interval between the last driven beat (S_1) and the first extrastimulus (S_2) was 110-120% of the previously-determined *VERP*, while the coupling interval of the S_2 and S_3 was further lengthened by 10% of *VERP*. The stimulation train (ST) (ie. 12 basic driven beats plus S_2 and S_3 extrastimuli) was repeated 10 times, and the quality of the evoked cardiac arrhythmias were defined on the basis of their ECG morphology and time duration by implementing the guidelines of Lambeth Conventions.

To determine the quantity of cardiac arrhythmias, all of them were counted in the interpacing period lasting 40 seconds (sec) subsequently to each train. The probability to generate cardiac arrhythmias was determined as the ratio (%) of train(s) followed by cardiac arrhythmia(s) and the total number of applied trains (n=10) and expressed in a percentage form. This latter value was termed as “Arrhythmia incidence”.

3.2. The „Hyperinsulinemia protocol” (Study design)

Thirty (N=30) *NZW* rabbits were used throughout the experiments. After a one-week long rehabilitation period following pacemaker electrode implantation, hyperinsulinemic state was induced in the experimental animals by the method of *Hyperinsulinemic Euglycemic Glucose Clamping* (*HEGC*). Animals were randomly

assigned into three groups (n=10 animals/group) on the basis of the concentration of continuous insulin infusion. One group was subjected to low rate of insulin infusion (5 mIU/kg/min), while the other was subjected to high rate of that (10 mIU/kg/min). As a control to these series, another group of animals was given *placebo* (isotonic saline) in the same volume and rate via the venous route as insulin and glucose infusion were applied during HEGC. The presence of euglycemic hyperinsulinemia was verified by the determination of glucose- and insulin concentration of the blood samples obtained during the experimental period. Blood glucose level was determined by glucose oxidase method (*Accu-Chek Active, Roche Diagnostics, Budaörs, Hungary*). Plasma insulin concentration was determined by means of *radioimmunoassay* (*RIA*) with commercially available *RIA kit* (*RK-400CT, Institute of Isotopes, Budapest, Hungary*).

The alterations of electrophysiological and hemodynamic parameters and the arrhythmia inducibility associated with the state of hyperinsulinemia were examined in the *steady state* period of *HEGC.* To investigate the potential mechanisms underlying arrhythmia development in euglycemic hyperinsulinemia, catecholamine (epinephrine and norepinephrine) levels were determined by *HPLC* (*Abl&E-Jasco HPLC, JASCO Corporation, Tokyo, Japan*) using p-catecolamines kit (Bio-Rad Laboratories GmbH, München, Germany) and plasma potassium levels were assessed by direct ion-selective electrode (*ISE*) method using Cobas Integra 800 analyzer (*Roche GmbH, Mannheim, Germany*).

Animals received high-rate (10 mIU/kg/min) of insulin infusion were administered a selective β-adrenoceptor blocking agent, *metoprolol* at an intravenous dose of 1 mg/kg during the state state period of *HEGC.* The main goal of drug delivery was to test preclinically a pharmacological approach that is capable to mitigate the elevated incidence of cardiac arrhythmias associated with hyperinsulinemia. The efficiency of the drug was examined via the alterations of electrophysiologic and hemodynamic parameters and arrhythmia inducibility.

3.3. The „Metabolic syndrome protocol” (Study design)

Fiftyfour (N=54) *NZW* rabbits were used throughout the experiments. Essential symptoms of metabolic syndrome were induced in the animals by an 8-week-long cholesterol-enriched (1,5%) diet (Bioplan Ltd, Isaszeg, Hungary). The development of metabolic syndrome was verified by the alteration of metabolic and hemodynamic parameters.

Animals were randomly assigned into three experimental groups according to the applied drug therapy. The first group of animals (n=24) were referred to as control and received *placebo* (empty gelatine capsule). The alterations of hemodynamic and electrophysiological parameters and arrhythmia inducibility associated with metabolic syndrome were determined on the controls by performing a comparison with the corresponding parameters obtained from the control animals in „*Hyperinsulinemia protocol*” (healthy animals). Furthermore, the former values served even as a base of comparison to evaluate the efficiency of the applied drug therapies. Rabbits assigned to the second group (n = 24) were treated with 50 mg/kg *cicletanine* (*Beaufour Ipsen Pharma*, *France*) twice a day. The delivery of *cicletanine* was established according to antihypertensive and insulin-sensitizing dose of that observed in the human clinical practice. Animals belonging to the third group (n=6) were administered with racemic, *d,l-sotalol* (*Sotalex Mite*, *Bristol-Myers Squibb Ltd*, *Hungary*) twice a day at the dose of 25 mg/kg. The exact dose of *sotalol* was calculated on the basis of its antiarrhythmic property observed in the human clinical practice. All the drug treatment procedures were performed *per orally* and lasted for 5 days. The main purpose of drug delivery was to test preclinically the pharmacological approaches capable to mitigate the elevated incidence of cardiac arrhythmias associated with metabolic syndrome. The efficiency of the drugs was examined via the alterations of electrophysiological and hemodynamic parameters and arrhythmia inducibility.

Prior to the electrophysiological and hemodynamic measurements, subgroups of the animals (n=12) participated in *placebo* and *cicletanine* treatment were

administered *methylene-blue* (*MB*) at the dose of 10 mg/kg, intravenously to determine the potential mechanisms responsible for the development of arrhythmias associated with metabolic syndrome and to examine the pivotal mechanism of action of the applied drug therapy (*cicletanine*). *Cyclic guanosine-3',5'-monophosphate* (*cGMP*), *cyclic adenosine-3',5'-monophosphate* (*cAMP*), and *nitric-oxide* (*NO*) levels were measured on the myocardial samples taken from the animals received *placebo* and *cicletanine* treatment in the last phase of the experiment. To determine the myocardial *cAMP* and *cGMP* concentration the method of *radioimmunoassay* (*RIA*) was implemented. The cardiac NO content was measured by employing *electronspin-resonance spectroscopy* (*ESR*).

3.4. Statistical analysis

All values shown are mean ± SEM of the number (n) of observations with exception of the "Arrhythmia incidence" which is expressed as percentage. Hemodynamic, metabolic and electrophysiological parameters, blood glucose, plasma insulin, catecholamine, and potassium levels were analyzed statistically by *one-way analysis of variance* (*ANOVA*) followed by *Bonferroni's post hoc test* for multiple comparisons. The incidence of arrhythmias was analyzed by using *Fisher's exact test*. *Kruskal–Wallis test* with the *Dunn post hoc test* for multiple comparisons were used for statistical analysis of cardiac *cyclic nucleotide* and *NO* values. Differences were considered statistically significant when $p<0.05$.

4. RESULTS

4.1. Results of the „Hyperinsulinemia protocol"

Blood glucose levels of each treatment groups remained constantly euglycemic in the *steady state* period of HEGC. Plasma insulin levels were increased significantly by both 5 and 10 mIU/kg/min insulin infusion rates as compared to those measured in the control group (35 ± 4,3; 103 ± 7,5 vs. 18,1 ± 4,4 μU/ml). Animals received high-rate

insulin infusion exhibited significantly higher plasma insulin levels than those participated in the low rate of that (103 ± 7,5 vs. 35 ± 4,3 μU/ml).

QT interval (187 ± 5; 195 ± 6,1 vs. 174 ± 7 ms) and QT_c (349 ± 14; 377 ± 15 vs. 308 ± 13) showed significant prolongation in response to both 5 and 10 mIU/kg/min insulin infusion rate as compared to the values measured in the control group. Assessing T_p–T_e interval, we found a significant lengthening (69 ± 12; 81 ± 11 vs. 49 ± 9 ms) of this measure of dispersion of repolarization in both insulin regimen groups comperd to the control values. QT_p interval (118 ± 4,3; 114 ± 2,3 vs. 125,5 ± 4 ms) and VERP (104,8 ± 2,9; 101,4 ± 1,7 vs. 110 ± 3,7 ms) showed significant shortening from the control values in response to euglycemic hyperinsulinemia evoked by either 5 or 10 mIU/kg/min insulin infusion rate, respectively. HEGC procedure induced elevation on *HR* as compared to control values, which reached the level of significance, when 10 mIU/kg/min insulin rate was applied (247 ± 33 vs. 227,5 ± 37,5 Hgmm). Euglycemic hyperinsulinemia (regardless 5 or10 mIU/kg/min insulin infusion rate) failed to evoke significant changes on MABP compared to values obtained from control animals.

In our current set of experiments, the animals responded to the PES protocol exclusively with ventricular premature beats (VPBs) and non-sustained ventricular tachycardia (NSVT). The category of VPBs consisted of soliter and coupled monomorphic ectopic beats. Neither bigeminy nor trigeminy could be observed. The category of NSVT comprised ventricular tachycardia (more than four ventricular premature beats persisting not more than 15 sec) and *torsade de pointes* ventricular tachycardia (terminating spontaneously within 15 sec). The incidence of VPBs and NSVT generated by PES was significantly higher in euglycemic hyperinsulinemic state obtained by either 5 or 10 mIU/kg/min insulin infusion rate as compared to those observed at the control animals. Sustained ventricular tachycardia (VT and TdP lasting for more than 15 sec) and ventricular fibrillation could not be noticed in response to PES in either treatment groups. The incidence of ventricular arrhythmias

induced by PES exhibited concentration-dependent relationship to plasma insulin levels.

Metoprolol at an intravenous dose of 1 mg/kg caused significant shortening on the previously prolonged *QT* (168,5 ± 14,1 vs. 195 ± 6,1 ms), *QTc* (309,7 ± 23,5 vs. 377 ± 15), and *Tp–Te* (59,5 ± 8,9 vs. 81 ± 11 ms) intervals attained by euglycemic hyperinsulinemia at 10 mIU/kg/min insulin infusion rate. *HR* responded with decrease to metoprolol treatment; however, this alteration did not reach the statistically-significant level.

The intravenous dose of 1 mg/kg metoprolol decreased significantly the incidence of VPBs and NSVT induced by PES in rabbits subjected to HEGC performed by 10 mIU/kg/min insulin infusion rate.

There were no significant changes on plasma levels of either epinephrine or norepinephrine in response to euglycemic hyperinsulinemia. Although hyperinsulinemia induced by either 5 or 10 mIU/kg/min insulin infusion caused a slight decrease on plasma potassium level compared to the control values, however, the evoked changes did not reach the statistically-significant level.

4.2. Results of the „Metabolic syndrome protocol”

QT interval (140,4 ± 10,1 vs. 174 ± 7 ms), QT_c (288,4 ± 23,22 vs. 308 ± 13), QT_p (100,8 ± 7 vs. 125,5 ± 4 ms) and *VERP* (100,2 ± 1,24 vs. 110 ± 3,7 ms) showed significant reduction in the control group of the „*Metabolic syndrome protocol*„as compared to the values measured in the control group of the „*Hyperinsulinemia protocol*” (healthy animals). T_p–T_e interval was even found to be shortened, however this change did not reach the statistically-significant level.

The state of metabolic syndrome induced elevation on *HR*, but the changes failed to be statistically significant as opposed to *MABP*, which elevation became considerably higher in response to metabolic syndrome compared to that observed in healthy animals (105,1 ± 3,6 vs. 76,9 ± 10,8 Hgmm).

The animals with metabolic syndrome responded to the PES protocol with VPBs, NSVT and SVT. The incidence of VPBs and NSVT generated by PES was significantly higher in the state of metabolic syndrome as compared to that observed in healthy condition. The category of VPBs consisted of soliter and coupled monomorphic ectopic beats, while the category of NSVT comprised ventricular tachycardia (more than four ventricular premature beats persisting not more than 15 sec) and *torsade de pointes* ventricular tachycardia (terminating spontaneously within 15 sec). SVT category was compiled of ventricular tachycardia and *torsade de pointes* ventricular tachycardia (both persisting more than 15 sec) and ventricular fibrillation.

Animals responded with significant lengthening of VERP to *cicletanine* treatment as compared with the control group (108,2 ± 10,1 vs. 100,2 ± 1,2 ms). *QT*, QT_c, QT_p and T_p–T_e interval exhibited prolongation to *cicletanine* therapy, however these changes did not reach the statistically-significant level. A considerable reduction could be observed in terms of *HR* (212 ± 13,1 vs. 253 ± 17,1 min^{-1}) and *MABP* (72,4 ± 4,3 vs. 105,1 ± 3,6 Hgmm) in the *cicletanine*-treated group as compared to those measured in the control group.

Sotalol treatment caused significant prolongation on *QT* interval (180,4 ± 10,6 vs. 140,4 ± 10,1 ms), QT_c (319 ± 9,7 vs. 288,4 ± 23,2) and *VERP* (124,8 ± 1,4 vs. 100,2 ± 1,2 ms) compared to the values observed in the control group. Significant reduction was measured on *HR* (187 ± 12,1 vs. 253 ± 17,1 min^{-1}) and *MABP* (79,6 ± 6,1 vs. 105,1 ± 3,6 Hgmm) in response to *sotalol* treatment in the study animals compared to the control values.

Animals in the „*Metabolic syndrome protocol*" responded to the PES protocol with VPBs, NSVT and SVT. The incidence of arrhythmias generated by PES was significantly decreased in animals treated with either *cicletanine* or *sotalol* as compared to that measured in the control animals. SVT was impossible to be induced by PES in rabbits treated with *cicletanine*. The incidence of VPBs and NSVT proved to be significantly lower in the *cicletanine*-treated animals than in those received *sotalol* treatment. In response to *MB* treatment the inducibility of arrhythmias became

slightly elevated in the control group. The incidence of arrhythmias was significantly increased by *MB* administered to the group treated previously with *cicletanine* as compared to the values observed at animals received solely *cicletanine* treatment.

Cicletanine administration elicited a significant increase on cardiac cGMP content as compared with that obtained from *placebo*-treated animals (0,16 ± 0,029 vs. 0,09 ± 0,013 pmol/mg x ww). *MB* application to the *cicletanine*-treated animals decreased significantly the cardiac cGMP increase observed in the *cicletanine*-treated group (0,03 ± 0,011 vs. 0,16 ± 0,029 pmol/mg x ww), which revealed to be even significant as compared to the values measured in the *placebo*-treated group (0,03 ± 0,011 vs. 0,09 ± 0,013 pmol/mg x ww). Conversely, cardiac cAMP level was significantly decreased in response to *cicletanine* treatment as compared to the values observed in the control group (1,46 ± 0,116 vs. 1,88 ± 0,099 pmol/mg x ww). Myocardial cAMP content was increased significantly in the *cicletanine*-treated group in response to the application of *MB* as compared to the values obtained in either *cicletanine*-treated group (2,27 ± 0,233 vs. 1,46 ± 0,116 pmol/mg x ww) or animals participated in *placebo* (2,27 ± 0,233 vs. 1,88 ± 0,099 pmol/mg x ww) administration. Application of *MB* attained significant increase on cardiac cAMP level in the control group (2,34 ± 0,196 vs. 1,88 ± 0,099 pmol/mg x ww) also.

Cardiac NO content was found to be significantly increased after *cicletanine* treatment as comapred to the values measured in the control group (0,51 ± 0,124 vs. 0,22 ± 0,03 nmol/g x ww). *MB* application decreased NO levels under the detection limit in both *placebo*- and *cicletanine*-treated animals.

5. DISCUSSION

5.1. Experimental induction of hyperinsulinemia and metabolic syndrome

Plasma insulin and blood glucose levels of the animals participated in the „*Hyperinsulinemia protocol*" verify the successful development of experimental hyperinsulinemia. Several distinct methods (eg.: bolus insulin injection, *Rapid Isulin Sensitivity Test* /*RIST*/) are suitable to induce experimental hyperinsulinemia, however

HEGC seemed to be cuurently the most proper procedure, when respecting the exact nature of the present research.

The induction of metabolic syndrome in rabbit as a species can be fulfilled succesfully by alimentaric way (ie. designed and calculated alteration of normal animal diet). The most notable advantage –except inexpensivity– of preclinical animal models created by alimentar way is the faithful patophysiological resemblence to human variants of the disease, since the main components of the diet applied to induce metabolic syndrome is particularly similar to *cafeteria diet*, which has an essential role in the high prevalence of the disease in the human population according to the most relevant studies.

5.2. Impact of hyperinsulinemia on cardiac arrhythmogenesis

In the current set of experiments we demonstrated that the state of hyperinsulinemia elevated considerably the incidence of arrhythmias generated by PES, moreover the inducibility of arrhythmias correlated strictly with the plasma insulin level.

Based on the considerable prolongation of the electrophysiologic parameters (*QT* interval, QT_c, T_p-T_e) induced by high plasma insulin level, we strongly suspect that the state of hyperinsulinemia can facilitate the inducibility of cardiac arrhytmias via the inhomogenous prolongation of myocardial action potential in a particular area of the ventricular wall (ie. transmural dispersion of repolarization /*TDR*/) observed previously in *LQTS* also.

We claim on the basis of the alteration of the electrophysiological parameters, the results of the biochemical measurements and the observed cardiac values in response to *metoprolol* treatment that the state of hyperinsulinemia can amplificate both directly and indirectly the inhomogeneity of myocardial repolarization in the employed preclinical animal model. Furthermore, we suggest that sympathetic activation may be considerably involved in the prolongation of the electrophysiologic parameters and increased arrhythmia inducibility in euglycemic hyperinsulinemia.

5.3. Impact of metabolic syndrome on cardiac arrhythmogenesis

In the current set of experiments we demonstrated that metabolic syndrome increased significantly the incidence of ventricular arrhythmias generated by PES.

We strongly suspect on the basis of our electrophysiological measurements that heterogenous abbreviation of the myocardial action potential can play an essential role in the development of cardiac arrhythmias associated with preclinically-induced metabolic syndrome.

The biochemical measurements prove that the deficit in myocardial cGMP, NO levels and increase on cAMP concentration is responsible for the elevated incidence of ventricular arrhythmias induced by PES in metabolic syndrome. Our hypothesis is even confirmed by the finding that reduced arrhythmia inducibility of the animals received *cicletanine* treatment was associated with elevated myocardial cGMP and NO content and reduced cAMP level. Furthermore, decrease on cardiac cGMP and NO content and increase on myocardial cAMP concentration in response *MB* application in the *cicletanine*-treated group enhanced considerably the incidence of ventricular arrhythmias in the preclinical model.

5.4. The therapeutical relevance of metoprolol in hyperinsulinemia

In the current set of experiments we demonstrated that *metorolol* can exert beneficial effect on the increased arrhytmogeneicity associated with hiperinsulinemia. Based on the restoration of the elevated incidence of arrhythmias in response to the drug treatment we strongly suspect that effects of sympathetic overactivity induced by hyperinsulinemia develop via the activiation of molecular pathways linked to β_1-adrenoceptors.

5.5. The therapeutical relevance of cicletanine in metabolic syndrome

Results of the biochemical measurements along with the reduced arrhythmia incidence observed in the *cicletanine*-treated group demonstrate clearly that

cicletanine's antiarrhytmic efficacy is based on the elevation of myocardial cGMP and NO level and decrease on cardiac cAMP content. This finding can be confirmed by the application of *MB* exhibiting both sGC and NOS inhibitor property, which increased the arrhythmia inducibility via the deficit of cardiac cGMP and NO content and elevated myocardial cAMP level.

6. NEW FINDINGS

The states of hyperinsulinemia and metabolic syndrome enhance considerably the inducibility of ventricular arrhythmias.

Amplification of *transmural dispersion of repolarization* plays essential role in the development arrhythmias observed in hyperinsulinemia and metabolic syndrome.

Systemic sympathetic overactivity is not involved in the enhanced arrhytmia inducibilty associated with hyperinsulinemia, nevertheless activation of the adrenergic system contributes considerably to the increased incidence of arrhythmias in hyperinsulinemia.

Deficit of myocardial cGMP and NO content and increase on cardiac cAMP level is particularly responsible for the enhanced arrhythmia inducibility in metabolic syndrome.

Metoprolol influences beneficially the increased incidence of ventricular arrhytmias associated with hyperinsulinemia via the selective adrenergic blockade.

Cicletanine possessing antihypertensive, anti-ischemic and insulin-sensitizing effects, is able to reduce significantly the elevated incidence of ventricular arrhythmias in metabolic syndrome by increasing myocardial cGMP, NO levels and decreasing cAMP concentration.

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8. APPENDIX

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List of publications related to the dissertation

1. **Drimba, L.**, Döbrönte, R., Hegedűs, C., Sári, R., Di, Y., Nemeth, J., Szilvássy, Z., Peitl, B.: The role of acute hyperinsulinemia in the development of cardiac arrhythmias.
Naunyn Schmiedebergs Arch. Pharmacol. 386 (5), 435-444, 2013.
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IF:2.647 (2011)

2. **Drimba, L.**, Hegedűs, C., Yin, D., Sári, R., Németh, J., Szilvássy, Z., Peitl, B.: Beneficial Cardiac Effects of Cicletanine in Conscious Rabbits With Metabolic Syndrome.
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List of other publications

3. **Drimba, L.**, Németh, J., Sári, R., Di, Y., Kovács, A., Szénási, G., Szilvássy, Z., Peitl, B.: In vivo preclinical evaluation of a promising antiarrhythmic agent, EGIS-7229.
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List of abstracts related to the dissertation:

Drimba L., Sári R., Németh J., Peitl B., Szilvássy Z.:
Hyperinsulinemia induces cardiac arrhythmias in conscious rabbits
Journal of Diabetes, 2013, 5 (Suppl. 1), 16-202

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Interaction between cicletanine and preconditioning in conscious rabbits with insulin resistance
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List of other abstracts:

Drimba L., Sári R., Németh J., Peitl B., Szilvássy Z.:
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