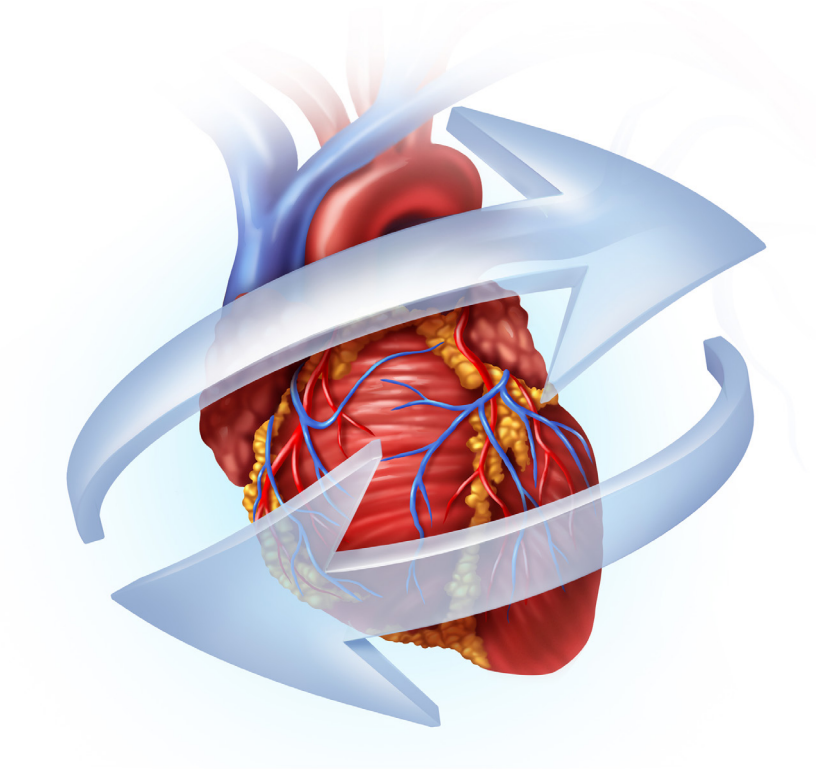
New Frontiers in Basic Cardiovascular Research

France – New EU Members



Program & Book of Abstracts

Bratislava, Slovakia

May 25 - 27, 2022

Organizers

Institute for Heart Research, Centre of Experimental Medicine
Slovak Academy of Sciences

Under the auspices of the:

Slovak Physiological Society
Slovak Society for Biochemistry and Molecular Biology

MINISTERSTVO ŠKOLSTVA, VEDY, VÝSKUMU A ŠPORTU SLOVENSKEJ REPUBLIKY

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Bussiness & Congress Hotel Saffron****

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Welcome address

Dear Colleagues and Friends,

The 14th meeting "New Frontiers in Basic Cardiovascular Research: France – New EU Members" will be held in Bratislava, Slovakia, on May 25-27, 2022.

The conference follows a long-lasting tradition of these meetings that started in Prague in 1994 as the initiative of prof. B. Ošťádal (Czech Republic) and prof. R. Fishmeister (France). The meetings were initially focused on the collaboration between researchers from France, Czech Republic and Slovakia, but since 2006, the conference embraced all countries of V4 region and New EU countries. So far, the conference has been organized in all V4 countries and in France.

Unfortunately, the 2-year period tradition of these meetings has been interrupted by COVID-19 pandemic. We are thus very happy that the actual COVID-19 situation enables organization of the postponed 14th meeting on site, and we are pleased to announce a "face to face" meeting that will take place in the conference hotel Saffron in Bratislava, Slovakia.

To continue the tradition of the "Frontiers" meetings, the meeting will feature basic scientific and clinical sessions in the field of cardiovascular research including lectures of invited keynote speakers and free oral communications selected from the submitted abstracts. We will provide various opportunities for young investigators to discuss their latest findings with the established investigators and to compete in both oral and poster sessions. In addition to an attractive scientific program we also promise to prepare an enjoyable social program.

We hope that despite the tight scientific schedule, there will be enough space for fruitful and stimulating discussions and chances to enjoy the city of Bratislava.

On behalf of the Organizing committee

Monika Barteková, Táňa Ravingerová, Barbora Kaločayová

LOCAL ORGANIZING COMMITTEE

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GENERAL INFORMATION

Venue and date

The meeting will be held in the hotel Saffron, Radlinského 27, 811 07 Bratislava on May 25-27, 2022. Website: www.hotelsaffron.sk/en/; GPS: 48.152466 17.116778

Registration

On the premises of the Hotel Saffron from Wednesday May 25th, 2022 from 9:00

Accommodation

The accommodation has been arranged in the venue hotel Saffron, Radlinského 27, 811 07 Bratislava.

Information for presenters

Oral presentations of invited speakers – 20 min including discussion
Oral presentations from submitted abstracts - 15 min including discussion
Posters should be mounted before 11:00 A.M. at the day of the respective Poster session.
The authors should be present during the Poster session.
Poster board size – 100 x 80 cm (vertical)

Day 1: Wednesday, May 25th

09:00 **REGISTRATION & REFRESHMENT**

10:30 **MEETING OPENING**

10:45 – 11:15 **PLENARY LECTURE**

Chair: T. Ravingerová (Bratislava, Slovakia)

10:45 – 11:15 **B. Ošťádal (Prague, Czech Republic)**
Developmental and sex differences in cardiac tolerance to oxygen deprivation

11:20 – 13:00 **SESSION 1.**
MECHANISMS TRIGGERING ARRHYTHMIAS AND ANTIARRHYTHMIC INTERVENTIONS

Chairs: I. Baczkó (Szeged, Hungary), M. Bébarová (Brno, Czech Republic)

11:20 – 11:40 **I. Baczkó (Szeged, Hungary)**
New transgenic rabbit models to predict drug-induced arrhythmias

11:40 – 12:00 **M. Bébarová, O. Švecová, L. Chmelíková, J. Hošek, M. Pásek, T. Bárta, J. Pacherník, I. Synková, T. Novotný (Brno, Czech Republic)**
Inherited arrhythmias: from gene variants to ionic channel dysfunctions

12:00 – 12:20 **A. Varró (Szeged, Hungary)**
Citrus alkaloids may enhance proarrhythmic risk

12:20 – 12:40 **J. Neckář (Prague, Czech Republic)**
Infarct size limitation triggered by excess ischemic arrhythmias in hypertensive rats

12:40 – 13:00 **N. Jost, Z. Kohajda, L. Virag, T. Hornyik, Z. Husti, A. Sztojkov-Ivanov, N. Nagy, J. Prorok, N. Toth, A.-L. Tamás, I. Koncz, S. Deri, V. Demeter-Haludka, B. Ordog, M. Patfalusi, L. Talosi, L. Tiszlavicz, I. Foldesi, I. Baczko, A. Varro (Szeged, Hungary)**
"New wine in an old bottle or old wine in a new bottle?" In vivo and cellular antiarrhythmic and cardiac electrophysiological effects of desethylamiodarone in dogs"

13:00 – 14:00 **LUNCH**

14:00 – 15:40 **SESSION 2.**
SUCCESS AND FAILURE OF CARDIAC THERAPY: WHAT FACTORS MATTER?

Chairs: A. Meli (Montpellier, France), M. Štěrba (Hradec Králové, Czech Republic)

14:00 – 14:20 **N. S. Dhalla (Winnipeg, Canada)**
Rationale for the prevention or therapy of sudden cardiac death in heart failure

14:20 – 14:40 **M. Souidi, J. Resta, Y. Sleiman, S. Reiken, P. Amedro, P. Meyer, A. Charrabi, O. Cazorla, M. Vincenti, S. Blot, F. Rivier, A. Parini, A. Marks, J. Mialet-Perez, A. Lacampagne, A. Meli (Montpellier, France)**
Patient-specific derived cardiomyocytes: will we be able to predict the cardiomyopathies in the dish tomorrow?

14:40 – 15:00 **M. Štěrba (Hradec Králové, Czech Republic)**
Pharmacological cardioprotection against chronic ANT cardiotoxicity – topoisomerase II beta targeting and beyond

15:00 – 15:20 **A. Görbe, Z. Giricz, P. Ferdinandy (Budapest, Szeged; Hungary)**
Hidden cardiotoxicity - cardiac safety testing in ischemic and comorbid conditions: development of preclinical test platforms

15:20 – 15:40 **F. Šimko (Bratislava, Slovakia)**
Evidence-based cardiovascular medicine: perspectives and disappointments

15:40 – 17:00 **COFFEE BREAK & POSTER SESSION I.**

17:00 – 19:00 **SESSION 3.**
NOVEL TARGETS FOR CARDIOPROTECTION

Chairs: B. Podesser (Vienna, Austria), J. Slezák (Bratislava, Slovakia)

17:00 – 17:20 **A. Adameová (Bratislava, Slovakia)**
RIP3 mediates necroptosis and non-necroptotic inflammatory response: an interesting pharmacological tool for treatment of heart diseases

17:20 – 17:40 **B. Podesser, A. Kiss, P. Pokreis, L. Szabo, C. Dostal (Vienna, Austria)**
Extracellular matrix remodeling under pressure overload

17:40 – 18:00 **P. Bencsik, T. Szabados, É. Kenyeres, K. Gömöri, G. Dormán, A. Görbe, P. Ferdinandy (Szeged, Dunakeszi; Hungary)**
Development of novel matrix metalloproteinase-2 inhibitors for cardioprotection against ischemia/reperfusion injury: from chemical design to preclinical proof-of-concept studies

18:00 – 18:15 **T. Ravingerová, Ľ. Lonek, L. Kindernay, V. Zohdi, A. Adameová (Bratislava, Slovakia)**
Non-invasive „conditioning“: potential mechanisms of antiischemic cardioprotection

18:15 – 18:30 **J. Slezak, M. Hulman, V. Hudec, J. Luptak, I. Olejarova, M. Ondrusek, I. Gasparovic, R. Sramaty, B. Szeiffova Bacova, M. Barancik, M. Sykora, L. Okruhlicova, N. Tribulova, R. Boli, B. Kalocayova, T. W. LeBaron, T. Ravingerova, L. Lonek, M. Zalesak, K. Andelova, B. Kura (Bratislava, Slovakia)**
Transplantation of the heart. Innovative method mitigating oxidative stress by molecular hydrogen

18:30 – 18:45 **PHOTO**

19:00 – 22:00 **WELCOME RECEPTION**

Day 2: Thursday, May 26th

9:00 – 11:00 **SESSION 4.**
RISING STARS & THEIR SCIENTIFIC DISCOVERIES - YOUNG INVESTIGATORS` COMPETITION

Chairs/Committee: I. Baczkó (Szeged, Hungary), A. Meli (Montpellier, France), J. Beltowski (Lublin, Poland)

9:00 – 9:15 **C. Horváth, A. Szobi, M. Young, I. Jarabicová, J. Hrdlička, J. Neckář, M. Lewis, F. Kolář, T. Ravingerová, M. S. Suleiman, A. Adameová (Bratislava, Slovakia)**
Necroptosis mediates cardiac damage under conditions of ischemia and reperfusion: emphasis on duration of reperfusion

9:15 – 9: 30 **B. Iaparov, I. Zahradník, A. Zahradníková (Bratislava, Slovakia)**
Determinants of RyR-RyR coupling strength in cardiac calcium release sites

9:30 – 9:45 **T. Jasenovec, D. Radošinská, M. Kollarová, N. Vrbjar, P. Bališ, S. Trubačová, Ľ. Paulis, L. Tóthová, J. Radošinská (Bratislava, Slovakia)**
Erythrocyte and plasma properties in monocrotaline model of pulmonary arterial hypertension

9:45 – 10:00 **M. Kluknavsky, A. Micurova, P. Balis, M. Skratek, J. Manka, I. Bernatova (Bratislava, Slovakia)**
Tissue- and strain-dependent differences in iron metabolism after single administration of iron oxide nanoparticles

10:00 – 10: 15 **M. Kollárová, M. Chomová, D. Radošinská, Ľ. Tóthová, J. Radošinská (Bratislava, Slovakia)**
Left ventricle remodellation in zucker diabetic fatty rats but also in zucker lean rats

10:15 – 10:30 **B. Kura, B. Kalocayova, B. Szeiffova Bacova, M. Sykora, N. Tribulova, V. Hudec, M. Ondrusek, I. Gasparovic, R. Sramaty, M. Hulman, J. Slezak (Bratislava, Slovakia)**
Beneficial effect of hydrogen gas on the heart that has undergone simulated heart transplantation. Possible new therapeutic agent?

10:30 – 10:45 **A. Micurova, M. Kluknavsky, S. Liskova, P. Balis, M. Skratek, L. Okruhlicova, J. Manka, I. Bernatova (Bratislava, Slovakia)**
Differences in distribution and biological effects of polyethylene glycol-coated iron oxide nanoparticles in normotensive and hypertensive rats - focus on vascular function and liver

10:45 – 11:00 **M. Miklovič, O. Gawryś, P. Kala, Z. Honetschlägerová, Š. Jíchová, Z. Vaňourková, Z. Husková, S. Kikerlová, H. Maxová, D. Sedmera, T. Mráček, V. Melenovský (Prague, Czech Republic)**
Effect of renal denervation on left and right ventricular function in transgenic hypertensive rats with heart failure induced by volume overload

11:00 – 11:15 **COFFEE BREAK**

11:15 – 13:10 **SESSION 5.**
INTRACELLUAR SIGNALING IN HEALTHY AND DISEASED HEART: FOCUS ON RECEPTORS

Chairs: A. M. Gomez (Châtenay-Malabry, France), Z. Papp (Debrecen, Hungary)

11:15 – 11:35 **M. Barthe, F. Lefebvre, E. Langlois, F. Lefebvre, X. Iturrioz, C. Llorens-Cortes, T. Ha-Duong, L. Moine, N. Tsapis, R. Fischmeister (Châtenay-Malabry, France)**
Distinct functions of cardiac β-adrenergic receptors in the T-tubule vs. outer surface membrane

11:35 – 11:55 **A. Val Blasco, L. Yin, P. Gerbaud, E. Zorio, R. Perrier, J. P. Benitah, A. M. Gomez (Châtenay-Malabry, France)**
Mechanisms of the RyR2R420Q CPVT mutation. Lessons human cardiomyocytes derived from induced-pluripotent stem cells

11:55 – 12:15 **D. Jezova, A. Puhova (Bratislava, Slovakia)**
Stress, catecholamines and beta3-adrenergic receptors

12:15 – 12:35 **M. Nováková, T. Stračina (Brno, Czech Republic)**
Sigma receptor as a potential target for cardiac remodelling

12:35 – 12:55 **Z. Papp, F. Sárkány, P. Polesello (Debrecen, Hungary)**
Potassium channels as potential targets in pulmonary hypertension complicating heart failure with preserved ejection fraction (PH-HFpEF)

12:55 – 13:10 **A. Zahradníková, B. Iaparov, I. Baglaeva, I. Zahradník (Bratislava, Slovakia)**
Effect of RyR gating on elementary calcium release of cardiac myocytes

13:10 – 14:00 **LUNCH**

14:00 – 16:00 **SESSION 6.**
"NONCONVENTIONAL" DIAGNOSTIC AND THERAPEUTIC APPROACHES

Chairs: R. Andriantsitohaina (Montpellier, France), J. Žurmanová (Prague, Czech Republic)

14:00 – 14:20 **R. Andriantsitohaina (Montpellier, France)**
Extracellular vesicles as biomarkers and targets in vascular consequences of metabolic syndrome

14:20 – 14:40 **Z. Giricz (Budapest, Hungary)**
EVs in oxidative stress and cardioprotection

14:40 – 15:00 **P. Ferdinandy (Budapest, Szeged; Hungary)**
Cardioprotection by microRNA therapeutics

15:00 – 15:20 **O. Pechanova, E. Dayar, A. Barta, M. Cebova (Bratislava, Slovakia)**
Combined therapy with simvastatin- and coenzyme Q10-loaded nanoparticles ameliorates PI3K-Akt-eNOS pathway in experimental metabolic syndrome

15:20 – 15:40 **J. Žurmanová, A. Marvanová, V. Tibenská, P. Kšík, A. Žbírková, B. Elsnicová, L. Hejnová, D. Horníková, P. Vodička, J. Novotný, B. Szeiffová Bačová, M. Sýkora, N. Tribulová, F. Kolář, O. Nováková (Prague, Czech Republic)**
Mild cold acclimation as a new cardioprotective intervention

15:40 – 16:00 **S. Liskova, P. Balis, A. Micurova, I. Bernatova (Bratislava, Slovakia)**
The effect of iron oxide nanoparticles on vascular function of the femoral artery of normotensive rats

16:00 – 17:00 **COFFEE BREAK & POSTER SESSION II.**

18:00 – 19:30 **CITY EXCURSION**

19:30 – 23:00 **GALA DINNER**

Day 3: Friday, May 27th

9:00 – 11:00 **SESSION 7.**
NOVEL MECHANISMS AND PLAYERS IN CARDIOVASCULAR AND CARDIOMETABOLIC DISEASES

Chairs: A. Kiss (Vienna, Austria), K. Javorka (Martin, Slovakia)

9:00 – 9:20 **A. Kiss, M. Sárközy, E. Acar, Z. Kovács, S. Watzinger, F. Márványkövi, G. Szücs, A. Siska, I. Földesi, A. Kriston, P. Horváth, G. Cseri, B. Kővári, L. Szabó, D. Abraham, T. Csont, B. Podesser (Vienna, Austria)**
Neuregulin-1 attenuates development of cardiac and kidney dysfunction in a rat model of chronic kidney disease

9:20 – 9:40 **M. Zeman, V. S. Rumanova, H. Šutovska, Z. Dzirbíková, L. Molčan, M. Okuliarová (Bratislava, Slovakia)**
Consequences of chronodisruption on the circadian control of cardiometabolic processes

9:40 – 10:00 **K. Javorka, M. Javorka, K. Maťašová, M. Zibolen (Martin, Slovakia)**
Mechanisms of cardiovascular changes of phototherapy in newborns with hyperbilirubinemia

10:00 – 10:20 **M. Hlaváčková (Prague, Czech Republic)**
FTO inhibition impairs cardiomyocyte tolerance to oxygen deprivation

10:20 – 10:35 **M. Javorka, R. Wiszt, B. Czippelová, J. Čerňanová Krohová, N. Mažgútová, Z. Turianiková (Martin, Slovakia)**
Stroke volume variation as an index of fluid responsiveness in conscious patients

10:35 – 10:50 **M. Cagalinec, A. Zahradníková Jr., J. Pavelková, M. Novotová, A. Zahradníková (Bratislava, Slovakia)**
Impairment of calcium dynamics and contractility in cardiomyocytes of Wolframin invalidated rats

11:00 – 11:20 **COFFEE BREAK**

11:20 – 12-45 **SESSION 8.**
3x STRESS: PSYCHOSOCIAL, OXIDATIVE AND WHAT ELSE?

Chairs: V. Veksler (Châtenay-Malabry, France), I. Bernátová (Bratislava, Slovakia)

11:20 – 11:40 **A. Zeb, V. Choubey, R. Gupta, V. Veksler, A. Kaasik (Châtenay-Malabry, France)**
How cells are able to eliminate mitochondria producing too much reactive oxygen species (ROS)?

11:40 – 12:00 **J. Beltowski (Lublin, Poland)**
Green tea polyphenol improves inflammatory phenotype of perivascular adipose tissue by oxidizing hydrogen sulfide (H_2S) to polysulfides (H_2Sn)

12:00 – 12:15 **I. Bernatova, M. Kluknavsky, A. Micurova, P. Balis, M. Skratek, M. Okuliarova, S. Liskova, J. Manka (Bratislava, Slovakia)**
Acute stress-induced alterations in expressions of genes involved in iron metabolism in the hearts and livers of normotensive rats

12:15 – 12:30 **N. Hlavacova, P. Solarikova, I. Brezina, D. Jezova (Bratislava, Slovakia)**
Decreased sympathetic activation during psychosocial stress in allergic patients

12:30 – 12:45 **I. Žila (Martin, Slovakia)**
Cardiovascular changes in rats with LPS-induced lung injury

12:45 – 13:15 **CLOSING REMARKS**

13:15 **LUNCH AND DEPARTURE**

POSTER PRESENTATIONS

Poster Session I. Wednesday, May 25 th 16:00 – 17:00

♣ Young Investigator Poster Competition
Chairs/Committee: M. Bébarová (Brno, Czech Republic), M. Javorka (Martin, Slovakia) P. Bencsik (Szeged, Hungary)

1. ♣ **L. Bartošová, C. Horváth, P. Galis, K. Ferenczyová, B. Kaločayová, A. Szobi, A. Duriš-Adameová, M. Barteková, T. Rajtík (Bratislava, Slovakia)**
   Quercetin improves diastolic dysfunction and reduces heart hypertrophy in diabetic ZDF rats

2. ♣ **S. Déri, T. Hartai, L. Virág, N. Jost, A. J. Labro, A. Varró, I. Baczkó, S. Nattel, B. Ördög (Szeged, Hungary)**
   MiRP2 rescues long QT syndrome type 5

3. ♣ **K. Ferenczyova, L. Kindernay, B. Kalocayova, M. Sykora, M. Jelemensky, P. Balis, A. Berenyiova, A. Zemancikova, J. Torok, S. Cacanyiova, T. Rajtik, M. Barancik, M. Bartekova (Bratislava, Slovakia)**
   Effects of polyphenol quercetin on selected cardiovascular parameters and ischemia-reperfusion injury of the myocardium in rats with type 2 diabetes

4. ♣ **O. Gawrys, I. Baranowska, A. Walkowska, Z. Husková, J.R. Falck, J.D. Imig, E. Kompanowska-Jezierska, L. Červenka (Prague, Czech Republic; Warsaw, Poland)**
   Antihypertensive activity of 20-HETE antagonist (AAA) and epoxyeicosatrienoic acid analogue (EET-A) in spontaneously hypertensive rats

5. ♣ **S. Golas, S. Cacanyiova, A. Berenyiova (Bratislava, Slovakia)**
   The effect of perivascular adipose tissue in interaction with endogenous and exogenous hydrogen sulfide in vasoactive responses of isolated thoracic aorta in normotensive and spontaneously hypertensive rats

6. ♣ **T. Hornyik, A. Castiglione, E. M. Wülfers, L. Giammarino, I. Edler, J. J. Jowais, M. Rieder, S. Perez-Feliz, Z. Bősze, A. Varró, M. Brunner, S. I. Liin, H. P. Larsson, K. E. Odening, I. Baczkó (Szeged, Hungary; Freiburg, Germany; Bern, Switzerland)**
   Beneficial repolarisation-normalizing effect of a polyunsaturated fatty acid, DHA in transgenic long QT type 2 rabbit model

7. ♣ **I. Jarabicová, C. Horváth, E. Veľasová, L. Bies Piváčková, J. Vetešková, J. Klimas, P. Křenek, A. Adameová (Bratislava, Slovakia)**
   The role of necrosis-like cell death modes in organ damage in experimental pulmonary arterial hypertension

8. ♣ **L. Kindernay, M. Pilchová, M. Jelemenský, K. Ferenczyová, T. Ravingerová (Bratislava, Slovakia)**
   The cardioprotective effect of remote ischemic preconditioning and protective signaling pathways in aging rats

9. ♣ **P. Kollárová, O. Lenčová, G. Karabanovich, J. Kubeš, Y. Mazurová, M. Adamcová, A. Jirkovská, T. Šimůnek, J. Roh, M. Štěrba (Hradec Králové, Czech Republic)**
   A new bisdioxopiperazine analogue provides promising protective effects against chronic anthracycline cardiotoxicity in vivo in rabbits

10. ♣ **F. Sárkány, A. P. Ráduly, A. Tóth, Z. Papp, Z. Csanádi, B. Horváth, N. Szentandrássy, A. Borbély (Debrecen, Hungary)**
    Investigation of the effect of the novel myosin activator danicamtiv on the contractility and Ca^{2+} transients of isolated left ventricular cardiomyocytes

11. **M. Adamcova, H. Kovarikova, I. Baranova, O. Lencova-Popelova, Y. Mazurova, M. Sterba (Hradec Králové, Czech Republic)**
    MiRNAs profiling of chronic anthracycline-induced cardiomyopathy in rabbits

12. **K. Andelova, M. Sykora, B. Szeiffova-Bacova, T. Egan Benova, V. Knezl, J. Slezak, N. Tribulova (Bratislava, Slovakia)**
    Distinct myocardial connexin-43 alteration due to cardiac hypertrophy and atrophy impact the vulnerability of the heart to malignant arrhythmias

13. **N. Andelova, I. Waczulikova, I. Talian, T. Ravingerova, M. Ferko (Bratislava, Slovakia)**
    Dichloroacetate and reduced oxygen utilization in the heart: regulation of the mitochondrial proteome

14. **B. G. Aydemir, A. Berenyiova, M. Drobna, S. Cacanyiova, S. Golas (Bratislava, Slovakia)**
    The vasoactive effect of hydrogen sulfide donor and chronic fructose intake in spontaneously hypertensive rats

15. **I. Baglaeva, M. Cagalinec, B. Iaparov, A. Zahradníková, A. Zahradníková Jr. (Bratislava, Slovakia)**
    Calcium transients in cardiomyocytes of sedentary and active rats

16. **P. Balis, A. Berenyiova, J. Radosinska, M. Kvandova, I. Bernatova, M. Kluknavsky, A. Puzserova (Bratislava, Slovakia)**
    High concentration of uric acid did not affect endothelial function of various - small, medium-sized and large arteries from aged WKY rats

17. **A. Berenyiova, S. Golas, M. Cebova, S. Cacanyiova (Bratislava, Slovakia)**
    Effect of the long-term fructose intake on the participation of nitric oxide and hydrogen sulfide signaling pathways in vasoregulation of rat thoracic aorta

18. **B. Boťanská, P. Sovík, M. Barančík (Bratislava, Slovakia)**
    Effect of sulforaphane on doxorubicin-induced toxicity in HEK 293 cells

19. **Z. Brnoliakova, V. Knezl, R. Sotnikova, Z. Gasparova (Bratislava, Slovakia)**
    Metabolic syndrome in hypertriacylglycerolemic rats: effects of antioxidants

20. **M. Cebova, A. Barta, O. Pechanova (Bratislava, Slovakia)**
    HMGB1 as a potential target for treatment after experimental myocardial infarction

21. **S. Čačányiová, S. Golas, M. Cebová, M. Majzúnová, H. Malínská, A. Berényiová (Bratislava, Slovakia)**
    The role of interaction between perivascular adipose tissue and hydrogen sulfide in vasoactive responses of thoracic aorta in hypertriglyceridemic rats

22. **V. Farkasova, L. Kindernay, L. Lonek, T. Ravingerova (Bratislava, Slovakia)**
    Nitric oxide as one of the triggering factors of cardioprotection induced by remote preconditioning

23. **K. Frimmel, J. Križák, R. Sotníková, V. Knezl, Ľ. Okruhlicová (Bratislava, Slovakia)**
    Lipopolysaccharide-induced changes in endothelial connexin-40 and occludin associated with macrophage infiltration in both normotensive and spontaneously hypertensive rats

Poster Session II.Thursday, May 26 th 16:00 – 17:00

24. **J. Gaburjakova, M. Gaburjakova, E. Krejciova, S. Nagy, D. Kosnac, M. Kopani (Bratislava, Slovakia)**
    Blocking effect of the ferritin nanoparticle on the cardiac ryanodine receptor

25. **K. Grossmannova, P. Belvoncikova, M. Barathova, V. Lauko, L. Csaderova, J. Tomka, T. Dulka, J. Pastorek, J. Madaric (Bratislava, Slovakia)**
    Presence of hypoxia marker carbonic anhydrase ix in human abdominal aortic aneurysm tissue and plasma

26. **J. Hrdlička, V. Olejníčková, F. Papoušek, M. Pešková, E. Zabrodská, J. Neckář (Prague, Czech Republic)**
    Sex differences in cardiac remodeling induced by early postnatal abdominal aorta constriction in rats

27. **K. Hrivikova, Z. Romanova, I. Riecansky, D. Jezova (Bratislava, Slovakia)**
    Cardiovascular response during acute stress in subjects with schizotypal personality traits

28. **D. Jarkovská, M. Miklovič, J. Švíglerová, L. Červenka, P. Škaroupková, V. Melenovský, M. Štengl (Pilsen, Czech Republic)**
    Trandolapril effect on the rat myocardium in experimental volume overload heart failure

29. **P. Karailiev, L. Karailievova, D. Jezova (Bratislava, Slovakia)**
    Adaptive changes in the left heart ventricle in a chronic stress model in rats

30. **O. Lenčová, P. Kollárová, M. Adamcová, Y. Mazurová, M. Štěrba (Hradec Králové, Czech Republic)**
    Role of pharmacological inhibition of ATM in the development of anthracycline cardiotoxicity

31. **A. Matloobi, T. Buday, M. Konarska, M. Brozmanova, J. Plevkova (Martin, Slovakia)**
    Cough as a cause and consequence of heart dysfunction

32. **A. Matloobi, T. Buday, M. Konarska, M. Brozmanova, J. Plevkova (Martin, Slovakia)**
    Stimulation and unloading of baroreceptors modulate cough in experimental conditions

33. **S. A. Mohammed, M. Naveed, L. Topal, G. Mohácsi, J. Prorok, I. Baczkó, L. Virág, N. Jost, A. Varró (Szeged, Hungary)**
    Orange flavonoid hesperetin prolonged action potential duration and inhibits the slow delayed rectifier potassium current (IKs) in dog and rabbit cardiac ventricular muscle preparations and isolated myocytes

34. **L. Molcan, H. Sutovska, L. Olexová, M. Morová, L. Kršková, M. Zeman (Bratislava, Slovakia)**
    Cardiovascular response of rats to behavioural stress measured in phenotyper

35. **M. Naveed, L. Topal, J. Prorok, B. Pászti, D. Csupor, I. Baczkó, L. Virág, N. Jost, A. Varró (Szeged, Hungary)**
    The electrophysiological effects of cannabidiol on action potential and transmembrane potassium currents in dog and rabbit cardiac preparations

36. **M. Novotová, A. Zahradníková Jr., I. Zahradník (Bratislava, Slovakia)**
    Growth-related activities at the plasmalemma in neonatal cardiac myocytes

37. **H. Šútovská, Ľ. Molčan, L. Kopkan, M. Zeman (Bratislava, Slovakia)**
    Effect of aldosterone antagonist, spironolactone, on non-dipping blood pressure rhythm in hypertensive Ren-2 transgenic rats

38. **O. Švecová, M. Bébarová, M. Šimurdová, J. Šimurda (Brno, Czech Republic)**
    Assessment of the fraction of t-tubular membrane in cardiomyocytes: a new and reversible approach

39. **B. Szeiffova Bacova, T. Egan Benova, M. Sykora, J. Zurmanova, V. Knezl, K. Andelova, N. Tribulova (Bratislava, Slovakia)**
    Effect of omacor against the increased incidence of malignant cardiac arrhythmias triggered by light pollution

40. **L. Topal, A. Polyák, N. Tóth, Z. Kohajda, S. Déri, J. Prorok, N. Nagy, L. Virág, N. Jost, A. Farkas, I. Baczkó, A. Varró (Szeged, Hungary)**
    Endurance training induced cellular electrophysiological remodeling in newly developed animal athlete's heart models

41. **J. Török, A. Zemančíková, P. Bališ (Bratislava, Slovakia)**
    Neurogenic regulation of splanchnic arteries in rats treated with high-fat diet in combination with high-fructose intake

42. **N. Tribulova, K. Andelova, B. Szeiffova Bacova, M. Sykora, T. Egan Benova (Bratislava, Slovakia)**
    Cardiac connexin-43 hemichannels and pannexin-1 channels: potential novel antiarrhythmic targets

43. **J. Vlkovicova, D. Snurikova, N. Vrbjar, B. Kura, J. Slezak, V. Hudec, M. Ondrusek, I. Gasparovic, R. Sramaty, J. Luptak, M. Hulman, B. Kalocayova (Bratislava, Slovakia)**
    Application of molecular hydrogen in the cardiac surgery-associated acute kidney injury

44. **A. Zahradnikova Jr., S. Kezmarova, M. Novotova, I. Zahradnik, A. Zahradnikova (Bratislava, Slovakia)**
    Formation of dyads during postnatal cardiac development in rats

45. **A. Zemančíková, J. Török, P. Bališ, P. Valovič, M. Chomová (Bratislava, Slovakia)**
    Sympathoadrenergic contractions in mesenteric arteries from zucker diabetic fatty rats: focus on perivascular adipose tissue

Abstracts of oral presentations

RIP3 MEDIATES NECROPTOSIS AND NON-NECROPTOTIC INFLAMMATORY RESPONSE: AN INTERESTING PHARMACOLOGICAL TOOL FOR TREATMENT OF HEART DISEASES

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Necroptosis has been identified in various cardiac diseases, however, very little is known about its underlying mechanisms. In addition to a canonical pathway involving RIP1-RIP3-MLKL axis, other molecules being associated with mitochondrial dynamics and function as well as oxidative stress have been proposed to be the signaling upstreams of RIP3. This protein kinase has also emerged as a convergence point of multiple signaling cascade involving non-necroptotic inflammation. Thus, RIP3 can be considered as an interesting and powerful pharmacological target. The approaches leading directly or indirectly to the lowering activity of RIP3 can possess remarkable cardioprotection due to the mitigation of various phenotypes of cardiac damage. The presentation will provide an experimental evidence of necroptosis activation in acute and subacute models of myocardial ischemia/reperfusion injury as well as in various failing hearts and highlight a role of active RIP3 in the pathomechanisms of such injuries. Finally, RIP3 released from the damaged heart will also be discussed with respect to its diagnostic and prognostic potential.

Keywords: RIP3, necroptosis, heart, ischemia, inflammation

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EXTRACELLULAR VESICLES AS BIOMARKERS AND TARGETS IN VASCULAR CONSEQUENCES OF METABOLIC SYNDROME

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Metabolic syndrome (MetS) is a worldwide public health problem, characterized by a cluster of risk factors including hyperglycemia, dyslipidemia, hypertension and obesity, leading to an increased risk of cardiovascular events. Endothelial dysfunction participates in the development of cardiovascular diseases associated with MetS. Extracellular vesicles (EVs) are involved in the pathogenesis and maintenance of cardiovascular and metabolic diseases.1,2 Two types of EVs have been described: microvesicles or large EVs (lEVs), and exosomes or small EVs (sEVs).

Regarding MetS, we have described that circulating lEVs from MetS (MetS-lEVs) patients induced endothelial dysfunction characterized by a decrease of nitric oxide (NO) production associated with the inhibition of endothelial NO-synthase, and an increase in oxidative and nitrative stresses. Furthermore, MetS-lEVs carry Fas-ligand and interacted with Fas in endothelial cells. This induced a temporal crosstalk between endoplasmic reticulum and mitochondria with respect to spatial regulation of reactive oxygen species (ROS) production via the neutral sphingomyelinase. These events led to a reduction of NO bioavailability accounting for the subsequent impairment of endothelium-dependent vasorelaxation.3 Proteomic analysis revealed that the small GTPase, Rap1 is overexpressed in lEVs from MetS patients. Rap1-lEV levels correlated with increased cardiovascular risks. MetS-lEVs promoted migration and proliferation of human aortic smooth muscle cells, and increased expression of pro-inflammatory molecules. Neutralization of Rap1 completely prevented the effects of lEVs from MetS patients. High fat diet-fed ApoE-/- mice displayed an increased expression of Rap1 in aortas, circulating lEVs and lEVs from plaque atherosclerotic lesions. Human atherosclerotic lesions were enriched in lEVs expressing Rap1. Thus, Rap1 carried by MetS-lEVs is a novel determinant of diagnostic value for cardiometabolic risk factors and a potential therapeutic target against the development of atherosclerosis.4 Regarding sEVs, circulating concentration and size of sEVs were, positively or negatively respectively, correlated with visceral obesity, hypertension, insulin resistance and dyslipidemia. Furthermore, sEVs from MetS patients decreased in vitro NO production in endothelial cells and impaired ex vivo endothelium-dependent relaxation. The decreased NO bioavailability induced by MetS-sEVs was associated with an increase of cytosolic and mitochondrial ROS production. Activation of TLR4 by LPS carried by MetS-sEVs account for the increased oxidative stress.5. In conclusion, we demonstrate that lEVs and sEVs are biomarkers and key players of both atherosclerosis, inflammation and metabolic disorders in MetS.

1-Martinez MC, Andriantsitohaina R Circ Res 2017; 2-Malloci M et al. Antioxid Red Signal 2019; 3-Safiedeen Z et al. Antioxid Redox Signal 2017; 4-Perdomo L et al. Circ Res 2020; 5-Ali S et al. Metabolism 2021

Keywords: extracellular vesicles, metabolic syndrome, vascular dysfunction, biomarkers, atherosclerosis

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NEW TRANSGENIC RABBIT MODELS TO PREDICT DRUG-INDUCED ARRHYTHMIAS

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Drug-induced proarrhythmia represents a potentially lethal adverse effect. This proarrhythmic side effect is often linked to the drug's potential to modulate repolarizing cardiac ion currents thereby causing a lengthening of the QT interval on the ECG. In spite of the sophisticated screening approaches in preclinical and clinical drug development, reliable prediction of proarrhythmia remains a largely unmet need. Drug-induced proarrhythmic events are often facilitated by pathological conditions that impair the patient's repolarization reserve, however, most cellular, tissue, and whole animal model systems used for current preclinical drug safety screening are based on normal, healthy tissues and animals. Several transgenic rabbit models for different types of long QT syndromes (LQTS) cauding impairments in repolarization reserve have been generated recently. The potential use of these models for screening/prediction of drug induced arrhythmia is discussed. Also, the electrophysiological characteristics of the available transgenic LQTS rabbit models are summarized along with proof-of-principle studies in these models – identifying certain advantages and disadvantages of rabbit LQTS models.

Keywords: proarrhythmia, repolarization reserve, long QT syndrome, transgenic rabbit

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DISTINCT FUNCTIONS OF CARDIAC B-ADRENERGIC RECEPTORS IN THE T-TUBULE VS. OUTER SURFACE MEMBRANE

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The membrane of cardiac transverse tubules (TTM) contains many receptors, channels or enzymes, such as β-adrenergic receptors (β-ARs), adenylyl cyclases or L-type Ca2+ channels (LTCCs). These membrane proteins are also present in the outer surface membrane (OSM), although often at a different density. Classical pharmacology allows to explore the function of a membrane protein in the whole cell membrane but not separately in OSM vs. TTM compartments.

Here, we developed a technology based on size exclusion to explore the function of β-ARs located in the OSM. We synthetized a PEG-Iso molecule by covalent linking isoprenaline (Iso) to a 5000 Da PolyEthylene-Glycol (PEG) chain to increase the size of the β-AR agonist and prevent it from accessing the TT network. The affinity of PEG-Iso and Iso on β1- and β2-ARs was measured using radioligand binding. Molecular dynamics simulation was used to assess PEG-Iso conformation and visualise the accessibility of the Iso moiety to water. Using confocal microscopy, we show that PEGylation constrains molecules outside the T-tubule network due to the presence of the extracellular matrix. The effects of PEG-Iso and Iso were measured in adult rat ventricular myocytes on cAMP and PKA activity by Förster resonance energy transfer, ICa,L by whole cell patch-clamp, and sarcomere shortening and Ca2+ transients with a Ionoptix® system. β-AR activation in OSM with PEG-Iso produced a lower stimulation of [cAMP]i than Iso but a larger stimulation of cytosolic PKA at equivalent levels of [cAMP]I and similar effects on excitation-contraction coupling parameters. However, PEG-Iso produced a much lower stimulation of nuclear PKA than Iso.

Thus, OSM β-ARs control mainly cytosolic cAMP/PKA pathway and contractility, while TTM β-ARs control mainly nuclear PKA and nuclear protein phosphorylation. Size exclusion strategy using ligand PEGylation provides a unique approach to evaluate the respective contribution of T-tubule vs. outer surface membrane proteins in cardiac cells.

Keywords: T-tubules - membrane compartmentation - size exclusion - β-adrenergic receptors - cyclic AMP signaling

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INHERITED ARRHYTHMIAS: FROM GENE VARIANTS TO IONIC CHANNEL DYSFUNCTIONS

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Inherited arrhythmias represent relatively rare, but life-threatening cardiac pathologies. The origin of the arrhythmias is heterogeneous. Variants in genes encoding cardiac ionic channels or associated proteins can be often detected. A complex clinical, genetic, and functional analysis is then needed to reveal if the identified genetic variant may cause the phenotype.

The long QT syndrome (LQTS), the most frequent type of inherited arrhythmia, is associated with various genetic variants, most often in the KCNQ1 gene (LQTS type 1, LQT1). This gene encodes the pore-forming subunit (Kv7.1) of slow delayed rectifier K+ (IKs) channels. We have recently characterized IKs dysfunction caused by two LQT1 variants identified in the Czech population, T309I and R562S. T309I resulted in a complete loss of function due to an impaired channel trafficking in the homozygous setting and a dominant-negative effect in the heterozygous setting (representing the situation in heterozygous carriers). R562S showed a preserved channel trafficking and, in the heterozygous setting, haploinsufficiency. The responsiveness to beta-adrenergic stimulation, an important regulator of IKs channel function namely at an increased sympathetic tone (e.g., at exercise), was preserved in T309I channels whereas it was completely missing in R562S channels. Using in silico simulations in the human ventricular cell model, delayed terdepolarizations were detected as the possible arrhythmogenic mechanism in both variants.

Variants in various genes encoding cardiac ionic channels can be also detected in some patients with idiopathic ventricular fibrillation (VF; both structural heart disease and any clinical signs of an inherited arrhythmia are missing). We have recently selected several identified genetic variants in our patients with idiopathic VF for the functional analysis. Patient-specific cardiomyocytes carrying a variant in the RYR2 gene (Y4734C) were prepared and a pilot investigation has been started (patch-clamp and microelectrode array). The first data showed an increased tendency of the patient-specific cardiomyocytes to irregular electric activity at specific conditions (e.g., increased temperature, decreased extracellular K+ concentration, activation of adrenergic receptors). Ongoing detailed analysis is aimed at elucidating the origin of the proarrhythmic activity in the patient.

The connection between genotype, clinical phenotype, and subcellular and cellular origin of the dysfunction is relatively clear in the “classic” inherited arrhythmias. In contrast, idiopathic VF is characterized by the absence of any typical clinical phenotype. Hence, ionic channel dysfunction resulting from an identified associated genetic variant is likely masked by compensatory mechanisms and can be revealed only under specific circumstances. Functional analysis of these variants is essential for a better understanding of the pathophysiology of this life-threatening disease.

Keywords: inherited arrhythmia, long QT syndrome, idiopathic ventricular fibrillation, electrophysiology, mathematical modelling

Funding: Supported by the grant project NU22-02-00348 provided by the Ministry of Health of the Czech Republic and by the Specific University Research Grant of the Masaryk University MUNI/A/1133/2021 provided by the Ministry of Education, Youth and Sports of the Czech Republic.

GREEN TEA POLYPHENOL IMPROVES INFLAMMATORY PHENOTYPE OF PERIVASCULAR ADIPOSE TISSUE BY OXIDIZING HYDROGEN SULFIDE (H_2S) TO POLYSULFIDES (H_2Sn)

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Background: Perivascular adipose tissue (PVAT) produces vasodilating and anti-inflammatory factors. However, in obesity/metabolic syndrome PVAT phenotype changes to pro-inflammatory one. Recently, it has been demonstrated that green tea polyphenols such as (−)-epigallocatechin (EGC) oxidize the gasotransmitter hydrogen sulfide (H_2S) to polysulfides (H_2Sn); the important signaling molecules (Redox Biol 2020; 37:101731). We examined the effect of EGC on periaortic adipose tissue (PAT) phenotype in rats fed high fat diet (HFD).

Methods.

Rats were fed regular diet or HFD for 1 month as well as were treated or not with EGC (10 mg/kg/day). The expression of pro- and antinflammatory factors was measured in PAT by qRT-PCR. H_2S and H_2Sn levels in PAT were measured by electrochemical sensor.

Results.

Expression/secretion of leptin, resisting, TNF-alpha, IL-6 and MCP-1 was higher and adiponectin was lower in PAT of obese rats which was accompanied by increased H_2S production. The H_2S and H_2Sn donors, Na_2S and Na_2S_4, had pro- and anti-inflammatory effects on PAT, respectively. Administration of EGC in HFD rats reduced the expression of leptin, resistin, TNF-alpha, IL-6 and MCP-1 and increased the expression of adiponectin. In addition, EGC reduced M1 macrophage marker, inducible NO synthase (iNOS), and increased the expression of M2 markers, IL-10 and arginase-1. The effects of EGC were mimicked by Na2S4. EGC decreased H_2S and increased H_2Sn in PAT of obese rats. The similar results were observed ex vivo in EGC-treated PAT explants

Conclusions.

EGC has the anti-inflammatory effect on perivascular adipose tissue mediated by oxidation of H_2S to H_2Sn. This effect may contribute to anti-atherosclerotic properties of green tea polyphenols.

Keywords: perivascular adipose tissue, hydrogen sulfide, polysulfides, adipokines, green tea plyphenols

DEVELOPMENT OF NOVEL MATRIX METALLOPROTEINASE-2 INHIBITORS FOR CARDIOPROTECTION AGAINST ISCHEMIA/REPERFUSION INJURY: FROM CHEMICAL DESIGN TO PRECLINICAL PROOF-OF-CONCEPT STUDIES

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Background: Matrix metalloproteinase-2 (MMP-2) has been demonstrated to play a crucial role in the development of ischemia/reperfusion injury due to its enhanced activation, which leads to increased infarcted area. However, we have previously shown that there is no need for complete inhibition of MMP-2 to achieve cardioprotection since its moderate (~20–25%) inhibition was sufficient to reduce infarct size in ex vivo and in vivo models of acute myocardial infarction (AMI). Therefore, we have designed novel MMP inhibitor compounds and performed a screening cascade to select potent MMP-2 inhibitors to test their cardioprotective effects in vivo.

Methods: We selected 568 novel substituted carboxylic acid derivatives based on imidazole and thiazole scaffolds from molecular libraries and then tested in a screening cascade for MMP inhibition. Initially, we used in silico docking to the 3D model of MMP-2 followed by an in vitro screening based on a fluorescent assay employing MMP-2 catalytic domain as well as gelatin zymography for MMP-2 inhibitory tests to determine IC50 values. Seven compounds were selected and tested in neonatal rat cardiac myocytes subjected to simulated I/R injury. Six compounds showed significant cardio-cytoprotection and the most effective compounds (MMPI-1154, -1260, and -1248) were tested in an in vivo rat model of AMI in the presence or absence of hypercholesterolemia.

Results: Ischemic preconditioning as positive control as well as MMPI-1154 and -1260 but not MMPI-1248 showed significant infarct size-limiting effects as compared to vehicle control at 1 and 3 μmol/kg, respectively, both in young healthy rats as well as in the age-matched controls of hypercholesterolemic group. However, in the presence of hypercholesterolemia, both inhibitors failed to reduce infarct size similarly to that of ischemic preconditioned group used as a positive control of cardioprotection.

Conclusion: We found that the MMP-inhibiting effects of imidazole and thiazole carboxylic acid-based compounds were superior in efficacy in comparison to the conventional hydroxamic acid-type MMP inhibitors. MMPI-1154 and -1260 reduced infarct size reproducibly in normocholesterolemic rat AMI model. Although, hypercholesterolemia abolished their infarct size-limiting effect, the cardioprotective potential of our novel MMP-2 inhibitors cannot completely be excluded even in hypercholesterolemic comorbid models.

Keywords: matrix metalloproteinase; drug development; ischemia/reperfusion injury; mypcardial infarction; cardioprotection

Funding: This project was supported by the New National Excellence Program of the Ministry of Human Capacities (ÚNKP-21-5-SZTE-543), and by the Hungarian National Scientific Research Fund (OTKA-138223) as well as by the Faculty of Medicine of the University of Szeged (SZGYA-2020). PB was supported by the János Bolyai Research Scholarships of the Hungarian Academy of Sciences Bolyai (bo_481_21).

ACUTE STRESS-INDUCED ALTERATIONS IN EXPRESSIONS OF GENES INVOLVED IN IRON METABOLISM IN THE HEARTS AND LIVERS OF NORMOTENSIVE RATS

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Iron is essential in many metabolic processes and chronic stress was shown to alter iron homeostasis. Stress is also considered to be an etiological factor in the development of cardiovascular diseases and metabolic disorders. In addition, there is also a crosstalk between the heart and liver: liver disorders trigger cardiovascular complications (and vice versa) with a significant clinical impact. We investigated if mild repeated acute stress alters the expressions of certain genes involved in iron homeostasis, nitric oxide and superoxide productions as well as the content of iron-containing compounds in the hearts and livers of Wistar-Kyoto rats.

Acute stress was induced by three sessions of 5-sec air jet (AJ, at 20, 50 and 130 min) during the 140-min experiment. Changes in relative iron content were determined using SQUID magnetometry (1 T hysteresis curve measured at -271.15 oC). Gene expressions of nuclear factor erythroid 2-related factor 2 (NRF2), inducible and endothelial nitric oxide synthase (iNOS, eNOS), superoxide dismutase 1 and 2 (SOD 1, SOD2), glutathione peroxidase 4 (GPx4), hepcidin (HAMP), ferroportin (FPT), divalent metal transporter 1 (DMT1), ferritin heavy chain 1 (FTH1), transferrin (TF) and transferrin receptor 1 (TFR1) were determined by qRT-PCR. Mean arterial pressure (MAP) was determined in the carotid artery continuously. AJ elevated MAP significantly during each session by about 50% vs. pre-stress levels. AJ also elevated plasma corticosterone and relative content of iron-containing compounds in the liver but not in the left heart ventricle (LHV). AJ failed to affect NO and superoxide productions in both tissues investigated. In the liver, expressions of NRF2, PPAR-gamma, iNOS, FTH1 and FPT were significantly elevated vs. controls. In the LHV, only FTH1 and SOD1 expressions were elevated, without the changes in expressions of the remaining abovementioned genes. There was significant positive correlation between the saturation magnetization and FTH1 expression in the liver but not in the LHV.

In conclusion, repeated, relatively mild, acute stress increased FTH1 expression in the LHV and liver while relative content of iron-containing compounds was elevated only in the liver. The results suggest rapid effect of acute psycho-emotional stress on iron accumulation in the liver that may provide the link between stress and liver diseases that may consequently result in cardiac function disturbances.

Keywords: iron metabolism, stress, nitric oxide, heart, liver

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IMPAIRMENT OF CALCIUM DYNAMICS AND CONTRACTILITY IN CARDIOMYOCYTES OF WOLFRAMIN INVALIDATED RATS

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Wolfram syndrome (WS) is a rare recessive disorder caused by mutations in the Wfs1 gene encoding the wolframin protein (Wfs1). Wfs1 is located in the membrane of the endoplasmic reticulum (ER) and is involved in ER stress, mitochondrial function, and calcium signalling [1]. Since calcium is the principal trigger of cardiomyocyte contraction and since Wfs1 is highly expressed in the cardiac tissue [2], we have evaluated calcium metabolism and contractility in the Wfs1 exon5-deleted (Wfs1-e5/-e5) cardiac myocytes using confocal microscopy, patch-clamp, transmission molecular biology and electron microscopy [3].

In four months old male rats, invalidation of Wfs1 resulted in a significant increase of the amplitude of myocyte contraction as well as in prolongation of contraction in field-stimulated myocytes. The Fluo-3/AM stained Wfs1-e5/-e5 myocytes, recorded simultaneously with contractions, showed significantly prolonged calcium transient, but no significant change in the amplitude. Moreover, sarcoplasmic reticulum calcium content was not changed in the absence of functional Wfs1, as revealed by caffeine application.

The change in contractility could result from a change in the calcium current (ICa, the trigger for calcium release) and/or from a change in calcium release itself. Calcium currents showed no statistically significant difference in voltage-dependent activation, inactivation, or peak amplitude. On the other hand, the extent of calcium release-dependent inactivation of ICa was significantly higher in Wfs1 e5/-e5 myocytes.

Expression of the plasma membrane sodium-calcium exchanger (NCX) in Wfs1-e5/-e5 myocytes was decreased both at the mRNA and the protein level, whereas the expression levels of L-type calcium channel, RyR2, SERCA2, and CSQ2 were unchanged [4]. The decreased NCX expression might slow down Ca^{2+}extrusion from myocytes, prolong calcium transients, and curtail ICa due to increased calcium release-dependent inactivation. This would explain the unchanged SR calcium content. Finally, electron microscopic examination of the cardiac myocyte ultrastructure revealed variability in diameter of t-tubule profiles in dyads and higher occurrence of small non-dyadic t-tubules near Z-lines in the Wfs1 e5/ e5 group.

In conclusion, the Wfs1 invalidation negatively impacts calcium handling and contractility of cardiac myocytes before the onset of diabetes at the organism level.

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Keywords: Wolfram syndrome, cardiac myocyte, calcium signalling, contractility, patch clamp, ultrastructure

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RATIONALE FOR THE PREVENTION OR THERAPY OF SUDDEN CARDIAC DEATH IN HEART FAILURE

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Although sudden cardiac death is considered to be a major mode of death in patients with heart failure, no satisfactory medical therapy is available for its prevention. Since over activation of sympathetic nervous system is invariably seen in subjects with high risk for sudden cardiac death, elevated levels of circulating catecholamine levels are considered to result in lethal ventricular arrhythmias and subsequent sudden cardiac death. However, experimental evidence from our laboratory has revealed that excessive amounts of circulating catecholamines are oxidized to aminochromes, which are highly reactive quinine compounds, in addition to producing oxidative stress. A marked increase in plasma adrenochrome level was seen in heart failure; patients with higher than mean value for adrenochrome showed greater mortality. The oxidation products of catecholamines (both adrenochrome and oxidative stress) have been demonstrated to produce subcellular alterations, intracellular Ca2+-overload, coronary spasm, myocardial cell damage, depletion of high energy stores and ventricular arrhythmias. Furthermore, catecholamine-induced arrhythmias and ventricular fibrillation were associated with elevated levels of plasma adrenochromes and these changes were markedly attenuated by treatment of animals with different antioxidants. The results suggest that oxidation of catecholamines under stressful conditions may play a critical role in the genesis of fatal ventricular arrhythmias. Accordingly, antioxidant therapy in heart failure may be helpful in preventing sudden cardiac death.

Keywords: sudden cardiac death, heart failure, arrhythmias, oxidative stress, catecholamines

CARDIOPROTECTION BY microRNA THERAPEUTICS

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Ischemic heart disease is a leading cause of mortality worldwide, therefore, identification of valid drug targets for cardioprotection is of great importance. The lack of successful translation of cardioprotection to clinical therapy after more than 3 decades of intensive research urge the need for novel therapeutics to treat this disease with molecular mechanisms. Indeed major cardiovascular comorbidities such as hyperlipidemia, diabetes, and their co-medications have been shown to interfere with most of the known cardioprotective mechanisms (see for reviews: Ferdinandy et al, *Pharmacol Rev*, 2014; 2022 upcoming) and to lead to manifestation of hidden cardiotoxicy of drugs (Ferdinandy et al, *Eur Heart J*, 2019; Brenner et al, *Cells*, 2020). Cardioprotectionand cardiotoxicity have been shown to affect global myocardial gene expression profile showing that cardioprotectionand cardiotoxicity trigger a complex network of signaling cascade rather than a single major pathway. Moreover, cardiovascular comorbidities have been also shown to affect global cardiac gene expression profile at the transcript level including the non-coding RNAmicroRNAs (Perrino et al, *Cardiovasc Res*, 2017; Makkos et al, Free Rad Biol Med, 2021). MicroRNS are found in all tissues and body fluids carried by extracellular vesicles (Slujiter et al, *Cardiovasc Res*, 2018), therefore, microRNA transcriptomics-based target identification may lead to development of novel microRNA therapeutics and diagnostics for cardioprotection against ischemic heart disease and cardiotoxicity.

EVs IN OXIDATIVE STRESS AND CARDIOPROTECTION

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Extracellular vesicles (EVs) are actively investigated for their role both in cardiac pathologies involving oxidative stress and in cardioprotective interventions that may alleviate oxidative stress-induced cardiac insults. Although EVs have been the focus of numerous cardiac research projects for decades, their isolation from bodily fluids still poses challenge that may influence results of studies as well as their reproducibility and translatability. Similarly, methods for production of EVs suitable for therapeutic goals and that are potentially applicable in industrial settings have to be developed for cardiovascular medicine.
In this lecture recent advances and remaining challenges in the field of EV isolation technologies will be summarized. The presentation will also include novel findings on cardioprotection achieved by EVs in models of ischemic cardiac diseases, and on methods being developed for cardioprotective EV production.

HIDDEN CARDIOTOXICITY - CARDIAC SAFETY TESTING IN ISCHEMIC AND COMORBID CONDITIONS: DEVELOPMENT OF PRECLINICAL TEST PLATFORMS

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Unexpected cardiac adverse events are one of the leading causes of interruption of clinical trials and drug withdrawals. It has been shown that cardiovascular risk factors and comorbidities (such as aging, metabolic diseases, etc) and their medications (e.g. nitrates, antidiabetic drugs, statins, etc) may interfere with cardiac ischemic tolerance and molecular signaling of endogenous cardioprotection. Indeed certain drugs may exert adverse events on the diseased heart that is hidden in the healthy myocardium. Hidden cardiotoxic effects of drugs may occur due to (i) enhancement of unwanted signaling due to ischemia/reperfusion injury and/or the presence of risk factors and/or (ii) inhibition of cardioprotective signaling pathways, both of which may lead to ischemia-related cell death and pro-arrhythmic events. This led to novel concept of "hidden cardiotoxicity", i.e. cardiotoxity seen only in the diseased heart, i.e. ischemia/reperfusion injury and/or its major comorbidities (Ferdinandy et al, Eur Heart J, 2018). Hidden cardiotoxicity cannot be revealed by the routinely used cardiac safety testing methods in "healthy" test systems, moreover, the mechanism of hidden cardiotoxocity is largely unknown. Therefore, we aimed to develop a preclinical in vivo and vitro platform and some examples of already withdrawn drugs with hidden cardiotoxic properties and new drugs with potential cardiotoxic properties. Here we summarize the current knowledge on hidden cardiotoxicity and urge the need for development of novel cardiac safety testing platforms for early detection of yet "hidden" cardiotoxicity.

Keywords: hidden cardiotoxicity, drug safety, comorbidity, cardioprotection

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FTO INHIBITION IMPAIRS CARDIOMYOCYTE TOLERANCE TO OXYGEN DEPRIVATION

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Ischemic heart disease is the leading cause of death worldwide. Cardiac tolerance to ischemia can be increased by adaptation to chronic hypoxia, which is associated with significant myocardial gene expression profile changes. Among the possible mechanisms of gene expression alterations are epigenetic modifications of RNA – epitranscriptomics. Our study focused on two of the most prominent marks – N6-methyladenosine (m6A) and N6,2'-O-dimethyladenosine (m6Am). We found that m6Am is more abundant in rRNA-depleted RNA isolated from rat cardiomyocytes than m6A. Hypoxic adaptation of rats affected the expression of m6A and m6Am regulators in the heart, including up-regulation of both demethylases – ALKBH5 (m6A) and FTO (m6Am and m6A). Based on these results, we studied the effects of FTO (the only m6Am eraser) inhibition on the proteome, metabolism, and also tolerance to oxygen deprivation of rat cardiomyocytes. FTO inhibition affected protein levels involved in crucial cellular processes such as gene expression, non-coding RNA processing, peptide biosynthesis, and cellular metabolism. Glycolytic and respiration rates of cardiomyocytes were increased after FTO inhibition. Most importantly, we found that FTO inhibition decreases the tolerance of cardiomyocytes to oxygen deprivation in vitro, supporting the possible role of epitranscriptomic regulations in the cardioprotective mechanisms.

Keywords: hypoxia, heart, epitranscriptomics, FTO, m6A, m6Am

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DECREASED SYMPATHETIC ACTIVATION DURING PSYCHOSOCIAL STRESS IN ALLERGIC PATIENTS

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Chronic stress events may result in worsening the quality of life and consequent pathological states. Accordingly, psychosocial stress may represent a factor involved in both the onset of allergic diseases and the exacerbation of the existing allergic disease. In patients with atopy, a decreased responsiveness of the hypothalamic-pituitary-adrenocortical axis to stress stimuli has been documented. Less consistent are the results on the autonomic nervous system function. The present study has focused on so far unexplored parameters related to the sympathetic nervous system activity, namely the salivary enzyme alpha-amylase and the stress hormone aldosterone. We test the hypothesis that the blunted cortisol response to psychosocial stress in atopic patients is associated with reduced salivary alpha-amylase activity and aldosterone concentrations. The sample consisted of 106 subjects of both sexes, 53 atopic patients suffering from allergic rhinitis, allergic asthma, or atopic dermatitis, and 53 age-, sex-, the menstrual cycle phase- and BMI- matched healthy controls. Volunteers were exposed to a laboratory model of psychosocial stress based on public speech. A substantially attenuated activity of alpha-amylase and reduced secretion of aldosterone during the psychosocial stress were observed in the whole sample of patients with atopy. Higher activity of alpha-amylase observed in the follicular compared to the luteal phase in healthy women was not present in atopic patients. In both males and females, atopy was associated with blunted cortisol response but unchanged heart rate. These findings provide evidence that patients with atopy exhibit insufficient alpha-amylase and aldosterone responsiveness to the psychosocial stressor, thus suggesting decreased sympathetic activity.

Keywords: stress, allergy, alpha-amylase, aldosterone, heart rate

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NECROPTOSIS MEDIATES CARDIAC DAMAGE UNDER CONDITIONS OF ISCHEMIA AND REPERFUSION: EMPHASIS ON DURATION OF REPERFUSION

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Since the discovery of necroptosis, this form of regulated necrosis mediated by receptor-interacting protein kinase 3 (RIP3) and mixed lineage domain kinase domain-like pseudokinase (MLKL), was implemented in various cardiovascular diseases, including ischemia/reperfusion (IR) injury. However, a potential relationship in the extent of necroptotic damage with respect to the duration of reperfusion phase remains elusive. Therefore, Wistar rat hearts were subjected to a 30-min ischemia followed by either a brief 10-min or longer 40-min reperfusion phase. Moreover, to elucidate necroptosis activation in a chronic model of IR mimicking post-ischemic heart failure (HF), regional 30-min ischemia followed by 42-day reperfusion was used. Although 10-min reperfusion impaired cardiac function, necroptosis activation via its either pThr231/Ser232-RIP3-MLKL canonical or non-canonical pathways, including Ca^{2+}/calmodulin dependent protein kinase II-mitochondrial permeability transition pore (CaMKII-mPTP) or phosphoglycerate mutase 5-dynamin-related protein 1 (PGAM5-Drp1) axes, was absent. Contrary, prolonged 40-min reperfusion caused both cardiac dysfunction and necroptotic damage evidenced by upregulation of RIP3, pSer229-RIP3 and MLKL. In support, the membrane fraction of these hearts was positive for MLKL translocation, thereby indicating robust evidence of necroptotic cell membrane disruption. Similarly, necroptosis was active in the infarcted area of postischemic HF which was accompanied by cardiac fibrosis. In contrast, increased level of pSer229-RIP3 in the non-infarcted tissue unlikely activated necroptotic cell loss and rather induced pro-inflammatory pyroptosis-like cell death. In summary, our findings suggest that prolonged reperfusion phase is needed to execute necroptosis in the heart while brief reperfusion may induce cardiac damage due to different mechanisms unlikely involving necroptosis activation. Therefore, limitation of necroptotic cell death might represent a cardioprotective strategy in the settings of chronic, but not acute myocardial IR injury.

Keywords: necroptosis, ischemia, reperfusion, heart failure, cell death

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DETERMINANTS OF RyR-RyR COUPLING STRENGTH IN CARDIAC CALCIUM RELEASE SITES

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Recent electron tomography and super-resolution microscopy of cardiac muscle cells has shown that the distribution of RyR in cardiac dyads is non-uniform in contrast to previous views that considered a checkerboard pattern of RyR placement.

To test how the placement of RyRs contributes to the formation of calcium sparks, which cannot be addressed experimentally, we performed in silico simulations of calcium release events (CREs) on a large parameter set of models of calcium release sites. The models covered the observed range of RyR number, density, and spatial placement in cardiac dyads. RyR activity in the models was approximated by a two-state gating model conforming to the published single RyR channel properties such as sensitivity to allosteric activation by Ca2+ ions and sensitivity to competitive/non-competitive inhibition by Mg2+ ions. Calcium dynamics in the calcium release site (CRS) model were approximated using the linearized buffered diffusion approximation and a constant single RyR channel calcium current. The spatial placement of RyRs in the calcium release site models was quantitatively characterized by RyR vicinity defined as an average reciprocal distance between RyRs normalized on the number of RyRs [1].

The simulated responses of CRS models to a random RyR channel opening, i.e., the calcium release events (CRE), were classified according to their amplitude histograms as a quark when no other RyR was activated, as a blip when few more RyRs were activated, and as a spark when many RyRs were activated. Each calcium release event was characterized by the amplitude and time to peak. The relative occurrence of individual CRE types was evaluated.

The characteristics of CREs did not correlate well with individual determinants of CRS models; however, they correlated in a dose-response manner with the coupling strength between RyRs of respective models defined as a weighted product of the RyR vicinity and single-channel calcium current amplitude [1]. This in silico observation explains how the distribution of RyRs in dyads affects their spontaneous calcium releasing activity in synergy with the single RyR calcium current.

From the physiological viewpoint, the simulations revealed how the sparse placement of RyRs or a small calcium current may lead to an increased spontaneous calcium leak from the sarcoplasmic reticulum and a decreased spontaneous spark occurrence during diastole.

The introduction of the concept of RyRs coupling strength made it possible to quantitatively estimate the spark probability for a given dyad. It revealed that under the modelling conditions, the resulting RyR coupling strength is dominated by the RyR vicinity over the single-channel calcium current.

[1] B. Iaparov, I. Zahradnik, A. Moskvin and A. Zahradnikova, J. Gen. Physiol. 153 (2021), e202012685.

Keywords: cardiac dyad, calcium spark, ryanodine receptor, calcium release site, mathematical modelling

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ERYTHROCYTE AND PLASMA PROPERTIES IN MONOCROTALINE MODEL OF PULMONARY ARTERIAL HYPERTENSION

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Introduction: Monocrotaline (MCT) is a toxic alkaloid used for the induction of pulmonary arterial hypertension (PAH) in experimental animals. After injection, MCT is bound to erythrocytes. In the oxygen-rich environment of pulmonary precapillary arterioles and capillaries, MCT is released and causes endothelial cell damage, leading to vascular remodelling and consecutively to PAH development. Modulations of renin-angiotensin-aldosterone system (RAS), and matrix metalloproteinases are involved in its pathogenesis. In patients with PAH, several parameters of erythrocytes deteriorate, such as erythrocyte deformability (ED) or aggregability. However, changes in other erythrocyte parameters have not yet been fully described in MCT model of PAH. Bosentan is an available medicament used in treatment of PAH. Its effect on erythrocytes remains unclear. Therefore, we focused on the characterization of the erythrocyte parameters, the RAS system in the MCT model, and the possible effect of bosentan treatment.

Methods: The experiment was carried out on 12-week-old Wistar rats (n = 30) divided into 3 groups: control group; MCT-treated group (60 mg/kg) and MCT- and bosentan-treated group (300 mg/kg/day). After 4 weeks of experiment, ED was determined by the filtration method and erythrocyte NO production using DAF-2 DA fluorescence probe. Plasma oxidation state parameters, erythrocyte osmotic resistance and erythrocyte Na,K-ATPase kinetic parameters were measured spectrometrically/spectrofluorometrically. The concentration of each component of RAS was determined by liquid chromatography-tandem mass spectroscopy.

Results: The administration of MCT decreased haematocrit. It also increased Vmax and decreased KNa kinetic parameters of Na,K-ATPase. Moreover, an activation of the alternative pathway of renin-angiotensin system (increased Ang I, Ang 1-7, and Ang 1-5 without changes in Ang II level) and down-regulation of aldosterone was also observed. Bosentan treatment improved ED, decreased AOPP and fructosamine level as well as increased GSH/GSSG ratio. Conclusion: Our findings don't entirely match with findings in patients with PAH reporting an increase in Ang II levels, increase in oxidative stress and deterioration in ED. Within the observed erythrocyte parameters, MCT administration only impaired Na+ binding properties of Na,K-ATPase, which probably led to a compensatory increase in the number of enzyme active molecules. Bosentan treatment enhanced ED in MCT-treated animals which can also contribute to the improvement of hemodynamics in the condition of PAH. This effect is most likely due to the antioxidant effect of bosentan, since several markers of oxidative state improved. To obtain more comparable erythrocyte parameters as in human PAH, adjustments to used model might be appropriate (e.g. different age of animal, MCT dosage, experiment duration).

Keywords: erythrocyte, pulmonary arterial hypertension, bosentan

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MECHANISMS OF CARDIOVASCULAR CHANGES OF PHOTOTHERAPY IN NEWBORNS WITH HYPERBILIRUBINEMIA

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During phototherapy (PT) of newborns, vasodilation occurs in the skin circulation compensated by vasoconstriction in the mesenteric and renal circulation, without significantly affecting the coronary and cerebral circulation. Furthermore, there is a slight decrease in cardiac systolic volume, blood pressure, an increase in heart rate and discrete changes in the heart rate variability (HRV).

Mechanisms of the skin vasodilatation: The primary change during phototherapy is vasodilatation mediated by multiple mechanisms:

Heat effect: One of the components of the light is the thermal influx. At higher irradiance intensities, body temperature increases, which promotes cutaneous vasodilation.

Photorelaxation: In both in vitro and in vivo animal experiments, light elicits a maximal response of the vessel diameter at the wavelength of ~430-460 nm, also used in PT (~450 ± 20 nm). The mechanisms of smooth muscle photorelaxation are being intensively studied. "Atypical opsins" have been found in the eye, but also in arteries of the systemic and pulmonary circulation. Of these, opsin4 (Opn4) - melanopsin is thought to play a major role in vascular photorelaxation. The signaling cascade of the photorelaxation via Opn4 is specific, independent of endothelium and NO (1).

Humoral regulation: Blood vessel diameter is influenced by endothelin 1 (ET-1) and nitric oxide (NO), among other substances. Abu Faddan et al. (2) found a rise in serum levels of NO but also ET-1 after phototherapy. The NO:ET-1 ratio increased, which may lead to a predominant effect of NO.

Neural regulation: A decrease in tonic sympathetic activity to the skin vessels during PT is expected.

Mechanisms of changes in cardiac function and blood redistribution:

Cardiac systolic output is slightly reduced from the beginning of phototherapy (3), which may be due to reduced venous return associated with skin vasodilation and decreased motor activity of the newborns.

Heart rate increases during PT. This response can arise through baroreflexes, the action of NO as well as the effect of increased body temperature on cardiac pacemaker. Changes in HRV assessed by methods of symbolic dynamics confirm increase in sympathetic activity.

Redistribution of blood into the skin circulation during PT is ensured by restriction of blood flow in the mesenteric and renal circulations. The presumed dominant regulator here are baroreflexes, which, when the systemic blood pressure decreases, increase vasoconstriction in the above mentioned regional circulations (3).

Conclusion: During neonatal phototherapy, cardiovascular changes occur which are important for maintaining vital functions as well as for improving the effect of PT by increasing skin blood flow. Complex and specific mechanisms of the hemodynamic changes during PT confirm the functioning regulation of the neonatal cardiovascular system, including baroreflexes. 1.Sikka et al. 2014; 2. Abu Faddan, et al. 2014; 3.Benders et al. 1999.

Keywords: Phototherapy, cardiovascular changes, photorelaxation, skin vasodilation, newborns

STROKE VOLUME VARIATION AS AN INDEX OF FLUID RESPONSIVENESS IN CONSCIOUS PATIENTS

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Cardiac stroke volume variation (SVV) measurement is an established technique to detect fluid-responsive hypovolemia in patients under mechanical ventilation. There is an ongoing effort to apply SVV for this purpose also in conscious patients. However, the effect of mental stress often occurring in conscious trauma patients as a potential confounding factor on SVV is not known. The aim of our study was to compare effect of simulated hypovolemia and mental stress on SVV in healthy volunteers in the context of potential confounders – breathing pattern, respiratory sinus arrhythmia magnitude and sex.

We examined 102 young healthy volunteers (58 females), mean age 18.6 years. Finger arterial blood pressure was recorded by volume-clamp photoplethysmographic method (Finometer Pro, FMS, Netherlands). From the blood pressure curve, a built in ModelFlow algorithm calculated stroke volume values (SV) for each heart beat. Respiratory volume was recorded using calibrated respiratory inductive plethysmography (RespiTrace, NIMS, USA). During four phases of examination protocol (supine rest, head-up tilt – HUT, supine recovery, mental arithmetic task – MA) we analyzed SVV related to respiratory activity. While during HUT we found an expected increase in SVV together with mean SV decrease, SVV significantly decreased during MA. The observed changes during MA could be attributed to an increased respiratory rate and/or decreased respiratory sinus arrhythmia. Sex related differences in SVV responses to HUT and MA were observed.

We conclude that mental stress together with respiratory sinus arrhythmia and respiratory pattern changes could significantly influence SVV as a potential index of fluid responsiveness in conscious patients. Sex differences and other confounding factors should be considered when interpreting SVV changes.

Keywords: stroke volume variation, fluid responsiveness, cardio-respiratory interactions, respiratory sinus arrhythmia, sex differences

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STRESS, CATECHOLAMINES AND BETA3-ADRENERGIC RECEPTORS

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Beta-adrenergic stimulation is known to be a significant regulatory mechanism in the heart. Beta3 subtype of adrenergic receptors localized in the heart may protect the myocardium against adverse effects of excessive catecholamine stimulation. Stimulation of cardiac beta3-adrenoceptors may lead to a decrease in heart contractility. In the adipose tissue, the main function of these receptors is to mediate lipolysis and thermogenesis. Their function in the brain is unclear. The sympathetic-adrenomedullary system is one of the two main stress systems. The action of catecholamines via beta-adrenoceptors is important under stress conditions. The stress-induced adrenaline and noradrenaline release depends on the type and intensity of the stress stimulus. We have tested the hypothesis that stress associated with the repeated immune challenge has an impact on beta3-adrenergic receptor gene expression in the adipose tissue and in the brain (1). Rats of both sexes were intraperitoneally treated with increasing doses of lipopolysaccharide (LPS) for 5 days. LPS treatment led to body weight loss, an increase in anxiety behavior, and a decrease in beta3-receptor gene expression in the white adipose tissue with higher values in males compared to females. In LPS-treated animals of both sexes, beta3-receptor gene expression was increased in the prefrontal cortex but not the hippocampus. Another chronic stressor evaluated was compulsory wheel running for three weeks (2). No changes in beta3-adrenergic receptor and cell proliferation measured by 5-bromo-2′-deoxyuridine incorporation in the left heart ventricle were observed in rats exposed to physical exercise compared to controls. In the brown adipose tissue, the gene expression of uncoupling protein-1 (UCP-1), which is an important mediator of thermogenesis, was increased. The gene expression of beta3-adrenergic receptors following prolonged wheel running increased in the white, while it decreased in the brown adipose tissue. The reduced beta3-adrenergic receptor but not enhanced uncoupling protein-1 gene expression supports the hypothesis of hypoactive brown adipose tissue in response to exercise.

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Keywords: autonomic nervous system, adipose tissue, heart

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"NEW WINE IN AN OLD BOTTLE OR OLD WINE IN A NEW BOTTLE?" IN VIVO AND CELLULAR ANTIARRHYTHMIC AND CARDIAC ELECTROPHYSIOLOGICAL EFFECTS OF DESETHYLAMIODARONE IN DOGS

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Aims: The aim of the present study was to study the antiarrhythmic effects and cellular mechanisms of desethylamiodarone (DEA), the main metabolite of the most powerful antiarrhythmic drug amiodarone, following 4-week oral treatment (25-50 mg/kg/day) in in vivo and cellular canine experiments.

Methods and Results: Acute and chronic administration of DEA and amiodarone exerted marked antiarrhythmic effects in atrial fibrillation models. DEA and amiodarone similarly influenced ECG parameters. DEA prolonged action potential duration in atrial and ventricular muscle without changing it in Purkinje fibre, and decreased the amplitude of several outward potassium currents such as IKr, IKs, IK1, Ito and IKACh. The L-type ICa and late INa inward currents were also depressed by chronic DEA treatment. Pharmacokinetic studies following a single intravenous dose of 25 mg/kg revealed better drug bioavailability and higher volume of distribution with DEA than with amiodarone treatment. Chronic toxicological investigations (91 days with 25mg/kg/day orally) showed no neutropenia and less severe pulmonary fibrosis following DEA compared to that of amiodarone treatment.

Conclusion: Chronic DEA treatment in animal experiments has similar antiarrhythmic and electrophysiological effects as its parent compound with better pharmacokinetics and lower tissue accumulation and related toxicity. These results suggest that the active metabolite, DEA should be tested in clinical trials as a possible new, more favorable option for the treatment of cardiac arrhythmias including atrial fibrillation.

Keywords: desethylamiodarone, atrial fibrillation, cardiac electrophysiology, canine

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NEUREGULIN-1 ATTENUATES DEVELOPMENT OF CARDIAC AND KINDEY DYSFUNCTION IN A RAT MODEL OF CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) is uremic cardiomyopathy characterised by left ventricular hypertrophy, (LVH), diastolic dysfunction and cardiac fibrosis. Inflammation and dysregulation of endothelium-derived neuregulin-1β (NRG-1β) signalling are known contributors to heart failure with different etiologies. Here, we aimed to clarify 1) the impact of CKD on the expression of NRG-1 and 2) recombinant human neuregulin-1 β (rhNRG-1β) treatment to alleviate cardiac and renal dysfunction in a rat model of CKD. Adult male Wistar rats were randomised into 3 groups: i) sham-operated group (iv. saline; 0.5 mL/kg/day), ii) 5/6-nephrectomy-induced CKD group (iv. saline; 0.5 mL/kg/day), and iii) rhNRG-1β-treated (iv. 10 μg/kg/day) CKD group. Treatments were administered daily from week 3 for 10 days. Ten weeks after the operations, cardiac and renal NRG-1β expressions were significantly reduced in CKD compared to the sham-operated group. In CKD, rhNRG-1 treatment markedly improved renal function and serum LDL cholesterol levels, suggesting its reno- and atheroprotective effects. In CKD, rhNRG-1β treatment alleviated concentric LVH, diastolic dysfunction and cardiac fibrosis as well as macrophage infiltration. Furthermore, rhNRG-1β treatment significantly reduced expression of molecular markers of fibrosis, inflammation and oxidative-stress in LV tissue, cardiac fibroblast and isolated cardiomyocytes. In conclusion, the rhNRG-1β treatment improved both uremic cardiomyopathy and renal function. Therefore, rhNRG-1β may represent a novel promising agent in prevention and therapy for uremic cardiomyopathy.

Keywords: heart failure, chronic kidney disease, neuregulin-1, cardioprotection

TISSUE- AND STRAIN-DEPENDENT DIFFERENCES IN IRON METABOLISM AFTER SINGLE ADMINISTRATION OF IRON OXIDE NANOPARTICLES

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We investigated the effect of polyethylene glycol-coated Fe3O4 nanoparticles (IONs) on mean arterial pressure (MAP) and heart rate (HR) in normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) after single intravenous administration of IONs (1 mg Fe/kg). In the liver and left heart ventricle (LHV) of both strains, we determined nitric oxide (NO) production, superoxide (O2•–) production, ION-originated iron and biogenic iron content. Finely, we investigated expression of genes involved in iron metabolism, antioxidant defence and NO production in both tissues.

IONs administration did not affect MAP and HR in both strains and led to increased deposition of ION-originated iron in tissues of WKY compared to SHR. Our findings showed strain differences of biogenic iron content in the liver (↓WKY and ↑SHR) and LHV (↑WKY and ↓SHR). In WKY, IONs treatment increased O2•– production in both tissues with unaltered NO production. In SHR, ION treatment reduced liver NO production without effect on O2•– production in both tissues.

In the liver, IONs administration increased expression of the hepcidin gene in both strains which product has a crucial role in iron regulation. IONs treatment also increased gene expression of antioxidants superoxide dismutase 1 and 2 (Sod1 and Sod2) in WKY with opposite trends in SHR. Strain differences after IONs treatment were also noted in the expression of the H-ferritin (Fth1; ↑WKY and ↓SHR) and transferrin (Tf; unchanged in WKY and ↓SHR) genes, whose products represent the major intracellular iron storage protein and circulating iron transporter.

In the LHV, IONs administration significantly decreased expression of antioxidant glutathione peroxidase 4 (Gpx 4) and transcription factor peroxisome proliferator-activated receptor gamma in WKY with similar trends in SHR. Strain differences after IONs administration were also noted in the expression of the transferrin receptor (unchanged in WKY and ↑SHR) which product is responsible for iron endocytosis.

Our study showed that despite increased expression of antioxidant genes (Sod1 and Sod2) and genes involved in iron utilization (Fth1), there is a risk of tissue damage in normotensive WKY which tended to accumulate and subsequently utilize exogenous iron despite increased oxidative stress. At the same time, we did not detected oxidative stress in hypertensive SHR after IONs administration, despite the reduced expression of antioxidant genes (Sod1, Sod2 and Gpx4). Unchanged oxidative status in SHR was probably associated with decreased exogenous iron accumulation and repression of iron utilization genes (Fth1 and Tf). However, questions arise about possible negative effects after repeated or prolonged administration of IONs, mainly in normotensive and partly in hypertensive individuals.

Keywords: nanoparticles, nitric oxide, iron metabolism

Funding: This research was funded by grants VEGA 2/0160/17, VEGA 2/164/17 and APVV-16-0263.

LEFT VENTRICLE REMODELLATION IN ZUCKER DIABETIC FATTY RATS BUT ALSO IN ZUCKER LEAN RATS

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The Zucker diabetic fatty (ZDF) rat is a widely used animal model of type 2 diabetes mellitus (DM2) that represents a major risk factor for cardiovascular diseases. In the pathogenesis of DM2, matrix metalloproteinases (MMPs) play a significant role. MMPs are proteolytic enzymes involved in the degradation and remodelling of the extracellular matrix that can serve as possible predictors of the treatment effectiveness or the development of DM2 complications. The hyperglycaemia generates oxidative stress in DM2 that promotes MMP activation. The aim of our study was to observe biometric and biochemical parameters and MMP activities in plasma and myocardial tissue of ZDF rats and compare them with two possible controls.

We worked with males of 3 rat strains: ZDF inbred rats (n=16), Wistar outbred (WIS, independent control, n=14), and Zucker Lean (LEAN, control, n=15). Body weight, blood glucose, plasma insulin and lipid levels of all experimental animals were recorded. Based on fasting glycaemia and insulin concentrations, ZDF rats were split into 2 phenotypes - obese (FAT) and diabetic (DIA). Markers of oxidative stress, antioxidant capacity, and carbonyl stress were analysed spectrophotometrically and fluorometrically in plasma, left and right ventricle. The activity of MMP-2 and MMP-9 was determined by gelatin zymography. FAT revealed stronger left ventricular hypertrophy and greater body weight, differentiating them from DIA. Oxidative stress and antioxidant status pointed to higher oxidative stress in ZDF rats, both DIA and FAT. MMP-2 activity measured in plasma samples was lower in FAT compared with DIA ($p=0.037$) and it was also lower in DIA ($p=0.011$) and FAT ($p<0.0001$) compared with LEAN. MMP-9 activity was higher in WIS compared with LEAN ($p=0.009$) and higher in DIA compared with FAT ($p<0.005$) and LEAN ($p<0.0001$). In the right ventricle, the activity of MMP-2 was higher in LEAN compared with WIS ($p=0.049$). Similarly, in the left ventricle, the activity of MMP-2 was higher in LEAN compared with WIS ($p=0.034$). MMP-2 was higher in DIA ($p=0.034$), and FAT ($p=0.008$) compared with LEAN.

Based on our study, the general characteristic of ZDF rats - hyperphagia, obesity, hyperinsulinemia, and insulin resistance, applies only to a part of ZDFs. Obese rats with a genetic load for diabetes, that have not yet developed severe hyperglycaemia, have lower plasma activity of MMP-2 and MMP-9 compared with rats assigned to a group characterized by hyperglycaemia and lower insulin levels. However, increased myocardial MMP-2 activity indicates left ventricular remodelling in both ZDF phenotypes. The greater left ventricular weight, increase in carbonyl stress in the left myocardial tissue and the increase in MMP-2 activity in both ventricles of LEAN, could indicate myocardial remodelling in comparison with WIS. The inhomogeneity of ZDF rats may be beneficial in the study of different aspects of this pathology.

Keywords: diabetes mellitus, hyperglycaemia, gelatinases, oxidative stress, heart

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BENEFICIAL EFFECT OF HYDROGEN GAS ON THE HEART THAT HAS UNDERGONE SIMULATED HEART TRANSPLANTATION. POSSIBLE NEW THERAPEUTIC AGENT?

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Background: Acute or chronic heart failure patients often have to undergo a heart replacement from a donor to save their lives. The surgeons must connect the patient to the extracorporeal circulation (ECC), stop the heart activity, and resume the heart activity after replacing it with a new heart. During this procedure, ischemia/reperfusion and the overproduction of reactive oxygen species occur despite the progress in heart preservation and improved surgical techniques. Molecular hydrogen (H_2) is a novel substance with significant antioxidant and anti-inflammation capacity used in many experimental models and clinical trials on various diseases.

Methods: The main goal of this study was to examine the effect of molecular hydrogen on the heart allograft of pigs (female Přeštice black-mottled pig, 4 months old). The simulation of heart transplantation consisted of occluding venae cavae and pulmonary veins, cross-clamping of ascending aorta, and connection to ECC. Cold crystalloid cardioplegia (Custadiol) was administered for three hours. After the time of cold arrest, the coronary arteries were flushed with Plasmalyte solution and the aortic clamp was released. This was followed by rewarming the heart. After 60 minutes of reperfusion, the pig was detached from ECC and the experiment was terminated. H_2 was administered in gas form during oxygenation of blood and anesthesia (50% O_2, 3% H_2). In this study, levels and activities of oxidative disbalance markers, levels of inflammation, and expression of microRNAs (miRNAs) were measured from blood plasma and left ventricle tissues colorimetrically, by Western blot, and by qPCR methods.

Results: According to the results from this study, simulated heart transplantation significantly increases activities and amounts of endogenic antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and molecules of oxidative stress damage (uric acid, malondialdehyde, lactate dehydrogenase, 8-hydroxy-2-deoxyguanosine). Opposite of this, the application of H_2 gas significantly modulates all selected parameters almost up to control pigs. The positive effect of H_2 gas was observed in markers of inflammation damage like tumor necrosis factor alpha and nuclear factor-kappa B. The transplantation causes significant changes also in the levels of selected miRNAs where the H_2 treatment had normalization effects either.

Conclusion: We can conclude that the addition of H_2 during heart transplantation could be a new potential therapeutic strategy for minimalizing the negative effect of schemia/reperfusion injury, leading to better recovery of patients.

Keywords: heart transplantation, inflammation, miRNA, molecular hydrogen, oxidative stress

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THE EFFECT OF IRON OXIDE NANOPARTICLES ON VASCULAR FUNCTION OF THE FEMORAL ARTERY OF NORMOTENSIVE RATS

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Superparamagnetic iron oxide nanoparticles have the potential to be used in various biomedical applications including drug delivery and magnetic hyperthermia for the treatment of diverse carcinomas. The iron oxide nanoparticles are administered intravenously, thus their administration may influence the vascular tone regulation. The aim of our study was to investigate the effect of polyethylene glycol-coated Fe3O4 nanoparticles (IONs) on vascular functions of femoral arteries in Wistar-Kyoto rats. We investigated control and ION-treated rats (single dose 1 mg Fe/kg i.v., suspended in saline, 30 nm core size, ~51 nm hydrodynamic size). Vascular function was determined 100 min after ION infusion. Mean arterial pressure (MAP) and heart rate (HR) were not significantly different at the beginning of the experiment. The i.v. administration of IONs has not influenced MAP and HR at the end of experiment. KCl- and serotonin-induced contractions were not different between control and ION-treated group. Maximal acetylcholine (ACh)-induced and sodium nitroprusside (SNP)-induced relaxations in the femoral arteries did not differ among the groups. After NOS inhibition with Nω-nitro-L-arginine methyl ester (L-NAME) the serotonin-induced contraction was increased in control group, but not in ION-treated group. The L-NAME-sensitive component of ACh-induced relaxation was enhanced in ION-treated group suggesting enhanced production of NO in the femoral artery after ION application. On the other hand, the sensitivity of vascular smooth muscle cells to sodium nitroprusside was decreased in ION-treated group showing an adaptation of the vascular smooth muscle cells of femoral arteries to enhanced NO bioavailability. In conclusion, our results showed that single i.v. administration of IONs altered vascular function by preventing the increase of serotonin-induced contraction after NOS inhibition and by increasing the L-NAME-sensitive component of ACh-induced relaxation indicating augmented NO release from the endothelium in femoral arteries.

Keywords: iron oxide nanoparticles, femoral artery, endothelium

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DIFFERENCES IN DISTRIBUTION AND BIOLOGICAL EFFECTS OF POLYETHYLENE GLYCOL-COATED IRON OXIDE NANOPARTICLES IN NORMOTENSIVE AND HYPERTENSIVE RATS - FOCUS ON VASCULAR FUNCTION AND LIVER

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We investigated the distribution and biological effects of polyethylene glycol (PEG)-coated magnetite (Fe_3O_4@PEG) nanoparticles (~30 nm core size, ~51 nm hydrodynamic size, 2 mg Fe/kg/day, intravenously, for two consecutive days) in the aorta and liver of Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Fe_3O_4@PEG reduced the blood pressure (BP) of Fe_3O_4@PEG-treated SHR (SHRu) significantly, compared to both Fe_3O_4@PEG-treated WKY (WKYu) and saline-treated control SHR (SHRc). The Fe_3O_4@PEG content was significantly elevated in the aorta and liver of SHRu vs. WKYu.In WKY rats, USPIONs were observed in the elastic layers of the aorta, while in the aorta of SHR, the USPIONs were localized in both the elastic layers and smooth muscle cells. Nitric oxide synthase (NOS) activity was unaltered in the aorta, but significantly increased in the liver of SHRu vs. SHRc. In the aorta, Fe_3O_4@PEG treatment increased eNOS, iNOS, NRF2, and DMT1 gene expression (considered main effects). In the liver,Fe_3O_4@PEG significantly elevated eNOS and iNOS gene expression in SHRu vs. SHRc, as well as DMT1 and FTH1 gene expression (considered main effects). Noradrenaline-induced contractions of the femoral arteries were elevated, while endothelium-dependent contractions were reduced in SHRu vs. SHRc. No differences were found in these parameters in WKY rats. In the arterial segments,USPIONs reduced the endothelium-dependent contractionsafter both applications of ACh in SHRu vs. SHRc, while no differences were found in WKY. In conclusion, the results indicated that the altered haemodynamics in SHR affect the tissue distribution and selected biological effects of Fe_3O_4@PEG in the vasculature and liver, suggesting that caution should be taken when using iron oxide nanoparticles in hypertensive subjects.

Keywords: nanoparticles, blood pressure, vascular response

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EFFECT OF RENAL DENERVATION ON LEFT AND RIGHT VENTRICULAR FUNCTION IN TRANSGENIC HYPERTENSIVE RATS WITH HEART FAILURE INDUCED BY VOLUME OVERLOAD

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Mechanical and chemical removal of neural fibers around renal arteries (RDN) destructs both afferent nerve fibers that regulate central sympathetic nervous outflow and efferent fibers that regulate the renal renin-angiotensin system and neprilysin. RDN has known hypotensive effects and experimental data show that this therapy can prolong the survival rate in animal models of heart failure. However, the effect of renal denervation on the function of the right and left ventricle in the course of heart failure is still poorly known. The aim of the study was to determine the effect of renal denervation on the function of the right and left ventricle in hypertensive rats with volume overload induced by aorto-caval fistula (ACF).

Methods: In the first week of the experiment, heart failure was induced in Ren-2 transgenic rats (TGR), a model of angiotensin II-dependent hypertension by ACF at the animal age of 8 weeks. Bilateral renal denervation was performed chemically and mechanically in the second week of the experiment. Two weeks after RDN, the functions of the left and right ventricle were measured by simultaneous biventricular pressure-volume analysis, and the animals were examined using echocardiography each week. After in vivo experiments, animals were decapitated and organs were collected for molecular analysis.

RDN in ACF rats significantly reduced lungs congestion and both left and right ventricular hypertrophy. In sham-operated rats without heart failure, RDN had an antihypertensive effect, which was reflected in decreased maximum pressure in the left ventricle (by 13.43 mmHg, $P < 0.05$). In ACF rats, RDN decreased the diameter and filling volumes in both systole and diastole of the left ventricle after two weeks. RDN in ACF rats significantly reduced end-diastolic pressure (by 1.52 mmHg, $P < 0.05$) and maximum pressure (by 5.71 mmHg, $P < 0.05$) in the right ventricle but not in the left ventricle. RDN in ACF rats also improved contractility in both ventricles which was observed as an increase in parameters of systolic function ESPVR and PRSW. In ACF rats we observed increased levels of norepinephrine in the kidney and decreased levels in both ventricles of the heart compared with sham-operated rats. We also investigated the norepinephrine metabolizing enzyme monoamine oxidase A, which was shown to be increased in both ventricles of ACF rats compared to sham group. RDN in ACF rats significantly reduced renal norepinephrine levels and increased cardiac norepinephrine levels. Furthermore, we focused on the neprilysin signaling pathway in kidneys and observed inhibition of neprilysin activity after renal denervation in ACF rats.

In conclusion, our results showed cardioprotective effects of renal denervation in hypertensive transgenic rats with heart failure. Our data suggest that renal denervation could be a promising method in the treatment of both right and left ventricular heart failure.

Keywords: heart failure, hypertension, renal denervation

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INFARCT SIZE LIMITATION TRIGGERED BY EXCESS ISCHEMIC ARRHYTHMIAS IN HYPERTENSIVE RATS

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The inflammatory response plays a crucial role in the pathophysiology of many cardiovascular disorders, including acute myocardial infarction and post-ischemic heart failure. Previous research has shown that C-reactive protein (CRP), a protein of the acute phase of inflammation, enhances the extent of myocardial damage associated with ischemic heart disease. In humans, increased CRP production is a predictive marker for future coronary events, recurrent myocardial infarction, chronic heart failure, and cardiovascular death. To investigate the effect of increased levels of CRP on cardiac susceptibility to ischemia/reperfusion injury we used adult spontaneously hypertensive rats (SHR) with an expressed human CRP transgene (SHR-CRP), a unique animal model of chronic inflammation. We found that transgenic expression of CRP predisposed SHR-CRP to increased severity of ischemic ventricular arrhythmias in an in vivo model of coronary artery occlusion. The proarrhythmic phenotype in SHR-CRP was associated with altered heart and plasma eicosanoids levels, the myocardial composition of fatty acids in phospholipids, and autonomic nervous system dysbalance. Excessive ischemic arrhythmias in SHR-CRP led to a significant reduction in infarct size compared with SHR. To explain this unexpected finding, we performed a metabolomic analysis of plasma before and after ischemia. We also determined cardiac ischemic tolerance in hearts subjected to remote ischemic perconditioning and in hearts ex vivo. Acute ischemia increased plasma levels of multiple potent cardioprotective molecules that could reduce infarct size at reperfusion in SHR-CRP. Remote ischemic perconditioning provided an infarct size-limiting effect in SHR that was comparable with myocardial infarction observed in SHR-CRP. In hearts ex vivo, myocardial infarction did not differ between the strains, suggesting that extra-cardiac factors play a crucial role in protection. Our study shows that transgenic expression of human CRP predisposes SHR-CRP to excess ischemic ventricular tachyarrhythmias associated with a drop in pump function that triggers myocardial salvage against lethal ischemia/reperfusion injury mediated by protective substances released to blood from hypoxic organs and tissue at reperfusion. This new form of myocardial protection, i.e. the infarct size limitation triggered by the heart itself without any external intervention, may represent a more general phenomenon. Elucidation of its mechanism may be beneficial in the search for novel protective strategies against acute myocardial ischemia/reperfusion injury.

Keywords: myocardial infarction, ventricular arrhythmias, C-reactive protein, metabolomics, remote ischemic perconditioning

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SIGMA RECEPTOR AS A POTENTIAL TARGET FOR CARDIAC REMODELLING

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Although sigma receptors were originally proposed as a new subtype of opioid receptors, intensive research revealed that they represent unique ubiquitous receptors with several subtypes, specific tissue distribution and pluripotent functions. Primarily, they are located on endoplasmic reticulum membrane, where they function as ligand-operated molecular chaperones. In addition, they interact with numerous proteins in other organelles. In the heart muscle, type 1 of sigma receptor dominates. Sigma 1 receptor modulates calcium signalling in cardiomyocytes, regulates response to endoplasmic reticulum stress, and eventually affects function of voltage-gated ion channels. Since recent studies identify crucial role of cardiac sigma receptors in the development of cardiac hypertrophy and heart failure, their targeting may shed light into the processes of cardiac remodelling.

Keywords: sigma receptor, cardiac remodelling, social isolation

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DEVELOPMENTAL AND SEX DIFFERENCES IN CARDIAC TOLERANCE TO OXYGEN DEPRIVATION

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Age and sex play essential role in the cardiac tolerance to ischemia/reperfusion (I/R) injury: cardiac resistance significantly decreases during postnatal maturation and female heart is more tolerant as compared with the male myocardium. It is widely accepted that mitochondrial dysfunction and particularly mitochondrial permeability transition pore (MPTP) opening plays a major role in determining the extent of cardiac I/R injury. We have observed that the MPTP sensitivity to the calcium load differs in mitochondria isolated from neonatal and adult myocardium as well as from adult male and female hearts. Neonatal and female mitochondria are more resistant both in the extent and in the rate of mitochondrial swelling induced by high calcium concentration. Our data further suggest that age- and sex-dependent specificity of the MPTP is not the result of different amounts of ATP synthase and cyclophilin D (CypD): neonatal and adult hearts, similarly as the male and female hearts contain comparable amount of MPTP and its regulatory protein CypD. We can speculate that the lower sensitivity of MPTP to the calcium induced swelling may be related to the higher ischemic tolerance of both neonatal and female myocardium.

Keywords: neonatal heart, female heart, ischemia/reperfusion injury, cardiac ischemic tolerance, mitochondrial permeability transition pore

POTASSIUM CHANNELS AS POTENTIAL TARGETS IN PULMONARY HYPERTENSION COMPLICATING HEART FAILURE WITH PRESERVED EJECTION FRACTION (PH-HFpEF)

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Vascular remodeling in pulmonary hypertension (PH) includes the proliferation and migration of pulmonary artery smooth muscle cells, alterations in endothelial cell function and the activation of inflammatory cells. Endothelial and vascular K^+ channels are dysregulated in PH: certain K^+ channel types are downregulated, others are upregulated consistent with a more fetal phenotype in the remodeled pulmonary vasculature. Accordingly, K^+ channel dysregulation has been targeted by different pharmacological strategies in PH with the aim of restoring normal K^+ channel activities and vascular function. Nevertheless, to date, these efforts have not brought the expected clinical benefits. The hurdles of K^+ channel targeting can be illustrated through an example of ATP-sensitive K^+ (K(ATP)) channels, as recent data revealed that both LoF and GoF mutations in genes encoding subunits of K(ATP) channels can result in PH. Collectively, the widespread involvement of K^+ channels in cardiovascular and other organ functions makes it difficult to deliver selective therapies for the pulmonary circulation via K^+ channel activators. Levosimendan is a Ca^{2+}-sensitizer inodilator evoking prominent reductions in pulmonary capillary wedge pressure. K(ATP) channel activation is involved in the vasodilator effects of Levosimendan, nevertheless, complimentary contributions of additional K^+ channels (e.g. BKCa and Kv), endothelial NO-dependent and cAMP-related mechanisms have been also implicated. In short, Levosimendan is a potent vasodilator augmenting tissue perfusions in most organs and vascular beds, although Levosimendan sensitivities are not uniform within the cardiovascular system. Results of the HELP phase 2B study (where low dose, repeated Levosimendan administrations were employed in PH-HFpEF patients) included improvements in exercise capacity and reductions in systemic venous pressure as well as pulmonary venous resistance in the absence of changes in arterial resistances (both in the systemic circulation and in the pulmonary system). Hemodynamic data of the HELP study are in line with Levosimendan-induced selective venodilation and partial redistribution of the circulating blood volume from the arterial side to the venous side within the circulatory system. Taken together, currently available preclinical and clinical data are consistent with the proposal that restoration of dysregulated K^+ channel function is a potential strategy in treating PH. Nevertheless, the widespread involvement of K^+ channels in biological processes complicate selective K^+ channel targeting in the vasculature of the lungs. The apparent benefit of drug induced modulation of circulating blood volume distribution requires further investigations in PH-HFpEF patients.

Keywords: pulmonary hypertension, heart failure with preservedn ejection fraction, K+ channels, levosimendan, treatment

COMBINED THERAPY WITH SIMVASTATIN- AND COENZYME Q10-LOADED NANOPARTICLES AMELIORATES PI3K-Akt-eNOS PATHWAY IN EXPERIMENTAL METABOLIC SYNDROME

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Metabolic syndrome is a serious medical condition that increases the risk of heart disease, diabetes, stroke, and atherosclerosis. Statins are among the drugs of the first choice used to treat the symptoms of metabolic syndrome. Besides the LDL-cholesterol-lowering effect, statins have pleiotropic beneficial effects on the cardiovascular system. However, long-term treatment with statins may be associated with serious side effects. With the aim to streamline the statin therapy, we studied the effects of simvastatin- and coenzyme Q10-loaded polymeric nanoparticles on lipid profile and nitric oxide (NO)/reactive oxygen species (ROS) balance in the heart and aorta of adult male obese Zucker rats. The rats were divided into the untreated group, group treated with empty nanoparticles, and simvastatin-, or coenzyme Q10 (CoQ10)-, or a combination of simvastatin-loaded and CoQ10-loaded nanoparticles (SIMV+CoQ10). After 6 weeks, lipid profile was determined in the plasma and concentration of conjugated dienes in the liver. Akt, eNOS, phosphorylated eNOS (p-eNOS), NADPH oxidase, and NF-kappaB protein expressions were measured in the heart and aorta. All simvastatin, CoQ10, and SIMV+CoQ10 treatments decreased plasma LDL levels, but only the combined SIMV+CoQ10 treatment increased the expression of Akt, eNOS, and p-eNOS in both heart and aorta. Interestingly, NADPH oxidase in the heart and NF-kappaB protein expression in the aorta were decreased by all treatments, including nanoparticles alone. In conclusion, only combined therapy using simvastatin-loaded together with CoQ10-loaded nanoparticles ameliorated PI3K-Akt-eNOS pathway in obese Zucker rats. Enhancing the pleiotropic effects of simvastatin with the antioxidant properties of CoQ10 may increase the activating effect on PI3K-Akt-eNOS pathway and improve NO/ROS balance which may represent a promising tool for the treatment of cardiometabolic diseases.

Keywords: statins, polymeric nanoparticles, PI3K-Akt-eNOS pathway, ROS, cardiometabolic diseases

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EXTRACELLULAR MATRIX REMODELING UNDER PRESSURE OVERLOAD

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Pressure overload has server consequences not only for the cardiomyocytes but also for the extracellular compartment, leading ultimately to fibrosis and functional impairment including heart failure with preserved ejection fraction (HFpEF). During recent years various experimental and clinical studies have describe the process of remodeling at the level of the organ, the cell and the sub cellular compartments and defined the important players in this process of adaptation. In contrast little is known when it comes to unloading the heart- the so call reverse remodeling. We and others have conducted preclinical work that shall give an insight into this process at all three levels, the organ, cell and sub cellular compartment. The presented data will allow to speculate on fundamental principles of remodeling and reverse remodeling, using classic physiological examination combined with modern imaging and molecular biology. Finally, the gained preclinical information with be highlighted with respect to clinical data.

Keywords: remodeling, reverse remodeling, pressure overload

Funding:Ludwig Boltzmann Institute, Vienna, Austria

NON-INVASIVE „CONDITIONING“: POTENTIAL MECHANISMS OF ANTIISCHEMIC CARDIOPROTECTION

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Although ischemic preconditioning (IPC) is the most robust adaptive intervention protecting the heart of all animal species against ischemia/reperfusion (I/R) injury, its application in humans is limited to elective (planned) operations due to technical requirements, such as chest opening, short-term duration and unpredictable occurrence of AMI. However, some other forms of “conditioning” do not require an invasive intervention. Among those, pharmacological PC, hypoxia, remote PC (RPC), or exercise (EPC) also confer strong cardioprotection.

We explored whether preventive interventions applied *in vivo* increase cardiac resistance to I/R prior to AMI *ex vivo* using non-invasive approaches in the adult male Wistar rats: voluntary exercise (2 weeks) and RPC, second phase (24 h after application, 2-RPC). For EPC, animals were placed in cages equipped with wheels for free running, while control sedentary animals stayed in the cages without wheels. RPC was induced by 3 cycles of 5min inflation (200 mmHg)/5min deflation of pressure cuff on the hind limb.Resistance to I/R after both interventions was tested in the Langendorff-perfused hearts exposed to 30 min ischemia/2 hrs reperfusion, focused on changes in post-I/R recovery of function, reperfusion arrhythmias and extent of lethal injury (infarct size, IS/AR, TTC staining). In parallel groups, heart tissue samples were obtained for the investigation of the levels and activity of several proteins involved in “pro-survival” RISK cascade and pro- and anti-apoptotic cascades.

Echocardiography revealed lower BW and LV mass, PW width and HR, without changes in ejection fraction and in LV/BW index in “runners” and no changes after RPC. Both EPC and 2-RPC significantly reduced contractile dysfunction, IS/AR and incidence and severity of reperfusion arrhythmias. Protective effects were associated with a significant up-regulation of selected RISK proteins (PKB, PKC, eNOS), higher levels of SOD, HSP and reduction of proapoptoticcascades (BAX/Bcl-2, Caspase-3).

Conclusions: Beneficial effects of non-invasive forms of “conditioning”, sub-chronic free running and RPC, suggest their potential in the management of ischemic heart disease in clinical conditions. Potential mechanisms may involve activation of proteins of “pro-survival” cascades, antiapoptotic and antioxidative effects.

Keywords: ischemia/reperfusion, cardioprotection, preconditioning, adaptation

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EVIDENCE-BASED CARDIOVASCULAR MEDICINE: PERSPECTIVES AND DISAPPOINTMENTS

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Major clinical trials have changed cardiology over the last thirty years. Although no one doubts that evidence-based therapy saves lives, there is still plenty of room for uncertainty. First, the level of benefit achieved, although statistically significant, is in many cases low. The comparison with placebo is no longer acceptable because the first evidence-based treatment was introduced decades ago; thus it is difficult to achieve additional benefits at the current level of pathophysiological knowledge. Second, in line with the previous point, the overall reduction in mortality is relatively low and is not generally considered as a primary but only a secondary endpoint in the current studies. Instead, the main target is mostly combined endpoint, such as the sum of general and cardiovascular deaths, hospitalizations, fatal and non-fatal cardiovascular events. Third, in order to achieve the required statistical power, a very large number of patients need to be involved and follow-up extended from two / three years to five / ten years or even decades. This is not only expensive but also technically very difficult to implement. Fourth, trial studies are different from population monitoring. The true cardiovascular population consists of rather old patients, who suffer from a number of additional pathologic conditions (eg. mental, motor or oncological diseases), using a number of different drugs that potentially interfere with basic cardiovascular treatment, thus reducing the benefit compared to clinical trials. Given all these conditions in mind, the question arises: how will the future of evidence-based cardiovascular medicine evolve? Evidence from large and well-prepared clinical trials is undoubtedly the best weapon and cornerstone of therapy and will certainly dominate cardiovascular treatment in the near future. However, it should be borne in mind that the negative results of clinical trials will become more and more frequent over the successful trials. And the call for a personalized approach to patient management - "tailor-made treatment" will cease to be an empty slogan, but it could become the basic wisdom of every cardiologist.

Keywords: evidence-based medicine, clinical trial, population

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TRANSPLANTATION OF THE HEART. INNOVATIVE METHOD MITIGATING OXIDATIVE STRESS BY MOLECULAR HYDROGEN

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Introduction: Although heart transplantation becomes a routine method of treatment, ischemic reperfusion damage to the heart after cold ischemic storage and subsequent reperfusion is the most critical part in restoring heart function as a pump. After the cold-induced ischemic asystole and cold storage, the initial reperfusion with warm oxygenated blood, increases reactive oxygen species (ROS) including highly toxic nitrosyl and the •OH radicals. Anesthetics and hyperoxia used during anesthesia, has also been shown to be involved in ROS formation and may represent an independent mortality risk factor. Moreover, often needed electric shocks and repeated defibrillations cause significant cellular destruction in the ventricular myocardium, oxidative stress, which can complicate success of transplantation producing lipid peroxides and other oxidative stress products that damage the cardiomyocytes and interfere with production of ATP by mitochondria. The intracellular movement of calcium is leading to alterations in graft function, ventricular.

Methods: Administration of hydrogen gas may mitigate the injury by selectively reducing the hydroxyl radical, a primary mediator of I/RI, decrease lipid peroxidation (malondialdehyde), inflammation (TNF-α), decreased activity of native antioxidant enzymes (Superoxide dismutase, Catalase, Glutathione peroxidase), and the improved resumption of pumping activity of 3 hours cold ischemia stored and implanted pig heart.

Results: Hydrogen-treated swine exhibited significantly less severe ventricular fibrillation than controls, and improved histopathology findings. Addition of 4% inhaled hydrogen gas to inspiratory gases before the heart is taken from the donor, its cold storage, and after its implantation and subsequent warming up during its reperfusion, significantly decreased oxidative stress, markers of ischemia, inflammation, and peroxidation. Conclusion: With proper precautions, hydrogen may be administered safely through conventional ventilators, in ECC oxygenators and may represent a complementary therapy that can be easily incorporated into current transplantation technique.

Keywords: heart transplantation, molecular hydrogen, oxidative stress, inflammation, pigs

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PATIENT-SPECIFIC DERIVED CARDIOMYOCYTES: WILL WE BE ABLE TO PREDICT THE CARDIOMYOPATHIES IN THE DISH TOMORROW?

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Aims: Duchenne Muscular Dystrophy (DMD) is an X-linked disorder characterized by progressive muscle weakness due to absence of functional dystrophin. DMD patients developed dilated cardiomyopathy (DCM). In the past, we have demonstrated that aberrant sarcoplasmic reticulum (SR) calcium (Ca^{2+}) handling is an early pathophysiological feature in DMD mouse (mdx), DMD canine (GRMD) as well as in human CMs. We have also evidenced that abnormal intracellular Ca^{2+} cycling is related to early-stage pathological remodeling of the ryanodine receptor channel (RyR2) leading to age-dependent DCM.

Methods and Results: Here, we used hiPSC-CMs from DMD patients selected by Speckle-tracking echocardiography and canine DMD cardiac biopsies to assess key early-stage DMD-associated DCM features including the cellular defects associated with SR Ca^{2+} leak. Independently of the clinical observations from the 3 DMD patients, our data reveal that dystrophin-deficiency quickly induces RyR2 remodeling and Ca^{2+} leak. When evaluating the SR Ca^{2+} handling, we revealed that all DMD hiPSC-CMs exhibited aberrant Ca^{2+} transients, diastolic leak and elevated diastolic Ca^{2+} level. By evaluating the contractile properties, we found that RyR2 leak causes hypocontractility in DMD hiPSC-CMs. This force defect was associated with hiPSC-CM hypertrophy, sarcomere disorganization and fibrosis. Furthermore, we demonstrated for the first time that DMD hiPSC-CMs and GRMD cardiac biopsies exhibit a profile of premature senescence, with higher gene and protein expression of the senescence markers SA-β-gal, p15/p16, nuclear enlargement and cell hypertrophy. Preventing RyR2 dysfunction, by stabilizing its closed state conformation, improves the intracellular Ca^{2+} dynamics and restores normal contractility and prevents fibrosis development and senescence.

Conclusions: We revealed that cellular damages are established earlier than cardiac clinical pathology in DMD patients, with major perturbation of the cardiac ECC. We revealed RyR2 as an early biomarker of DMD-associated cardiac damages. The progressive and later DCM onset could be linked with the increased fibrosis and premature senescence, eventually causing cell death and further cardiac fibrosis in a vicious cycle leading to further hypocontractility as a major feature of DCM. Finally, our work reinforces the interest of using hiPSC-CMs as an optimal tool to dissect molecular mechanisms of the early stage of dystrophin deficiency leading to the development of DMD-associated-DCM.

Keywords: DMD, hiPSC-derived cardiomyocytes, ryanodine receptor, calcium, senescence

Funding:AFM

PHARMACOLOGICAL CARDIOPROTECTION AGAINST CHRONIC ANT CARDIOTOXICITY – TOPOISOMERASE II BETA TARGETING AND BEYOND

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Anthracycline (ANT) cardiotoxicity resulting in cardiomyopathy and heart failure continues to be a significant issue for current clinical cardio-oncology. The only drug approved for protection of the heart against ANT toxicity is a bisdioxopiperazine derivative dexrazoxane (DEX). It has long been believed to act through metal chelating effects of its metabolite, which should prevent iron-catalyzed oxidative stress, but recently published data from our laboratory has refuted this hypothesis using direct experimental evidence. We have shown instead that the parent DEX molecule acts as a catalytic inhibitor of topoisomerase II beta (TOP2B), which prevents poisoning of the enzyme by ANTs in the cardiomyocytes and thereby overcomes ANT-induced DNA damage and DNA damage signaling in the heart. The validity of the TOP2B hypothesis has been supported by our numerous in vitro and in vivo experiments with DEX and its derivatives. The DEX derivatives, which were ineffective as catalytic inhibitors of TOP2B, lacked a cardioprotective potential in the chronic cardiotoxicity model despite even very minor changes in chemical structure and preserved iron chelating activity. Bisdioxopiperazine derivatives, more potent than DEX as TOP2B catalytic inhibitors, were also more potent as cardioprotectants. These cardioprotective effects correlated closely with the ability of these DEX derivatives to prevent p53-mediated DNA damage response (DDR) signaling in the heart induced by a single clinically relevant ANT dose. Furthermore, we have demonstrated that ACE inhibitors, which do not prevent TOP2B-dependent and p53-mediated DDR, can provide only temporal, but not long-lasting cardioprotection against chronic ANT cardiotoxicity, which contrasted with effects obtained with DEX on the same model. In addition, we observed that a selective inhibition of ATM, a key molecule located at the apex of DDR, can also blunt the ANT-induced DDR signaling in the heart. However, the addition of the ATM inhibitor to the chronic ANT treatment had an opposite impact on the ANT cardiotoxicity development than DEX – it augmented the severity of ANT-induced cardiotoxicity in the same experimental model. In conclusion, while the catalytic inhibition of TOP2B with DEX and its derivatives is a promising cardioprotective strategy, the ATM inhibition blocking downstream DDR signaling seems to have a rather detrimental impact on chronic ANT cardiotoxicity development.

Keywords: Cardiotoxicity, cardioprotection, topoisomerase II beta, DNA damage response, anthracyclines

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MECHANISMS OF THE RyR2R420Q CPVT MUTATION. LESSONS HUMAN CARDIOMYOCYTES DERIVED FROM INDUCED-PLURIPOTENT STEM CELLS

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Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) is an inherited disease manifested as syncope or sudden death in apparently healthy children or young adults. The RyR2 R420Q mutation was identified in a 14-year-old boy who died suddenly due to emotional stress. Firsts analyses in RyR2 R420Q in heterologous systems, showed loss of function at high $[Ca^{2+}]i$ and gain of function at low $[Ca^{2+}]i$, and when this mutation was present in mice, the Ca^{2+} sparks in pacemaker cells were markedly prolonged. We propose to elucidate the $[Ca^{2+}]i$ handling in cardiomyocytes derived from pluripotent stem cells from patients with CPVT.

We differentiated human induced-pluripotent stem cells into cardiomyocytes (h-iPS-CM) from men and women of the mentioned family, ones exhibiting the mutation RyR2R420Q and others without the mutation used as control. First, we measured the action potentials (AP) with microelectrodes and found that woman CPVT h-iPS-CMs presented longer cycle length and increased AP amplitude whereas man CPVT h-iPS-CMs showed smaller cycle length and similar AP characteristics. Then, we found that Ca^{2+} transients amplitude were higher for woman CPVT cells compared to control cells, whereas no changes were found for man h-iPS-CMs; however, Ca^{2+} transients were similar after ISO perfusion. SR Ca^{2+} load presented no differences between woman groups but the time decay constant of caffeine-evoked Ca^{2+} transients were significantly faster in woman CPVT h-iPS-CMs. In contrast, man CPVT h-iPS-CMs presented reduced SR Ca^{2+} load compared to control cells. In addition, analysis of Ca^{2+} pro-arrhythmogenic events were significantly augmented in CPVT h-iPS-CMs compared to wt h-iPS-CMs both in man and woman cells and it was accentuated after ISO perfusion. The RyR2 R420Q mutation in CPVT h-iPS-CMs represents well the Ca^{2+} handling characteristics seen in patients and provides a reliable model to study CPVT in human context.

Keywords: arrhythmias, cardiomyocyte, calcium handling, iPS-CM, ryanodine Receptor, CPVT

Funding:ANR

CITRUS ALKALOIDS MAY ENHANCE PROARRHYTHMIC RISK

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Citrus fruits like lemon, orange or grapefruit are part of the Mediterranean diet and are generally considered beneficial, and as such can increase life expectancy. Important constituent of citrus fruits are the flavonoids. Many studies indicated that flavonoids have anti-ischaemic or anticancer effects. It was however, also reported that consumption of grapefruit and orange juice increase the time of cardiac repolarization manifested as lengthening of the QTc interval in the surface ECG recordings. In addition, grapefruit consumption inhibits important enzyme playing role in the metabolism of several drugs including those which themselves prolong QTc (terfenadine) and were reported that occasionally elicited severe arrhythmias sometimes resulting sudden cardiac death, The exact reason of the effect of the citrus fruits on cardiac repolarization and possible proarrhythmic complication are not well explored and still matter of debate. Some limited number of studies reported that naringenin and hesperidin two alkaloids present relatively high concentration in orange and grapefruit inhibited HERG potassium current in mammalian cell lines, but solid evidence is still missing how these alkaloids affect native cardiac ion channels and action potentials. In this presentation the author makes an attempt to briefly overlook the most important data can be find in the literature in this topic and show some preliminary unpublished data regarding the cellular cardiac electrophysiologic effect of hesperidin on ventricular action potential in dog right ventricular preparation obtained by the conventional microelectrode technique. In addition, by applying the whole cell configuration of the patch-clamp technique effects of hesperidin will be also presented in ventricular myocytes on the rapid and slow delayed rectifier outward potassium currents.

Keywords: proarrhytmia, repolarization reserve, citrus flavonoids, naringenin, hesperidin

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EFFECT OF RyR GATING ON ELEMENTARY CALCIUM RELEASE OF CARDIAC MYOCYTES

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Calcium release in cardiac myocytes is provided by ryanodine receptors (RyRs) clustered in calcium release sites (CRSs) of dyads. The physiological trigger for RyR activation is an increase in the local Ca^{2+} concentration. In the absence of elevated [Ca^{2+}], RyR opening frequency is extremely low due to tonic inhibition of the RyR by Mg^{2+} ions that compete with Ca^{2+} ions at the RyR activation site and also directly inhibit RyR at the inhibition site [1]. Of importance are indications that RyR dysfunction in some pathophysiological states may be due to changes in the regulation of RyR activity by Mg^{2+} [2].

To elucidate the effect of Ca^{2+} and Mg^{2+} binding on RyR activity, and the consequences it has on the formation of calcium release events (CREs), we developed a mathematical model of RyR gating that incorporated the allosteric interaction between the binding of Ca^{2+} or Mg^{2+} to the RyR activation site and channel opening, as well as the inhibition by Mg^{2+} at the RyR inhibition site. The parameters of the model were extracted from published experimental data and tested by simulations [3]. Changes in Mg^{2+} binding had a prominent effect on RyR open probability, mean open time, and mean closed time, as well as on the rate of activation. Mg^{2+} acted as a strong inhibitor of RyR opening, and a change in Mg^{2+} binding and unbinding rates had a profound effect on RyR gating kinetics, open probability, and rate of activation.

The RyR gating model was inserted into an in silico model of the calcium release site, based on a quantitative description of RyR placement and calcium diffusion. Calcium release events (CREs) were simulated at different strengths and kinetics of Mg^{2+} binding to RyRs and analysed [3]. The characteristics of CREs reacted sensitively and specifically to changes in the effective coupling strength, a parameter that jointly characterized RyR placement, Ca^{2+} flux, and RyR gating. Increased Mg^{2+} unbinding rate from the RyR activation site and decreased Mg^{2+} binding rate to the RyR inhibition site contributed the most to the increased effective coupling strength and the increased frequency of spontaneous sparks.

The results of simulations revealed the role of Mg^{2+} ions as a protector of the CRS from spontaneous activation in the absence of an external stimulus. This finding is of principal physiological importance since excessive calcium release may cause not only arrhythmias but also glucose intolerance and neuronal disorders [4].

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Keywords: calcium release, cardiac myocyte, ryanodine receptor, calcium release site, mathematical model

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HOW CELLS ARE ABLE TO ELIMINATE MITOCHONDRIA PRODUCING TOO MUCH REACTIVE OXYGEN SPECIES (ROS)?

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Although low ROS levels can be beneficial in normal physiological functions, the excessive ROS accumulation, often generated by defective mitochondria, can lead to cell oxidative damage. This is particularly important for cells like cardiomyocytes or neurons, which are very rich in mitochondria and where tissue regeneration is almost absent. Mitophagy is essential to remove "worn-out" mitochondria and to improve the mitochondrial population quality. It is well known how depolarized mitochondria that have reached the end of their life cycle are removed by Pink1/Parkin-dependent mitophagy. However, inner membrane depolarization is not the only prerequisite for mitophagy. Dysfunctional mitochondria could keep their membrane potential, and this potential favors excessive ROS production damaging the host cell. Thus, to survive, the cell should have a mechanism(s) to remove these internal killers, which are not yet depolarized. The present study unravels a mechanism of KEAP1-dependent mitophagy induced by even moderate cell ROS level.

KEAP1 is one of the primary cellular ROS sensors. We tested various proteins that possess the KEAP1 binding site and were known to interact with or stabilize PINK1. Among them, only mitochondrial phosphatase PGAM5 did accumulate in response to moderate mitochondrial ROS production and, at the same time, induced PINK1/PRKN dependent mitophagy.

PGAM5 is found in cells in two forms: a full-length and a short form. After being imported into mitochondria, PGAM5 is either inserted into the mitochondrial outer membrane (full-length form) or cleaved by the inner mitochondrial membrane (IMM)-resident proteases and then released back to the cytosol (short form). Only the full-length PGAM5 was co-immunoprecipitated with KEAP1, suggesting that KEAP1, being localized only in the cytosol, should interact with PGAM5 before it is imported to the mitochondria or/and when it is already inserted into the outer mitochondrial membrane. To dissect these two possibilities, we overexpressed fluorescently tagged PGAM5 and followed its fate in cells. Although the fluorescent PGAM5 was normally localized in the mitochondria, it started to accumulate in the cytosol when its proteasomal degradation was blocked. Western blot analysis demonstrated that it was specifically the full-length form of PGAM5 accumulating in the cytosol in response to proteasomal inhibition. Taken together, this suggests that KEAP1 controls the degradation of cytosolic full-length PGAM5 before it is inserted into the outer mitochondrial membrane.

We speculate that PGAM5 might directly affect PINK1 processing. PINK1 and PGAM5 are both cleaved by the same IMM-resident proteases and competition between the substrates would favor PINK1 stabilization (followed by Parkin recruitment) when ROS favor PGAM5 accumulates. This mechanism serves as fine-tuned feedback allowing cells to sense when the quality of their mitochondrial population is declining and to trigger mitophagy.

Keywords: mitochondria, ROS, mitophagy, membrane potential

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CONSEQUENCES OF CHRONODISRUPTION ON THE CIRCADIAN CONTROL OF CARDIOMETABOLIC PROCESSES

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Circadian rhythms allow organisms to predict regular changes in the environment and prepare to them in advance. They are important for the efficient functioning of the cardiovascular system and metabolism, which both exhibit pronounced daily rhythms. Artificial light at night (ALAN) is a new anthropogenic factor, increasing dramatically recently with possible adverse effects on health. Therefore, we explore the effects of ALAN on daily rhythms in cardiovascular parameters, selected metabolites, hormones, and clock and clock-controlled genes to evaluate the underlying mechanisms.

We used normotensive and spontaneously hypertensive rats (SHR), which were exposed either to the control 12/12 light/dark cycle or to dim light (~2 lux) at night (ALAN) for 2 and/or 5 weeks. Bodyweight, food and water consumption were monitored, and metabolites and hormones were measured in plasma. Blood pressure (BP) and heart rate (HR) were measured continuously by radiotelemetry (DSI, USA) and circadian oscillations were evaluated. The expression of genes involved in the control of metabolism was measured in the brain, liver and abdominal fat over 24 h. In the central oscillator, suprachiasmatic nucleus (SCN) of the hypothalamus, we found the attenuated clockwork after ALAN exposure, because circadian rhythms of several clock genes had lower amplitude or were lost. Importantly, the rhythmic pattern of vasopressin, the dominant neurotransmitter and an output signal of the SCN, was suppressed in the SCN and eliminated in the circulation, which was reflected in disturbances of drinking behavior. Glucose and lipid metabolism is under strong circadian control and therefore, we determined daily rhythms of plasma glucose, triacylglycerols, cholesterol and metabolic hormones, and the expression levels of genes involved in the control of metabolism. All three plasma metabolites lost their rhythms after 2 weeks of ALAN. Moreover, melatonin rhythm, which transmits information about the natural night over the internal milieu of the body, was lost and the plasma corticosterone rhythm was suppressed and phase-advanced after ALAN exposure. These complex changes can have serious consequences on the timing of physiological processes and stress response, with negative consequences on health. Further, ALAN attenuated insulin sensitivity in SHR rats, which are genetically insulin resistant, therefore diabetic patients might be more sensitive to chronodisruption than healthy individuals. Moreover, rhythmic response of BP and HR to the norepinephrine challenge was attenuated by ALAN, which enhanced the pressor response after 5 weeks of exposure.

In conclusion, functioning of the circadian system was compromised by ALAN, showing the impact on the cardiovascular system and metabolism. Chronodisruption can have more pronounced consequences for hypertensive individuals and therefore comorbidities should be considered when consequences of light pollution on human health are evaluated.

Keywords: circadian, daily, melatonin, corticosterone, clock

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CARDIOVASCULAR CHANGES IN RATS WITH LPS-INDUCED LUNG INJURY

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The study aimed to evaluate heart rate variability (HRV) as an index of cardiac autonomic control in rats with lipopolysaccharide (LPS)-induced lung injury. Anaesthetized rats (Wistar, n=20, 320±10g) were intratracheally instilled with bacterial lipopolysaccharide (LPS, 500 μg/kg b.w., 2.2 ml/kg, E. coli, O55:B5) to induce lung injury. Controls received saline (2.2 ml/kg). Animals were mechanically ventilated with frequency of 60/min, a fraction of inspired oxygen (FiO2) 0.4, inspiratory time 40%, tidal volume of 6ml/kg. Lung injury was specified as reduction of dynamic compliance >30% or decrease of a ratio between paO2 and FiO2 <40 kPa. ECG recordings were done before and 30, 60, 120, 180 and 240 min after LPS or saline administration. HRV magnitude was quantified by time and frequency-domain analysis (mean RR interval, SDRR, RMSSD, spectral powers in low (LF) and high frequency (HF) bands. After 4 hrs of artificial ventilation, inflammatory markers, galectin-3 and oxidative stress in homogenized heart and lactate in plasma were evaluated. Increased plasma lactate and oxidative stress parameters were found in LPS rats. Pro-inflammatory markers IL-1β, IL-5, IL-12p70 and anti-inflammatory IL-13 were increased after 4 hrs from LPS administration. Galectin-3 concentration raised in LPS animals compared to controls. HRV analysis did not exhibit any significant change, but tendency to decrease in HRV magnitude was present in rats with LPS-induced lung injury. Absence of correlation between HRV and hyper-cytokinemia might suggests pleuripotency of these cytokines possessing both pro-inflammatory and anti-inflammatory features at the same time.

Keywords: heart rate variability, lung injury, lipopolysaccharide, cytokine

MILD COLD ACCLIMATION AS A NEW CARDIOPROTECTIVE INTERVENTION

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Despite the progress in research and therapy, ischemic heart disease remains the most common cause of death and comorbidity worldwide. Moreover, many promising targets that have been demonstrated in animal models have failed in clinical trials (1). Recently, we demonstrated a unique cardioprotective model of mild cold acclimation (CA, 8±1 ºC, 5 weeks) that reduced the extent of myocardial infarction, improved mitochondrial resistance to Ca^{2+}-overload, and persisted for another 2 weeks after recovery (CAR). Under these conditions, the negative side effects such as hypertension and hypertrophy were excluded(2,3) as the temperature was set just below the threshold of shivering thermogenesis in rats and below the threshold of cold-induced hypertension (4). Three subtypes of β-adrenergic receptors (β-ARs) are expressed in the left ventricular myocardium in the following ratio: β1-ARs (~70%), β2-ARs (~27%), and β3-ARs (~3%). All three β-ARs can activate Gαs. β1-ARs are only coupled to the stimulatory G protein (Gs) and are required for hormone-stimulated cAMP generation by adenylyl cyclase. The major target protein stimulated by cAMP is cAMP-dependent protein kinase A (PKA). We found that the CA-elicited cardioprotective phenotype is not sensitive to metoprolol administration, preserves the function of adenylyl cyclase signalling and the potential role lies in the upregulated β2/β3-AR pathways. Further study revealed the cardioprotective effect of CA and that persisting for 2 weeks CAR engages in different mechanisms. The inhibitory Giα1/2 and Giα3 proteins were upregulated and the protein kinase B(Akt) was activated only in the CAR group. Acute administration of β2-AR inhibitor ICI-118551 abolished the protective effect in the CAR group but had no effect in the control and CA groups. Then we wondered, what is the shortest time of the cold exposure to achieve an improvement in cardiac ischemic tolerance. Based on preliminary data, we chose 1-3-10 days of the cold exposure and characterized the time course of brown adipose tissue activation (mitochondrial biogenesis, AMPK activation, UCP1, and FGF21 levels), inflammatory markers, and myocardial responses at physiological and cellular levels to determine the principle of cold-elicited cardioprotection at the early stages of the acclimation.

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Keywords: cardioprotection, mild cold acclimation, adrenergic signalling

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Abstracts of posters presentations

MiRNAs PROFILING OF CHRONIC ANTHRACYCLINE-INDUCED CARDIOMYOPATHY IN RABBITS

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MicroRNAs are small, non-coding RNA molecules involved in regulation and fine-tuning of gene expression. The present study aims to determine changes in miRNAs on the well-established experimental model of chronic anthracycline (ANT) cardiotoxicity at two distinct stages of cardiotoxicity development. Cardiotoxicity was induced in rabbits treated with daunorubicin (DAU, n=9; 3 mg/kg, weekly; for 5 and 10 weeks) and compared with the control (n=7, saline in the same schedule). The 1st analysis was done after the five DAU cycles (cumulative dose ~250 mg/m2) when we found first signs of cardiotoxicity, i.e., significantly increased levels of plasma cardiac troponin T (cTnT 0.018±0.003 μg/L vs. 0.006±0.001 μg/L; $p< 0.001$), but yet without any change in LV systolic function. Histological examination revealed only minor degenerative changes in cardiomyocytes without distinct fibrosis. The 2nd analysis was performed after the ten DAU cycles (cumulative dose ~500 mg/m2) which induced significant LV systolic dysfunction (FS 41.2 ± 0.4 % vs 29.0 ± 2.9 %; $p<0.001$ and dP/dtmax 8714 ± 275vs 5341 ± 499 mm Hg; $p<0.001$) and typical histopathological hallmarks of chronic ANT cardiotoxicity.

Based on results obtained from TaqMan® Advanced miRNA Human A and B Cards we selected 32 miRNAs for confirmation by Real-time PCR with specific assays (TaqMan® Advanced miRNA Assay systems). After 5 weeks, 10 miRNAs were significantly up-regulated: miR-let-7f-2-3p ($p<0.05$), miR-20b-5p ($p<0.05$), miR-21-3p ($p<0.05$), miR-21-5p ($p<0.05$), miR-34a-3p ($p<0.001$), miR-34a-5p ($p<0.001$), miR-34c-5p ($p<0.01$), miR-142-3p ($p<0.05$), miR-155-5p ($p<0.001$) with dominant change in miR-1298-5p (29-fold change, $p<0.01$) related to VSMC. The best correlation was found between cTnT and miR-155-5p (0.82; $p<0.001$), reflecting both DNA repair and the activation of macrophages; and cTnT and miR-34a-5p (0.765; $p<0.001$), which is related to p53-mediated DNA damage signalling. After 10 weeks only miR-504-3p ($p<0.01$) was significantly down-regulated and 11 of miRNAs were significantly up-regulated: miR-21-3p ($p<0.01$), miR-21-5p ($p<0.001$), miR-34a-3p ($p<0.01$), miR-34a-5p ($p<0.001$), miR-34c-5p ($p<0.001$), miR-142-3p ($p<0.01$), miR-155-5p ($p<0.001$), miR-223 ($p<0.001$), miR-433-3p ($p<0.05$), miR-1298-5p ($p<0.001$) with the dominant change in 34a-5p (76-fold change). Most of miRNAs measured after 10 weeks of the treatment very significantly positively correlated with cTnT and negatively with parameters of systolic dysfunction (LVFS and dP/dtmax). The best correlation has been achieved between miR-21-5p and LVFS and dP/dtmax, respectively and (-0.959; $p<0.001$, resp. -0.890; $p<0.001$) and miR-223-3p (-0.911; $p<0.001$; resp.- 0.803; $p<0.001$), which are probably involved in the alteration of cross-bridge cycling and fibrosis.

To our knowledge, this is the first study describing the changes of miRNAs profile in chronic ANT cardiotoxicity with precisely defined stages of cardiomyopathy development.

Keywords: anthracyclines, cardiotoxicity, miRNA, cardiac troponin, systolic dysfunction

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DISTINCT MYOCARDIAL CONNEXIN-43 ALTERATION DUE TO CARDIAC HYPERTROPHY AND ATROPHY IMPACT THE VULNERABILITY OF THE HEART TO MALIGNANT ARRHYTHMIAS

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Heart diseases related myocardial structural remodelling deteriorate heart function as well as increases susceptibility of the heart to malignant arrhythmias. One of the key factor involved in development of malignant arrhythmias are abnormalities of connexin-43 (Cx43) channels that ensure electrical coupling among cardiomyocytes to allow action potential propagation and synchronised contraction.

We aimed to explore whether myocardial Cx43 protein expression and its topology differ in hypertrophied and atrophied heart as well as the impact of Cx43 changes on the susceptibility of the heart to develop ventricular fibrillation (VF).

Experiments were performed using male, 2-4-month-old rats. Hypertrophied left ventricles of the of spontaneously hypertensive rats (SHR) and hyperthyroid rats (TH) as well as in atrophied left ventricle of diabetic rats (DM) and hypothyroid (HY) rats were analysed. Cx43 topology was examined using immunofluorescence labelling, while western blotting was used to determine Cx43 protein levels and its phosphorylated status related to PKC-ε. Isolated perfused heart was used to test its vulnerability to electrically inducible VF.

Comparing to healthy controls, the left ventricular hypertrophy was associated with increased while the ventricular atrophy with decreased susceptibility of the heart to electrically inducible VF. Total Cx43 levels and its functional phosphorylated forms along with PKC-ε were decreased in hypertrophied myocardium while increased in atrophied ones. Moreover, there was pronounced pro-arrhythmic localisation of Cx43 on lateral sides of hypertrophied cardiomyocytes of SHR and TH rat hearts, while not in atrophied cardiomyocytes of HY and DM rat heart.

Findings suggest that down-regulation of Cx43 and its altered topology in hypertrophied myocardium increase susceptibility of the heart to malignant arrhythmias. In contrast, up-regulation of Cx43 and its normal topology in atrophied myocardium may hamper development of life-threatening arrhythmias.

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DICHLOROACETATE AND REDUCED OXYGEN UTILIZATION IN THE HEART: REGULATION OF THE MITOCHONDRIAL PROTEOME

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Myocardial compensatory mechanisms stimulated by reduced oxygen utilization caused by streptozotocin-induced diabetes mellitus (D) and treated with the metabolic modulator dichloroacetate (DCA) are presumably associated with the regulation of mitochondria. Processes of inhibiting mitochondrial permeability transition pores (mPTP) opening lead to the maintenance of adequate ATP production and so represent an essential and beneficial cardioprotective strategy. Here we aimed to expand knowledge about key signaling pathways linked to regulation of mPTP opening, ROS and calcium signaling in the conditions of D treated with the DCA.

Isolated Wistar rat heart mitochondria were used for fluorescence-spectroscopy measurements and quantitative label-free LC-MS/MS proteomic analysis focused on the proteins forming and regulating mPTP complex and proteins linked to ROS production. The acute 8-day D model (65 mg/kg streptozotocin i.p.) was exposed to DCA administered to animals (150 mg/kg and 75 mg/kg i.p.) 60 minutes and 15 minutes before heart excision.

We revealed significantly upregulated protein amine oxidase [flavin-containing] A (AOFA) in D mitochondria, indicative of oxidative damage. DCA in diabetic animals (D+DCA) downregulated AOFA and stimulated thioredoxin-dependent peroxide reductase, a protein with antioxidant function. D group exhibited stimulation to the highest aggregated abundance of the mPTP proteins. Furthermore, the D condition was associated with mitochondrial resistance to calcium-overload through mPTP regulation, despite an increased protein level of voltage-dependent anion-selective protein (VDAC1). In contrast, D+DCA influenced ROS levels and downregulated VDAC1 and VDAC3 when compared to D alone.

Characterization of the combined effect of D+DCA is a novel finding showing that DCA acted as an effector of calcium uptake regulation and ROS production. Overall, the achieved results expanded the available knowledge about mitochondrial signaling pathways in the rat heart that can lead to cardioprotection during reduced oxygen utilization induced by D.

Keywords: cardiac mitochondrial proteome, cardioprotective signaling, reduced oxygen utilization, mPTP, dichloroacetate

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THE VASOACTIVE EFFECT OF HYDROGEN SULFIDE DONOR AND CHRONIC FRUCTOSE INTAKE IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hydrogen sulfide (H_2S) represents an important gaseous transmitter which could interfere with etiopathogenesis of different cardiovascular and metabolic diseases. However, H_2S in interaction with nitric oxide (NO) may also trigger compensatory vasoactive effects to counterbalance pathologically increased vascular tone in both arterial hypertension and metabolic syndrome. We aimed to study the role of sulfide signal pathway in vasoactive responses of mesenteric artery (MA) in spontaneously hypertensive rats (SHR) fed with fructose.

12 weeks-old SHR were divided into three groups: control rats, rats treated with 10% fructose in drinking water for 8 weeks and rats treated with fructose and during last three weeks with H_2S donor, GYY-4137 (266 µg/kg/day, i.p.). Vasoactivity of MA was recorded as changes of isometric tension. H_2S inhibition was performed by acute incubation with DL-propargylglycine (PPG, 10 mmol/l). Acute incubation with NG-nitro-L-arginine methyl ester (L-NAME, 10-5 mol/l) was used for inhibiting of NO production.

The chronic fructose intake significantly increased plasma level of triacylglycerols and the body adiposity, expressed as retroperitoneal fat weight to tibia length ratio. H_2S donor did not affect these parameters. The SBP was increased in fructose-fed rats, however 3-week-long treatment with GYY-4137 decreased the SBP, even to a lower level than in SHR. We observed that fructose intake enhanced endothelium-dependent vasorelaxation and decreased adrenergic contraction of MA, along with the sensitivity to noradrenaline remained unchanged. While GYY-4137 administration did not significantly affect vasorelaxant responses, it partially restored reduced contractility in fructose-fed rats.The acute pretreatment with PPG, enhanced endothelium-dependent vasorelaxation in control un-treated SHRs only. The acute pretreatment with L-NAME, inhibited the vasorelaxant response in all groups, however significantly more in treated compared to control rats.

Our results suggest that the sulfide signaling pathway could take a significant role in modulation of mesenteric vasoactive properties of hypertensive rats with metabolic disorder: a) endogenously produced H_2S could, probably in interaction with NO signaling, trigger compensator effects to maintain endothelial function; b) slow H_2S releasing donor could recover altered contractility.

Keywords: Hydrogen sulfide, Nitric Oxide, Metabolic syndrome, Spontaneously Hypertensive Rats

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CALCIUM TRANSIENTS IN CARDIOMYOCYTES OF SEDENTARY AND ACTIVE RATS

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The key process of myocardial function is the excitation-contraction coupling (ECC) by which the electrical excitation triggers mechanical contraction of the heart. The coupling agent in cardiac ECC are calcium ions, which also regulate the contraction of myocytes. Therefore, the final performance of cardiac muscle depends on the proper balance of all these components.

Physical exercise influences the cardiac output in all mammals [1]; however, the exact mechanisms are not sufficiently understood neither in physiological nor in pathological conditions. We inspected differences between the sedentary and voluntarily running rats in kinetic parameters of calcium transients in isolated cardiomyocytes. The rats were housed in individual cages with or without access to a running wheel. After two weeks, animals were euthanized, and left ventricular cardiomyocytes were isolated using standard procedures [2]. Changes in the intracellular calcium concentration were recorded using laser scanning fluorescence confocal microscopy in the line scan mode as transient changes in fluorescence of the calcium-sensitive dye Fluo-3. Recorded cardiomyocytes were field stimulated at frequency of 1.0 Hz for about 60 seconds and then recorded for further 20 seconds. The amplitude ($\Delta F/F0$) and the kinetic parameters of individual calcium transients in the recorded traces - the time to peak (TTP), the rise time (20-80%), the decay time (80-20%), and the duration at half-amplitude (FDHM) - were determined from fluorescence signals using custom software. The software identified individual transients, fitted their time course, and reported the parameters in a sequence of automatic algorithms. The verification on theoretical model traces provided reliable estimation of the model parameters in a broad range of signal-to-noise ratios.

Preliminary analysis of the dataset of 8 cardiomyocytes from two animals revealed a tendency for acceleration in all kinetic parameters of calcium transients in cardiomyocytes of rats with access to voluntary running. The distribution of the parameter estimates showed occasional deviation from the normal distribution and a large variation between individual myocytes isolated from the same animal. The parameter FDHM was distributed normally, with a significantly shorter duration in the exercising group.

These preliminary results suggest that even two weeks of voluntary exercise may affect parameters of calcium signalling in the rat ventricular myocytes, although the final conclusion will need verification and expansion of the recorded dataset. Analysis of further data is ongoing.

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Keywords: cardiac myocytes, calcium transients, voluntary exercise

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HIGH CONCENTRATION OF URIC ACID DID NOT AFFECT ENDOTHELIAL FUNCTION OF VARIOUS - SMALL, MEDIUM-SIZED AND LARGE ARTERIES FROM AGED WKY RATS

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Aim: The long-term increase in uric acid (UA) concentration represents a new cardiovascular risk factor constantly attracting scientific attention. It is however still not clear whether UA is a causative risk factor in endothelial dysfunction, an inert marker, or an endothelium-protective molecule concerning its antioxidant properties. Nevertheless, the minority of studies have been conducted to investigate its effect on vascular endothelium-dependent relaxation. Therefore, in our in vitro study, we focused on the effect of high concentrations of UA on arterial endothelial function of old normotensive rats.

Methods and results: For the study, male Wistar-Kyoto (WKY) rats were used. The acute effect of UA was analyzed by vascular reactivity measurements, using in vitro isometric tension studies by wire myograph - small mesenteric arteries (SMAs) and femoral arteries (FAs), and using organ chamber - aorta. Endothelium-dependent vasorelaxation using acetylcholine (ACh) test was determined before and after 1-hour incubation of isolated arteries with UA: 600 μmol/l (arteries from 68-week-old rats) and 1200 μmol/l (57-week-old). 20-week-old WKY were used as the corresponding control to aged animals. Impaired endothelial function was determined in FAs and aortas from old (57- and 68-weeks) WKY as compared with arteries from young 20-weeksold rats. On the other hand, we did not observe any acute effects of high concentrations of UA on endothelial function. ACh-induced vasorelaxation and pD2 values in the condition of high concentration of UA were not changed as compared to vasorelaxations before incubations.

Conclusion: Acute exposure of the resistant SMAs, FAs, and aortas isolated from aged WKY rats to a high concentration of UA did not provoke changes in endothelial function. Thus, the role of UA in the worsening of the endothelial dysfunction of aged rats is not supported by our data. Therefore, further studies are needed to determine whether hyperuricemia per se is causally associated with endothelial dysfunction.

Keywords: uric acide, vasorelaxation, hyperuricemia, old normotensive rats

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QUERCETIN IMPROVES DIASTOLIC DYSFUNCTION AND REDUCES HEART HYPERTROPHY IN DIABETIC ZDF RATS

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Introduction: Quercetin (QUE) is bioactive flavanol substance with radical scavenging, anti-apoptotic and anti-inflammatory properties both *in vitro* and *in vivo.* Its role in diabetes is not sufficiently elucidated, however, hypoglycemic and hypolipidemic activities have been experimentally observed. Nevertheless, there is still lack of information about its role on the heart functions. It has been showed that quercetin potentially inhibits adverse remodeling and improves heart function after myocardial infarction, however, for the type 2 diabetes mellitus (DM2) model such activity was not reported yet.

Methods:1-year Zucker Diabetic Fatty male rats were randomized into two groups: non-treated sham animals (Dia) and quercetin-treated animals (DiaQ). As controls male Wistar rats were used, also non-treated (C) and quercetin-treated (CQ). QUE was given orally for 6-weeks in dose 20mg/kg/day. Heart functions were measured echocardiographically (GE HealthcareVivid E9) before the onset of the treatment and at the end of experiment. The total level of collagen was measured by hydroxyproline assay and evaluated spectrophotometrically in the tissues of left ventricles (LV). For the protein analysis of remodeling pathways immunoblot analysis was used.

Results: QUE-treated animals exhibited decreased E/A wave ratios which suggest improvement of diastolic dysfunction along with reduced intraventricular septum diameter (IVSd) and left ventricular posterior wall (LVPWd) thicknessresulting in reduced overall relative mass thickness (RWT). This effect was further confirmed at the level of total collagen content in LV with significantly decreased collagen in DiaQ when compared to Diahearts. On the protein level myocyte enhancer factor-2 (MEF2) was significantly decreased in DiaQ when compared to Dia group.

Conclusions: We have shown that QUE-treatment is capable of improvement of diastolic dysfunction of heart accompanied with overall reduction of ventricular mass. This effect was further supported by decreased collagen content and decreased expression of remodeling-associated transcriptional factor MEF2 in LV, indicating potential anti-remodeling effect of QUE in experimental DM2.

Keywords: quercetin, diabetes mellitus 2, diastolic dysfunction, remodeling

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EFFECT OF THE LONG-TERM FRUCTOSE INTAKE ON THE PARTICIPATION OF NITRIC OXIDE AND HYDROGEN SULFIDE SIGNALING PATHWAYS IN VASOREGULATION OF RAT THORACIC AORTA

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Based on recent studies we can assume that the endogenous NO and H_2S signalling pathways could be involved in the metabolic disorders caused by high saccharide intake. However, the data related to their vasoregulatory mechanisms in this circumstance are still limited.

The main goal of the present study was to describe the effect of eight-weeks-lasting administration of 10% fructose solution to adult Wistar Kyoto (WKY) rats on plasma and biometric parameters, systolic blood pressure (SBP) measured by tail cuff plethysmography; vasoactive properties of the thoracic aorta (TA) followed by sensors of isometric tension Total NO synthase (NOS) activity was determined in crude homogenates of by measuring the formation of [3H]-L-citrulline from [3H]-L-arginine. The expression of enzymes producing NO and H_2S was determine by Western blotting.

Eight weeks of fructose administration did not affect SBP, glycaemia, or the plasma levels of total cholesterol or low-density and high-density lipoprotein; however, it significantly increased the levels of γ-glutamyl transferase and alanine transaminase in plasma. Chronic fructose intake deteriorated endothelium-dependent vasorelaxation (EDVR) and increased the sensitivity of adrenergic receptors to noradrenaline. Acute NOS inhibition evoked a reduction in EDVR that was similar between groups; however, it increased adrenergic contraction more in fructose-fed rats. CSE inhibition decreased EDVR in WKY but not in fructose-fed rats. The application of a H_2S scavenger evoked a reduction in the EDVR in WKY rats and normalized the sensitivity of adrenergic receptors in rats treated with fructose. Chronic increased fructose intake had no effect on the vasoactive responses induced by the H_2S donor. However, acute NO deficiency significantly increased the relaxant part of the dual vasoactive response to the H_2S donor in control WKY rats but not in rats treated with fructose solution. Fructose intake did not change NOS activity but reduced the expression of eNOS and CBS in the TA and CSE and CBS in the left ventricle.

In summary, although chronic increased fructose intake did not initiate changes characterizing metabolic syndrome, it impaired endothelium-dependent vasorelaxation, predicting possible cardiovascular complications. Moreover, we could assume that this reduction was probably not directly associated with decreased production of NO but rather with impairment of the NO-H_2S interaction. We confirmed that fructose altered the vasomotor manifestation of this interaction at least at two different levels: i) the contribution of endogenous H_2S to NO-mediated vasorelaxation and ii) the contribution of endogenous NO to the vasoactive effect of the H_2S donor.

Keywords: fructose, vasoactive responses, NO-H_2S interaction

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EFFECT OF SULFORAPHANE ON DOXORUBICIN-INDUCED TOXICITY IN HEK 293 CELLS

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Introduction: Sulforaphane (SFN) is natural antioxidant found in various cruciferous vegetables, especially in broccoli. Its important functions, including antioxidant, anticancer, and cytoprotective, have been described. SFN is also known as a potential activator of Nrf-2 (Nuclear factor-erythroid factor 2-related factor 2) signaling pathway.

Aim: The aim of our study was to study the impact of SFN on Dox-induced effects in human HEK 293 kidney cells. In this, we investigated the influence of both substances on proteins associated with the regulation of redox signaling and autophagy.

Methods: Dox and SFN cytotoxicity was analyzed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT test), changes in specific proteins were determined by Western blot.

Results: We found that SFN administration significantly increased Nrf-2 protein levels, but Dox administration had no significant effect on Nrf-2. Importantly, preincubation of cells with SFN resulted in a significant increase in Nrf-2 levels compared to cells exposed to Dox alone. In case of Keap-1, we found increased protein levels after Dox administration. SFN treatment did not significant influence Dox-induced effects. Next, we found changes in Beclin-1 protein, a key regulator of autophagy. Dox administration, similarly to Keap-1, significantly increased Beclin-1 levels compared to the control group. However, SFN administration had no significant effect on the levels of this protein. Finally, we observed the changes in heat shock proteins (Hsp40 and Hsp60), proteins which are activated in cell responses to stress stimulus. Different changes in levels of these Hsps indicate their specific role in cell responses to SFN and/or Dox administration. While SFN stimulated increase of Hsp40 protein levels, Dox administration had the opposite effect on Hsp40. SFN administration did not significantly influence Dox-induced effects on Hsp40. In contrast to Hsp40, co-administration of SFN with Dox significantly increased Hsp60 protein levels relative to all experimental groups.

Conclusions: The obtained results point to a role of Nrf-2 signaling pathway and autophagy in effects of SFN and Dox. Obtained data may help to better understand the interactions between Nrf-2 signaling pathway and autophagy under oxidative stress conditions.

Keywords: sulforaphane; doxorubicin; HEK 293 cells; Nrf2

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METABOLIC SYNDROME IN HYPERTRIACYLGLYCEROLEMIC RATS: EFFECTS OF ANTIOXIDANTS

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Long-lasting disturbances in lipid and glucose metabolism present in metabolic syndrome (MetS) lead to serious cardiovascular diseases. Inflammation and oxidative stress are closely related to MetS, therefore agents possessing both anti-inflammatory and anti-oxidative properties should have the potential in regulating MetS. The study was aimed to evaluate the effect of vitamin E (VitE) on basal biochemical and physiological parameters characterizing MetS and on the changed function of the heart and the aorta. Furthermore, the possible potentiation of VitE effect by synthetic antioxidant SMe1EC2 (SMe) was also tested. MetS was induced in hereditary hypertriacylglycerolemic rats (HTG) by the 5 weeks administration of a high-fat diet (1% cholesterol, 7,5% pork lard) with additional 10% fructose (HFFD). Controls were fed a standard diet (SD). VitE (100mg/kg/daily) alone or in combination with SMe (15mg/kg/daily) was administered p.o. After 5 weeks, rats were killed and biochemical analyses, as well as functional studies on the heart and the aorta, were performed. The biochemical parameters assessed in serum by ELISA methods: total cholesterol (TC), low-density lipoproteins (LDL), high-density lipoproteins (HDL), triacylglycerols (TG), thiobarbituric acid reactive substances (TBARs), N-acetyl glucosaminidase (NAGA) and blood glucose (Glu), as well as the oral glucose tolerance test (oGTT) were performed. The physiological status of rats was monitored (weight gain, diet consumption). The functional state of the aorta was tested in vitro under isometric conditions as endothelial-dependent relaxation of the phenylephrine-precontracted preparations by acetylcholine. The heart function was tested using Langendorff preparation (under constant pressure). The functional parameters of isolated heart, dysrhythmias and evoked fibrillations were evaluated in conditions of ischemia-reperfusion. The HFFD diet increased the body weight gain and serum levels of TC and LDL. A tendency towards the increase of serum NAGA was found. The endothelial function of the aorta was disturbed. The HFFD diet significantly increased the heart flow and the force of contraction, compared to SD. The evoked fibrillation was not influenced by HFFD. The HFFD caused a decreased number of serious dysrhythmias (ventricular tachycardia-VT; ventricular fibrillation-VF) while the number of ventricular premature beats (VPB) was increased. The fortification of the HFFD diet with VitE, either alone or in combination with SMe, decreased body weight gain, depressed blood pressure, improved particular biochemical parameters, and ameliorated endothelium-dependent relaxation. The combination of VitE and SMe also suppressed the occurrence of serious dysrhythmias VT and VF. Our data indicate that the HFFD diet-related disturbances led to alterations within pathophysiology in HTG rats. The results showed that a combination of antioxidants might have the potential to amend disorders accompanying MetS.

Keywords: metabolic syndrome, HTG rats, vitamin E, SMe1EC2, Langendorff preparation

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HMGB1 AS A POTENTIAL TARGET FOR TREATMENT AFTER EXPERIMENTAL MYOCARDIAL INFARCTION

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Myocardial infarction (MI) remains a leading cause of morbidity and mortality among all cardiovascular diseases over the world. High mobility group box 1 (HMGB1) is a DNA-binding protein with multiple cardioprotective effects. Besides its nuclear role, HMGB1 released during heart ischemia participates in interaction including the production of proinflammatory cytokines. The aim of the study was to evaluate the effects of anti-HMGB1 protein on biochemical and morphological parameters after experimentally induced MI in 12-week-old male WKY rats. In vivo model of experimental MI was induced by ligation of the left descending coronary artery and lasted 20 min. Prior to reperfusion anti-HMGB1 protein was administrated i.v. 7 days after MI, nitric oxide synthase (NOS) activity was determined by conversion of 3[H] Arginine to 3[H] Citrulline in the aorta and ischemic, border and non-ischemic region of the heart. NFκB, iNOS and eNOS expression was determined by Western blot. For morphological parameters, the hearts were excised and used for TTC-staining procedure. Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma. Concentration of conjugated dienes was measured spectrophotometrically in the heart.

Anti-HMGB1 protein increased NOS activity in both ischemic and border heart zone, as well as in the aorta, on the other hand NOS activity was not changed in non-ischemic part of the heart. The same pattern was found in eNOS expression level. Anti-HMGB-1 protein administration decreased iNOS and NFκB expression in the ischemic zone as well as TNF-alpha and IL-6 level in plasma. Moreover, anti-HMGB-1 protein decreased the level of conjugated dienes in the heart. Simultaneously, anti HMGB1 protein decreased ischemic part as well as border region of the heart.

Considering the results by using a rat model of experimentally induced MI, HMGB1 protein is a promising molecule for reduction the negative effects of the myocardium infarction, for the amelioration of inflammation, as well as a promising approach for treatment of cardiovascular diseases.

Keywords: myocardial infarction, HMGB-1 protein, nitric oxide

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THE ROLE OF INTERACTION BETWEEN PERIVASCULAR ADIPOSE TISSUE AND HYDROGEN SULFIDE IN VASOACTIVE RESPONSES OF THORACIC AORTA IN HYPERTRIGLYCERIDEMIC RATS

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Dysfunction of arterial smooth muscle cells and perivascular adipose tissue (PVAT) as main sources of vasoactive substances such as hydrogen sulfide (H_2S) interferes with the ethiopathogenesis of different pathological stages, such as hypertension or metabolic syndrome. However, the mutual relationship between activity of PVAT and H_2S effects has not been fully investigated to date. In spontaneously hypertensive rats, we confirmed that H_2S signal pathway in interaction with PVAT could serve as reserved mechanism of NO deficiency. The aim of this study was to evaluate the mutual relationship among PVAT, endogenous and exogenous H_2S in vasoactive responses of isolated thoracic aorta in hypertriglyceridemic (HTG) rats used as model of metabolic syndrome. 18-20-weeks-old male normotensive Wistar and HTG rats were used. Systolic blood pressure (sBP) was measured by plethysmography. Serum levels of glucose and lipids were measured using commercially available kits. Endothelium-dependent vasorelaxation induced by acetylcholine (Ach) and noradrenaline (NA)-induced contraction of TA with preserved PVAT+ or denuded PVAT- were recorded as changes in isometric tension. The pre-treatment with propargylglycine (PPG, 10 mM) was used to inhibit H_2S producing enzyme. $Na_2S \cdot 9H_2O$ was used to evaluate the vasoactive effect of exogenous H_2S. Statistical significance was determined using an ANOVA followed by a Bonferroni post hoc test on raw data. Compared to Wistar rats, in HTG rats mild hypertension was associated with glucose intolerance, dyslipidemia, increased amount of retroperitoneal fat, increased arterial contractility, and endothelial dysfunction associated with arterial wall injury, which was accompanied by decreased nitric oxide (NO)-synthase activity, increased expression of H_2S producing enzyme, and an altered oxidative state. In HTG, endogenous H_2S participated in the inhibition of endothelium-dependent vasorelaxation regardless of PVAT presence; on the other hand, aortas with preserved PVAT revealed a stronger anticontractile effect mediated at least partially by H_2S. Although we observed a higher vasorelaxation induced by exogenous H_2S donor in HTG rats than in Wistar rats, intact PVAT subtilized this effect. Our results confirmed that in hypertriglyceridemic rats, endogenous H_2S could manifest dual effect depending on the type of triggered signaling pathway. H_2S produced in the vessel wall contributed to endothelial dysfunction; however, the anti-contractile action of PVAT was associated with H_2S activity as probable part of compensatory mechanisms. In experimental model of metabolic syndrome, the presence of PVAT determined the character (pathological vs compensatory) of effect of endogenously produced H_2S.

Keywords: hydrogen sulfide, perivascular adipose tissue, thoracic aorta, vasoactivity, hypertriglyceridemic rats

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MiRP2 RESCUES LONG QT SYNDROME TYPE 5

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Long QT Syndrome (LQT) is a disorder characterized by delayed cardiac repolarization resulting in the prolongation of the QT interval. LQT5 is caused by loss of function mutations in the KCNE1 gene encoding minK, a regulatory subunit of a voltage-gated ion channel which is conducting the slow component of the cardiac delayed rectifier potassium current (IKs).

The minK-related peptides (MiRPs), also encoded by members of the KCNE gene family, are expressed in cardiomyocytes and can also modulate the function of IKs channels in vitro.

In this study, we aimed to investigate the possible effect of MiRP2, encoded by the KCNE3 gene, on the development of the LQT5 phenotype.

Plasmid constructs carrying cDNAs of KvLQT1 the pore forming subunit of IKs, WT-minK, LQT5-minK variant (G52R-minK) and MiRP2 were generated by standard molecular cloning techniques. These plasmid constructs were co-expressed in different combinations in CHO cells. Whole cell currents were characterized by patch clamp technique. The NanoBiT protein:protein interaction assay was applied to explore whether MiRP2 and minK are represented in a distinct ion channel population or they co-assemble in the same ion channel complex.

Mean current densities were similar in group 1 (KvLQT1+WT-minK) [74.9 pA/pF, 95% CI (54.7-95.2), n=22] and group 2 (KvLQT1+WT-minK+MiRP2) [62.33 pA/pF, 95% CI (48.2-76.5), n=22], while average current density was significantly lower in group 3 (KvLQT1+WT-minK+G52R-minK) [31.7 pA/pF, 95% CI (23.8-39.5), n=29] compared to the group 1 and 2. However, the mean current density in the presence of MiRP2 was significantly increased in group 4 (KvLQT1+WT-minK+G52R-minK+MiRP2) [54.3 pA/pF, 95% CI (38.2-70.5), n=27] compared to the group 3. Varying amount of MiRP2 was co-expressed with KvLQT1 and minK for the NanoBiT experiments. Average relative luminescence (RLU) was 194 in group 1 (KvLQT1:minK:MiRP2, cDNA ratio 1:2:0) which was similar to group 2 (1:2:1) (129.3 RLU). However, mean RLU was significantly lower in group 3 (1:2:2) (96.7 RLU, p=0.0085) compared to the group 1.

We conclude that MiRP2 has a rescue effect on the LQT5-minK variant which suppresses the IKs channel in vitro. Furthermore, MiRP2 is probably able to replace minK within the macromolecular complex of the IKs ion channel, therefore, MiRP2 possibly modulate the development of the LQT5 phenotype in patients.

Keywords: long QT syndrome type 5, slow component of the cardiac delayed rectifier potassium channel, minK- related peptide 2, NanoLuc® Binary Technology

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NITRIC OXIDE AS ONE OF THE TRIGGERING FACTORS OF CARDIOPROTECTION INDUCED BY REMOTE PRECONDITIONING

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Mechanisms of protection induced by remote preconditioning (RPC) represent a complex cascade of initial triggering in the remote tissue, communicationbetween the distant and the target organ, and end-effects responsible for the induction of the protective phenotype. The potential candidates for RPC triggering molecules includes, but is not limited to, nitric oxide (NO).

The aim of the study was to investigate the roleof NO as a triggering molecule in the cardioprotective effect of RPC in male Wistar rats aged 3-4 months. The NOS inhibitor L-NAME (60 mg/kg i.p.) was applied to the rats 20 minutes before the anesthesia with/without RPC induction (RPC+L-NAME/C+L-NAME). The RPC protocol consisted of three cycles of 5 minute occlusion (ischemia) and 5 minute reperfusion of hind limb. Subsequently, the rat hearts were isolated for Langendorff perfusion and subjected to 20min stabilization, 30min global ischemia and 120min of reperfusion for the evaluation of post-ischemic contractile dysfunction, reperfusion arrhythmias and size of myocardial infarction.

The recovery of LVDP (% of preischemic values) was significantely increased in RPC animals compare to Controls (58.5%±5.8 vs. 38.1%±4.7; $p<0.05$). Application of L-NAME suppressed this cardioprotective effect of RPC and LVDP recovery decreased in RPC+L-NAME group (RPC+L-NAME 37.3%±6.8 vs. RPC 58.5%±5.8; $p<0.05$) to the level observed in Controls. Similarly L-NAME inhibited the anti-infarct effect of RPC. According to our observation we can conclude that NO molecules play rolein triggering cardioprotective effect of RPC in Wistar rats. Inhibition of NO triggering action led to an inability to further transmit the signal from the distant organ to the target tissue/organ and thus suppressed potential RPC-induced protection.

Keywords: remote preconditioning, cardioprotection, nitric oxide, L-NAME

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EFFECTS OF POLYPHENOL QUERCETIN ON SELECTED CARDIOVASCULAR PARAMETERS AND ISCHEMIA-REPERFUSION INJURY OF THE MYOCARDIUM IN RATS WITH TYPE 2 DIABETES

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Quercetin (QCT) is a natural polyphenolic antioxidant that has been studied for its promising cardioprotective potential. In our previous studies QCT exerted cardioprotective effects on ischemia-reperfusion (I/R) injury in healthy young animals. The aim of the current study was to reveal potential beneficial cardio- and vasculoprotective effects of QCT in diabetic type 2 rats.

QCT (20 mg/kg/day, 6 weeks) was administered to 6-month- and 1-year-old lean (fa/+) and obese (fa/fa) ZDF (Zucker diabetic fatty) rats. Blood pressure was measured by tail-cuff pletysmography before the start and at the end of QCT administration. Isolated perfused hearts were exposed to global I/R (30/120min). Cardiac function was determined by echocardiography in 1-year old ZDF rats. Endothelium-dependent vasorelaxation was measured in isolated aortic rings pre-contracted with phenylephrine (10-6mol/l) followed by application of acetylcholine (10-9–10-5mol/l). Molecular mechanisms of QCT effects in the heart were analyzed by Western Blot monitoring protein expression of RISK (Reperfusion Injury Salvage Kinases) signaling pathway.

QCT significantly lowered blood pressure in lean and obese 6-month-old ZDF rats but had no effect on blood pressure in 1-year-old rats. Echocardiography revealed that QCT improved diabetes-induced diastolic dysfunction and reduced left ventricular mass. On the other hand, QCT exerted no cardioprotective effect against I/R injury in 6-month-old rats and even worsened post-ischemic recovery of heart function in 1-year-old rats. QCT prevented diabetes-induced impairment of vasorelaxation in obese 6-month-old rats, but accelerated impairment of vasorelaxation in obese 1-year-old rats. QCT had no effect on vasorelaxation in lean rats of both ages. QCT increased eNOS expression in hearts of 6-month-old rats and PKC-ε in 1-year-old ZDF rats but did not induce global activation of the RISK pathway.

QCT appears to have beneficial effects on blood pressure and endothelium-dependent vascular relaxation in type 2 diabetic rats but progression of diabetes and/or ageing may impair these vasculoprotective effects. QCT prevents diabetes-induced diastolic dysfunction and hypertrophy but is ineffective in prevention of cardiac I/R injury in ageing type 2 diabetic rats. QCT ineffectiveness in preventing I/R injury might be due to incomplete activation of RISK pathway, which is considered as one of major signaling pathways of several cardioprotective interventions. Taking together, the presence of comorbidities and ageing might act as confounding factors for beneficial effects of QCT in cardiovascular diseases.

Keywords: quercetin, ischemia-reperfusion injury, endothelium-dependent vasorelaxation, echocardiography, molecular mechanisms

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LIPOPOLYSACCHARIDE-INDUCED CHANGES IN ENDOTHELIAL CONNEXIN-40 AND OCCLUDIN ASSOCIATED WITH MACROPHAGE INFILTRATION IN BOTH NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Bacterial endotoxin (LPS) and hypertension were considered as an important pro-atherogenic factor targeting mainly vascular endothelium. The aim of our study was to investigate the effect of LPS on expression of connexin Cx40 (endothelial/conduction system), Cx43 (mainly myocardial) and endothelial tight junctions protein occludin in left ventricle (LV) Wistar (normotensive) and SHR (spontaneously hypertensive) rats. We used adult Wistar and SHR rats injected with a bolus of LPS (E. coli, 1mg/kg, i.p.). LV samples were taken 10 days after. We measured selected inflammatory markers (TNF-a, NFkB) and CD68 as a macrophage marker. Expression of Cx40, occluding and Cx43 were obtained by western blot method and localized with immunohistochemistry method. LPS application to SHR reduce expression both connexins and change redistribution of Cx43 accompanied with extensively increased macrophage infiltration and NFκB and TNF-α expression. Total occludin expression was increased in both SHR groups compared to Wistar, while SHR-LPS was higher compared to SHR. Administration of LPS to Wistar rats increased tissue infiltration by macrophages, decrease Cx40 expression. Our results are suggestive of regulation of Cx43, Cx40 and occludin expression with macrophages-related inflammation in the LV of SHR rats. The results also indicate that LV vascular endothelium of SHR rats can be more sensitive to LPS than are normotensive Wistar rats.

Keywords: LPS, hypertension, tight junction, connexin

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BLOCKING EFFECT OF THE FERRITIN NANOPARTICLE ON THE CARDIAC RYANODINE RECEPTOR

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Iron, an essential element for most living organisms, participates in a wide variety of physiological processes. Disturbance in iron homeostasis has been associated with numerous pathologies, particularly in the heart and brain, which are the most susceptible organs. Given a critical role of iron in cell functioning, its labile concentration in the cytoplasm has to be tightly controlled and regulated to prevent both iron deficiency and overload. The cellular iron is stored and sequestered in ferritin complexes (nonheme-protein), mainly located in the cytoplasm. Ferritin consists of a roughly spherical protein shell with an outer diameter of 12–13 nm and with an inner cavity diameter no greater than 8 nm, where iron is kept in a non-toxic and bioavailable form.

Under iron-overload conditions, the generation of reactive oxygen species leads to impairment in Ca^{2+} signaling, fundamentally implicated in cardiac physiology. Since iron excess is accompanied by increased expression of ferritin, we examined whether ferritin functionally interacts with the cardiac ryanodine receptor (RYR2), which is one of the major components of Ca^{2+} signaling in the heart.

Using the method of planar lipid membranes, we show that cytosolic ferritin induced an abrupt, permanent blockage of the RYR2 channel. The ferritin effect was strongly voltage dependent and competitively antagonized by cytosolic TEA+, an impermeant RYR2 blocker. Our results collectively indicate that monomeric ferritin highly likely blocks the RYR2 channel by a direct electrostatic interaction within the wider region of the channel permeation pathway. Magnetic field as a contributing factor was excluded, because we calculated that energy of even bigger clusters of ferritin nanoparticles in the Earth`s magnetic field is at least one order smaller than the thermal energy. We tested a wide range of ferritin concentrations (0.005–0.30 μg/ml) covering physiological and iron-overload situations. Although, ferritin caused RYR2 blockage in both cases, it seems highly unlikely that ferritin-RYR2 interaction occurs when iron homeostasis is maintained. Otherwise, it could significantly weaken or even abolish Ca^{2+} release that is required for cardiac contraction. As yet, there has been no precise examination of ferritin localization in the cytoplasm. Under iron-overload conditions, however, there is a greater chance of collisions between the ferritin nanoparticles and RYR2 channels, because ferritin concentration becomes increased. Thus, we can conclude that RYR2 blockage by ferritin might significantly contribute to abnormal cardiac Ca^{2+} signaling.

Keywords: cardiac ryanodine receptor, ferritin, ion channel blockage, iron homeostasis

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ANTIHYPERTENSIVE ACTIVITY OF 20-HETE ANTAGONIST (AAA) AND EPOXYEICOSATRIENOIC ACID ANALOGUE (EET-A) IN SPONTANEOUSLY HYPERTENSIVE RATS

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Hypertension is one of the most significant risk factor for all-cause morbidity and mortality in the world according to WHO. The death toll of hypertension and associated disorders reaches approximately ten million deaths globally and is still on the rise despite decades of research. Increasing evidence suggest the important role of cytochrome P-450 dependent metabolites of arachidonic acid (AA) in the regulation of blood pressure. The epoxyeicosatrienoic acids (EETs) act as vasodilators, they exhibit anti-inflammatory properties and decrease sodium reabsorption. On the other hand 20-HETE is an AA metabolite with both pro- and anti-hypertensive activity. Thus we decided to explore the antihypertensive efficiency of EET-A, a stable analogue of 14,15-EET, and AAA, a novel antagonist of 20-HETE receptors. To test the antihypertensive potential of both substances we employed spontaneously hypertensive rats (SHR), which are currently considered to mimic human essential hypertension to the best extend and are widely used in pre-clinical research to develop new drug targets.

Male SHR in the established stage of hypertension (16 week old) were treated for four weeks with either EET-A, AAA or the combination of EET-A and AAA and compared to age-matched untreated SHR. Both substances were administered in drinking water in a dose of 10 mg/kg/day (n=6-10). Systolic blood pressure (SBP) was measured by telemetry. Once a week observations in metabolic cages were performed; urine, blood and tissue samples were collected for further analysis. The combination of EET-A and AAA was also administered to young SHR (6 week old) in pre-hypertensive stage to evaluate the preventive potential of the new treatment.

EET-A had no significant effect on blood pressure of SHR. The AAA showed some antihypertensive potential, but only the combined treatment with AAA + EET-A significantly lowered the blood pressure in adult SHR (SBP day -2: 181±4 vs day 27: 162±5 mmHg, $p<0.05$). Additionally, combined AAA and EET-A attenuated cardiac hypertrophy, increased natriuresis, reduced ANG II level in the kidney and increased the excretion of nitric oxide metabolites. Considering our beneficial results in adult rats we decided to test the potential of combined treatment in the prevention of hypertension development. The treatment with AAA and EET-A proved to be very beneficial for young SHR, which remained normotensive during the four-week observation (SBP on day 27: 134±2 vs 156±5 mmHg in control group, $p<0.05$). Taking into account all the beneficial impact of the combined treatment with EET-A and AAA on cardiovascular and renal function of SHR we suggest that it constitutes a promising antihypertensive strategy, but further research are necessary to elucidate the exact mechanism of its action.

Keywords: Hypertension, SHR, cytochrome P-450 dependent metabolites of arachidonic acid, AAA, EET-A

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THE EFFECT OF PERIVASCULAR ADIPOSE TISSUE IN INTERACTION WITH ENDOGENOUS AND EXOGENOUS HYDROGEN SULFIDE IN VASOACTIVE RESPONSES OF ISOLATED THORACIC AORTA IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Perivascular adipose tissue (PVAT) plays an important role in the regulation of cardiovascular system. One of the crucial substances produced by PVAT is hydrogen sulfide (H_2S) with biphasic vasomotor effect on cardiovascular system. The aim of this study was to evaluate the mutual relationship between PVAT and exogenous and endogenous H_2S in the vasoactive responses of thoracic aorta (TA) isolated from adult normotensive (Wistar) rats and spontaneous hypertensive rats (SHRs). The changes in isometric tension were evaluated after application of exogenous acetylcholine (Ach), noradrenaline (NA) and exogenous H_2S donor (Na_2S) in TA with preserved or denuded PVAT. To inhibit the endogenous H_2S production, the inhibitor of cystathionine γ-lyase, propargylglycine, was used.

Regardless of the strain, PVAT revealed anti-contractile effect on the vasoconstrictor responses induced by exogenous NA. In both, Wistar and SHRs, PVAT worsened the endothelial-dependent vasorelaxant response induced by Ach. In Wistar rats, H_2S produced by the vascular wall as well as PVAT did not participate on the vasoactive response induced by exogenous NA and Ach. However, in SHR, H_2S produced by both, the vascular wall and PVAT had a pro-contractile effect on the vasoconstrictor response induced by exogenous NA. On the other hand, even if H_2S produced by PVAT did not contribute to endothelium-dependent vasorelaxant responses, H_2S produced by the vascular wall had a pro-relaxant effect in SHR.

In the SHRs, regardless of the presence of PVAT, we confirmed an increased maximal vasorelaxant phase of the Na_2S-induced response compared with that in the Wistar rats. In Wistar rats, PVAT did not affect the biphasic vasoactive response induced by H_2S; however, in SHRs, the rings with PVAT revealed significantly increased vasorelaxation induced by exogenous H_2S.

We confirmed that although PVAT of TA in SHRs aggravated endothelial function, it revealed an anticontractile effect mediated by the release of unknown factors and an increased vasorelaxant response to H_2S donors. Endogenously produced H_2S manifested a dual effect depending on the type of signaling pathway triggered. H_2S produced by PVAT, and the vascular wall had pro-contractile effects and could contribute to pathological changes in essential hypertension. However, H_2S produced by the vascular wall had a pro-relaxation effect and could represent a form of vasoactive compensatory mechanism to balance impaired vascular tone regulation.

Keywords: Perivascular adipose tissue, H_2S, SHR

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PRESENCE OF HYPOXIA MARKER CARBONIC ANHYDRASE IX IN HUMAN ABDOMINAL AORTIC ANEURYSM TISSUE AND PLASMA

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Abdominal aortic aneurysms (AAA) are a significant cause of premature deaths worldwide. Since there is no specific treatment for reducing AAA progression, it is crucial to understand the pathogenesis leading to aneurysm wall weakening/remodeling and identify new proteins involved in this process which could subsequently serve as novel therapeutic targets. Although many of the mechanisms leading to AAA development still remain unclear, recent studies have shown that key pathophysiological features of AAA include chronic inflammation, extracellular matrix degradation, vascular smooth muscle cell (VSMCs) phenotype modulation, VSMCs apoptosis, and hypoxia.

We analyzed the presence of the hypoxia-related proteins hypoxia-inducible factor 1alpha (HIF-1 alpha) and carbonic anhydrase IX (CA IX) in the human AAA tissues. Additionally, we used a blood-based assay to examine soluble CA IX (s-CA IX) levels in the plasma of AAA patients.

Using western blotting, we detected CA IX protein in 80% of AAA tissue samples. On the contrary, a CA IX signal was absent in control aortas. Five out of 12 CA IX-positive samples showed a high level of this protein. CA IX levels were not influenced by age, gender, or cigarette smoking status. The mean diameter of all CA IX positive aneurysms was 61 +/- 13 mm, the mean diameter of five aneurysms with the highest level of CA IX was 56 +/- 4 mm, while the mean diameter of three CA IX negative aneurysms was 72.7 +/- 6.7 mm. Immunohistochemistry staining proved CA IX expression in the media of the aneurysmal wall suggesting its presence in vascular smooth muscle cells. We also confirmed the presence of HIF-1alpha in three AAA protein lysates. Using ELISA, we determined the concentration of s-CA IX >20 pg/mL in 13 out of 15 AAA patients. Results obtained from in silico analysis of CA9 and aneurysm-associated genes suggest a role for CA IX in matrix degradation and altered smooth muscle cells phenotype/proliferation—an active remodeling of the vascular wall.

Our results prove the presence of hypoxia-related proteins CA IX and HIF-1 alpha in AAA tissues and elevated s-CA IX concentrations in AAA patient plasma specimens. Although the exact mechanism is not fully elucidated, our results open a new insights leading to an understanding of AAA development and to clarify the role of CA IX in diseases associated with decreased oxygen levels in the cellular microenvironment. CA IX and the mechanisms responsible for its increase could become potential targets of future therapeutic interventions in patients with AAA.

Keywords: abdominal aortic aneurysm, carbonic anhydrase IX, hypoxia

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BENEFICIAL REPOLARISATION-NORMALIZING EFFECT OF A POLYUNSATURATED FATTY ACID, DHA IN TRANSGENIC LONG QT TYPE 2 RABBIT MODEL

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INTRODUCTION: Current standard therapies in various LQTS subtypes are only symptom-directed (beta-blockade, ICD) and fail to prevent arrhythmic events in up to 40% of the patients. New, more efficient therapeutic strategies are therefore needed. Docosahexaenoic acid (DHA), a polyunsaturated fatty acid activates the repolarizing IKs current if both α- (KvLQT1) and β (KCNE1) –subunits to IKs are functionally intact. PURPOSE: The potential beneficial (repolarisation-normalizing) effects of DHA in transgenic LQT1 (KCNQ1-Y315S, loss of IKs), LQT2 (HERG-G628S, loss of IKr), LQT5 (KCNE1-G52R, decreased IKs) and LQT2-5 (loss of IKr/decreased IKs) rabbits were investigated. METHODS: In vivo telemetric ECG analyses in wild-type (WT), LQT1, LQT2, LQT5, and LQT2-5 rabbits were performed at baseline and after 10μM/kg DHA i.m. to assess changes in heart rate corrected QT (QTc) and short-term variability of QT (STVQT). Ex vivo monophasic action potential measurements in Langendorff-perfused hearts were carried out to investigate DHA-induced (20μM) changes in action potential duration (APD75) and action potential (AP) triangulation (APD90 - APD30). RESULTS: At baseline, QTc (ms±SEM) was significantly longer in LQT1, LQT2 and LQT2-5 rabbit models (166±3.8, n=8, 165±3.7, n=6, and 167±12.1, n=8; $p<0.05$ vs. WT) than in LQT5 (137±5.3, n=8) and WT (144±14.3, n=6). STVQT (ms±SEM), a (proarrhythmia)marker for the temporal - beat-to-beat - instability of repolarisation, was increased in LQT2 (LQT2 8.5±1.9, n=6 vs. WT 4.8±1.0, n=6; $p<0.05$ vs). DHA proved itself to be a potent IKs-activator: it shortened QTc (ms±SEM) in vivo only in rabbits with functionally intact alpha- and beta-subunits of IKs, i.e., in WT (-12.0±1.9, n=6, $p<0.01$) and more pronouncedly in LQT2 (-20.7±1.7, n=6, $p<0.01$), while had no effect on QTc in LQT1, LQT5 and LQT2-5 rabbits, that harbor loss-of-function mutations in KCNQ1 or KCNE1. Furthermore, in LQT2, DHA administration flattened QT/RR curve (QT/RR steepness: 'baseline' 0.61, 'after DHA' 0.49, n=6, $p<0.05$) and normalized STVQT (ΔSTVQT in ms±SEM: -2.3±0.6, n=6, $p<0.05$ before vs. after DHA). Similarly, ex vivo, DHA significantly shortened APD75 (ms±SEM) in WT (-12.3±2.2, n=7, $p<0.01$) and in LQT2 rabbits (-18.1±3.5, n=6, $p=0.019$), but had no effect on APD75 in LQT1, LQT5, and LQT2-5. Moreover, DHA significantly decreased APD triangulation in LQT2 (-5.80±1.83, n=5, $p<0.01$ before vs. after DHA). Importantly, spatial dispersion of repolarisation (QT and APD75 dispersion) was not increased by DHA. Conclusion: DHA exerts a genotype-specific beneficial shortening effect of QTc, STVQT, APD, and AP triangulation through activation of IKs in LQT2 rabbits

but has no effects if either α- or β-subunits to IKs are functionally impaired. DHA could thus represent a novel therapeutic tool in LQT2 syndrome. This work was supported by a grant from the Hungarian National Research, Development, and Innovation Office (NKFIH K-128851).

Keywords: cardiac electrophysiology, long QT syndrome, impaired repolarization reserve, transgenic LQT rabbit models, polyunsaturated fatty acid, docosahexaenoic acid (DHA)

Funding: Hungarian National Research, Development, and Innovation Office (NKFIH K-128851)

SEX DIFFERENCES IN CARDIAC REMODELING INDUCED BY EARLY POSTNATAL ABDOMINAL AORTA CONSTRICTION IN RATS

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Pressure overload-induced cardiac remodeling leads to changes in myocardial structure and function and it can result in life-threatening arrhythmias and progression of heart failure. Early postnatal abdominal aorta constriction (AAC) is a unique experimental model of gradual pressure overload that can be used for understanding the role of neonatal cardiac plasticity in cardiac remodeling. Here we examine the impact of increased pressure load imposed briefly after the birth on the development of left ventricle (LV) function and geometry in male and female Wistar rats. Newborn pups were subjected to the surgical induction of AAC or sham operation at postnatal day 2 under light ether anesthesia. A silk ligature was tied around the aorta (in the subdiaphragmatic, suprarenal region), and a hypodermic needle of 0.25 mm in outer diameter. In sham-operated littermates that were used as age-matched controls, the aorta was exposed, but not constricted. Cardiac function and geometry were assessed at postnatal days 21 and 90 using echocardiography. Animals were sacrificed and the hearts were harvested for further analysis.

At the end of the study, the relative heart weight was higher in females than in males compared to corresponding sham-operated animals (5.23 ± 0.50 vs. 3.61 ± 0.11 mg/g and 4.35 ± 0.40 vs. 2.71 ± 0.06 mg/g, respectively). In adult rats, AAC led to a gradual increase in LV diastolic diameter by 7% in females and 12% in males compared to sham-operated controls. Diastolic LV anterior and posterior wall thicknesses were increased by 12 % and 10 %, respectively in females, and by 33 % and 31 %, respectively in males. LV systolic function (expressed as fractional shortening) at day 21 was decreased in both female and male hearts with AAC compared to sham-operated animals (41.2 ± 2.6 vs. 46.1 ± 1.5 % and 41.8 ± 2.1 vs. 45.7 ± 2.9 %, respectively). Further decrease in fractional shortening was observed at day 90 in males (to 35.2 ± 2.9) but not in females (41.1 ± 3.4 %) with AAC.

Our data suggested, that male Wistar rats are more susceptible to AAC-induced cardiomegaly than female rats. This can be partially explained by differences in body growth. However, further investigation should focus on potential sex differences in myocardial structure, conduction system, and metabolic changes.

Keywords: cardiac remodeling, sex differences, neonatal rats

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CARDIOVASCULAR RESPONSE DURING ACUTE STRESS IN SUBJECTS WITH SCHIZOTYPAL PERSONALITY TRAITS

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Several factors, including major mental disorders such as schizophrenia, determine the cardiovascular and endocrine responses to acute stressors. The aim of the present study was to test the hypothesis that individuals with schizotypal personality traits exhibit an altered reactivity of the cardiovascular system during stressful situations. We have examined volunteers of both sexes, who were divided into a group with schizotypal personality traits present and a group without them (control group) based on their scores in the Schizotypal Personality Questionnaire. Probands were exposed to an acute stress stimulus in the form of public speech. The stressfulness of the model was assessed using a questionnaire for subjective stress perception. We have observed significant increases in systolic as well as diastolic blood pressure, and heart rate over time in individuals with schizotypal personality traits which was comparable to those in the control group. However, the perceived stress was higher in the schizotypal group compared to control subjects. We failed to find a difference in stress perception between men and women, but systolic blood pressure levels were higher in men compared to women, supporting known sex differences in the cardiovascular system activity. In conclusion, individuals with a possible predisposition to the development of schizophrenia exhibit an adequate activation of the cardiovascular system during stressful situations.

Keywords: schizotypy, blood pressure, heart rate, stress perception

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THE ROLE OF NECROSIS-LIKE CELL DEATH MODES IN ORGAN DAMAGE IN EXPERIMENTAL PULMONARY ARTERIAL HYPERTENSION

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Cell loss due to both necroptosis and pyroptosis has been shown to underlie the pathogenesis of various diseases, however, the role of such cell death modalities as well as their potential linkage in pulmonary arterial hypertension (PAH) remains not well understood. Therefore, detailed molecular analysis of both necroptosis and pyroptosis in the lung and right ventricular (RV) tissue affected by PAH was performed. The circulating levels of receptor-interacting protein kinase 3 (RIP3), associated with both investigated cell death forms, were also examined. In male Wistar rats, PAH was induced by a single injection of monocrotaline (MCT) (60 mg/kg, s.c.). The animals were sacrificed after 28 days (MCT group) or prematurely due to signs of rapid disease progression as a group with terminal stage of PAH (ptMCT group). The development of PAH was confirmed by changes in vital functions, increase in cardiac hemodynamic stress markers and RV hypertrophy. Western Blotting analysis revealed elevated expression of pThr231/Ser232-RIP3 as well as pSer345-mixed linkage kinase domain-like protein (pSer345-MLKL) in the RVs of both PAH stages, indicating necroptosis execution. Contrary, in the diseased lungs, upregulated pThr231/Ser232-RIP3 did not proceed to necroptosis but was likely associated with the activation of NLR family pyrin domain containing 3 (NLRP3) inflammasome resulting in pyroptosis execution. PAH caused an increase in the circulating RIP3 levels which were more evident in the terminal stage and positively correlated with RV hypertrophy marker – Fulton index, but not with cardiac hemodynamic stress marker – N-terminal pro-B-type natriuretic peptide (NT-proBNP). Taken together, this is the first study indicating that different necrosis-like cell death modes associated with RIP3 might underlie organ damage in PAH and that the circulating RIP3 might serve as an additional diagnostic and prognostic marker of RV hypertrophic injury under conditions of PAH.

Keywords: necroptosis, pyroptosis, pulmonary arterial hypertension, receptor-interacting protein kinase 3

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TRANDOLAPRIL EFFECT ON THE RAT MYOCARDIUM IN EXPERIMENTAL VOLUME OVERLOAD HEART FAILURE

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Heart failure (HF) is currently defined as a clinical syndrome with symptoms and/or signs caused by a structural and/or functional cardiac abnormality and corroborated by elevated natriuretic peptide levels and/or objective evidence of pulmonary or systemic congestion. The renin-angiotensin-aldosterone-system plays a significant role in HF progression which is proved by the beneficial effect of the drugs affecting this signaling pathway, e.g. angiotensin-converting enzyme (ACE) inhibitors. Rats with chronic volume overload due to aortocaval fistula (ACF) represent a well-characterized, reproducible and simple model of heart failure. This study aimed to investigate the effect of ACE inhibition on various remodeling processes occurring in rats with volume overload induced by ACF.

The 62 Hannover Sprague-Dawley male rats were divided into three groups: sham-operated (n = 25), ACF (n = 20) and ACF with ACE inhibitor trandolapril treatment (n = 18). ACF was induced surgically using a needle technique in 8 weeks old animals. In the trandolapril group, the treatment (6 mg/l of trandolapril in drinking water) started 4 weeks after the operation and continued for 20 weeks until the end of the experiment. 24 weeks after the surgery, the animals had been anesthetized before the ECG recording and echocardiography were performed. The rats were sacrificed by cervical dislocation and their hearts were excised and used for further experiments. Membrane potential and contraction force were recorded on multicellular preparations from the left ventricle. The calcium transients and sarcomeric shortening were measured on enzymatically dissociated ventricular cardiomyocytes using a fluorescent dye Fura-2.

In ACF rats, trandolapril treatment significantly improved survival rate, however, did not influence cardiac hypertrophy. In the ACF group, QRS complex and ventricular action potential prolongation together with spontaneous activity in isolated ventricular cardiomyocytes were observed. All these proarrhythmic electrophysiological changes were attenuated by trandolapril treatment which was associated with a lower mortality rate in this group. On the other hand, ACE inhibition did not have any effect on increased cardiac output, eccentric cardiac hypertrophy and intracellular calcium levels in ACF animals. The volume overload and the pharmacological treatment did not induce any changes in contractility in vitro on both multicellular and cellular levels.

ACE inhibition was shown to effectively suppress the proarrhythmic remodeling while not affecting the cardiac hypertrophy in volume overload.

Keywords: heart failure, cardiac remodeling, renin-angiotensin-aldosterone system, trandolapril, rat

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ADAPTIVE CHANGES IN THE LEFT HEART VENTRICLE IN A CHRONIC STRESS MODEL IN RATS

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Chronic stress is known to play a role in the development of cardiovascular diseases. However, stress exposure can induce adaptive changes and provide cardiovascular benefits as well. Previous studies using animal models have demonstrated that repeated stress stimuli can induce Akt kinase activation coupled with an increase of anti-apoptotic proteins (Bcl-2) and a down-regulation of pro-apoptotic proteins (cleaved caspase-3) in rat hearts (Barteková et al., 2015). Another protein expressed in the heart with possible cardioprotective effects is the angiotensin converting enzyme 2 (ACE2), which cleaves angiotensin II into angiotensin 1-7, leading to decreased blood pressure. ACE2 in the heart during stress has not been studied as of yet. Interestingly, increased mitochondrial respiratory chain complex activities were observed under chronic stress, resulting in increased ATP production, which is crucial for cardiomyocyte activity (de Souza Mota et al., 2017). The mechanism underlying these changes is not yet understood. We hypothesise that the production of ACE2 and UQCRFS1, the catalytic component of mitochondrial respiratory chain complex III, is increased in the heart ventricle during chronic stress. Male Sprague Dawley rats were left undisturbed or exposed to chronic mild stress for five weeks. We measured the gene expression of both proteins and protein abundance of ACE2 by quantitative PCR and ELISA, respectively. Our results showed no significant changes in the mRNA or protein production of ACE2 in the left heart ventricle. We found a significant increase in UQCRFS1 mRNA production in the heart as a result of chronic stress exposure. Our results allow us to suggest that chronic stress most likely leads to adaptive changes in the composition of the mitochondrial respiratory chain in the left heart ventricle. How these changes affect the cardiovascular system during chronic stress needs further exploration.

Keywords: stress, heart, ACE2, UQCRFS1

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THE CARDIOPROTECTIVE EFFECT OF REMOTE ISCHEMIC PRECONDITIONING AND PROTECTIVE SIGNALING PATHWAYS IN AGING RATS

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The effect of age on the reduced tolerance to ischemia-reperfusion (I/R) injury and myocardial adaptative mechanisms has been demonstrated in several studies in human and also in animal hearts. However, the onset of this unfavorable phenotype and cellular mechanisms behind are less known. Nevertheless, some studies are controversial and show that cardioprotection was preserved even in older age. Currently, one of the most actively studied forms of cardioprotection is remote-ischemic preconditioning (RIPC), mainly for its possible clinical use. In many studies, a positive effect of RIPC has already been found in elderly patients. However, little is known about the effect of RIPC and its molecular basis in elderly animals. Therefore, our work focuses on clarifying the effect of RIPC on the resistance of the heart against I/R injury and on identifying proteins involved in protective pathways of RIPC in aging 13 months old rats. In Langendorff-perfused hearts exposed to 30-min I/120-min R without or with prior RIPC. RIPC (3 cycles of 5-min I/5-min R) was applied on the hind limb of anesthetized rats (pressure cuff inflation (200 mmHg)/deflation). We measured infarct size (IS), susceptibility to ventricular arrhythmias and recovery of contractile function (left ventricular developed pressure - LVDP). In parallel groups, left ventricle tissue was sampled for the detection of protein levels of RISK pathway and pro/anti-apoptotic cascades (Western blot analysis). Remote preconditioning provided the adaptation of the heart to conditions occurring during reperfusion of the ischemic heart, thereby improving its response to the development of lethal I/R injury. Myocardial infarct size decreased and LVDP recovery was improved after I/R, although there was no change in incidence of reperfusion arrhythmias. Positive effect of RIPC was also associated with increased phosphorylation of glycogen synthase kinase 3 beta (GSK3β) and expression of endothelial nitric oxide synthase (eNOS). However, increasing age is likely to have caused a premature increase in protein kinase B (Akt) phosphorylation, that's why following RIPC could no more result in its increase. By induction of RIPC, the expression of proteinkinase C epsilon (PKCε) did not alter, while the apoptotic activity of the myocardial cells was decreased (Bax/Bcl-2 ratio). As a result, the application of RIPC provided protection of the heart against I/R injury to some degree in the 13 months old rats. Therefore, even at this age, RIPC appears to be still an effective and clinically easy-to-use form of cardioprotection.

Keywords: ischemia-reperfusion injury, preconditioning, protective cell signaling, aging

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A NEW BISDIOXOPIPERAZINE ANALOGUE PROVIDES PROMISING PROTECTIVE EFFECTS AGAINST CHRONIC ANTHRACYCLINE CARDIOTOXICITY IN VIVO IN RABBITS

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Chronic anthracycline (ANT) cardiotoxicity manifesting itself as heart failure is a feared complication of cancer chemotherapy. The only drug approved for its prevention is a bisdioxopiperazine agent dexrazoxane (DEX). Although its mechanism of action is still incompletely understood, the recent findings suggest the key role of catalytic inhibition of topoisomerase IIβ (TOP2B) which should prevent ANT-induced DNA damage caused by poisoning of the enzyme. We and others have studied dozens of DEX derivatives, but only a close derivative of DEX (ICRF-193) appeared more effective than DEX in vitro. However, poor water solubility of ICFR-193 precluded its testing beyond in vitro level and thus its prodrug (compound GK-667) was synthesized and selected for in vivo examination.

The aim of this study was to investigate whether compound GK-667 can provide effective and dose-dependent cardioprotection against chronic ANT cardiotoxicity in a DEX-validated in vivo model. Furthermore, molecular aspects of cardiotoxicity and cardioprotection were investigated. The cardiotoxicity was induced in rabbits by daunorubicin (DAU, 3 mg/kg, i.v., weekly for 10 weeks, n=10) and GK-667 (1 or 5 mg/kg, i.v., n=10 in each group) was administered 30 min before each DAU dose in the combination groups. Other groups received GK-667 alone (5 mg/kg, n=7) and saline (n=10). At the end of the study, cardiac troponin T was analysed in plasma and cardiac function was assessed by echocardiography and left ventricular (LV) catheterization. Administration of DAU resulted in 20% mortality, blood congestion in about one third of the animals and significant systolic dysfunction as examined by both echocardiography and LV catheterization. All these events were completely prevented by co-treatment with both doses of GK-667. Dose dependency of cardioprotective effects of GK-667 was notable on molecular markers of cardiac damage and dysfunction – particularly cardiac troponin T in plasma and expression of ANP in the LV myocardium. Chronic DAU treatment also induced p53-mediated DNA damage response (DDR) which was also found dose-dependently preventable by GK-667 co-treatment. Moreover, DAU induced a marked acute DDR even after single dose, and even here GK-667 induced dose-dependent prevention. Interestingly, another close DEX derivative, which has been previously found ineffective as both TOP2B inhibitor and cardioprotectant, was unable to affect the acute DAU-induced DDR in the heart.

In conclusion, these results suggest that GK-667 is to the best of our knowledge the most potent bisdioxopiperazine cardioprotective agent against chronic ANT cardiotoxicity. Its cardioprotective effects can be attributed to the prevention of DAU-induced and p53 mediated DNA damage response in the heart via catalytic inhibition of TOP2B by ICRF-193 released from the prodrug. Overall, GK-667 is an interesting drug candidate for further research and development.

Keywords: anthracycline, cardiotoxicity, cardioprotection, dexrazoxane, ICRF-193

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ROLE OF PHARMACOLOGICAL INHIBITION OF ATM IN THE DEVELOPMENT OF ANTHRACYCLINE CARDIOTOXICITY

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Anthracycline (ANT) cardiotoxicity resulting in cardiomyopathy and heart failure is still the main limitation of the clinical use of ANT anticancer drugs. Many mechanisms have been suggested to be involved in ANT-induced cardiac damage, but topoisomerase II beta (TOP2B)-dependent DNA damage in cardiomyocytes has become one of the leading theories. However, DNA damage response (DDR) downstream of TOP2B poisoning and its role in the pathogenesis of ANT cardiotoxicity remains elusive. This study aimed to use pharmacological inhibition of ATM to clarify whether this pathway is involved in DDR signaling in the heart after ANT exposure and whether the ATM-orchestrated signaling leads to the development of ANT cardiotoxicity or the opposite is true. For this purpose, chronic cardiotoxicity was induced in male rabbits by daunorubicin (DAU, 3 mg/kg, weekly for ten weeks). The highly selective and potent ATM inhibitor AZD0156, which is currently in clinical evaluation, was administered at a dose of 0.5 mg/kg either alone or 30 min before each dose of DAU. The results were compared with groups receiving saline or vehicle for AZD0156. In another set of animals, the DDR in the heart was studied 6 hours after a single administration of the same drugs. Our results show that ATM inhibitor AZD0156 effectively prevented the acute DAU-induced increase of p53 levels and up-regulation of p53 target genes (p21 and many others) in the left ventricular (LV) myocardium. Furthermore, chronic co-treatment of rabbits with AZD0156 and DAU resulted in markedly increased severity of cardiotoxicity and end-stage congestive heart failure as compared to DAU alone. This finding was documented by a markedly increased incidence of DAU-induced blood congestion (hydrothorax 80 % vs. 40 % in the DAU-alone group), heart failure-related mortality (60 % vs. 20 % in the DAU-alone group), steep rise in plasma levels of cardiac troponin T before the observed deaths and profound decrease in LV fractional shortening (LVFS) determined by echocardiography. The last measured LVFS values in the AZD0156+DAU group were significantly lower than in the DAU-alone group. AZD0156 alone was well tolerated and had no significant impact on any studied parameter. In summary, it seems that ANT-induced DDR signaling in the heart appears to be ATM-dependent, and pharmacological inhibition of ATM may sensitize rabbit hearts to the development of ANT-induced chronic cardiotoxicity and heart failure.

Keywords: Anthracyclines; cardiotoxicity; DNA damage signaling; ATM; pharmacological modulation

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COUGH AS A CAUSE AND CONSEQUENCE OF HEART DYSFUNCTION

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The cough reflex is an airway defensive process and it is also very common symptom that lead patients to seek medical attention. A bidirectional relationship between cough and heart dysfunctions exist. While the effect of robust switches of intrathoracic pressure during cough on haemodynamic and electrophysiological parameters are well known, mechanisms of so called “cardiogenic cough” are less understood. Cough induced by cardiac pathologies (mainly arrhythmias) is interesting and underestimated phenomenon. This condition is usually associated with the presence of abnormal heart rhythms and ceases with successful treatment of arrhythmia either by pharmacotherapy or by radiofrequency ablation of arrhythmogenic substrate. The two main hypotheses on heart-cough relationships (reflexogenic and haemodynamic) are discussed alongside with some uncommon cases of cardiogenic cough.

Keywords: cough, arrhythmia, reflex, plasticity, treatment

STIMULATION AND UNLOADING OF BARORECEPTORS MODULATE COUGH IN EXPERIMENTAL CONDITIONS

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Cough, as the main airway defensive process is modulated by multiple secondary sensory inputs from the respiratory system and outside of it. This modulation is one of the mechanisms that contribute to sensitization of cough related pathways at the peripheral and /or central level via neuroplasticity and it manifests in clinical settings most often as augmented coughing.
Some circumstantial and inconsistent findings from the older studies in animal models resulted to more sophisticated studies focusing on both stimulation and unloading of baroreceptors on cough in experimental conditions and computational simulations. These studies confirm that baroreflex alters coughing evoked in experiment even though the evolutional and/or physiological purpose of such regulations, nor clinical relevance of these findings are not understood at the moment.

Keywords: cough, baroreceptors, neurons, animal studies

ORANGE FLAVONOID HESPERETIN PROLONGED ACTION POTENTIAL DURATION AND INHIBITS THE SLOW DELAYED RECTIFIER POTASSIUM CURRENT (IKs) IN DOG AND RABBIT CARDIAC VENTRICULAR MUSCLE PREPARATIONS AND ISOLATED MYOCYTES

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Background: Hesperetin is the main flavonoid in oranges and can be found in orange juice at concentrations up to 720 mmol/L. It was stated that a high intake of dietary flavonoids, which are abundant in fruits, tea ,vegetables, and wine, is known to reduce cardiovascular mortality, however, the effects of flavonoids on cardiac electrophysiology may have both antiarrhythmic and proarrhythmic consequences as they can attenuate the repolarization reserve.

Aim: The combined effect of Hesperetin and IKr-blocking antiarrhythmic drugs on action potential duration (APD) has not been studied. Therefore, the present work aimed to study the additive inhibitory effect of Hesperetin on the repolarization of the action potential in dog and rabbit ventricular preparations with normal and attenuated repolarization reserve. The effects of the drug on transmembrane slow delayed rectifier K^+ currents (IKs) were also investigated.

Method: Action potentials were recorded in right ventricular trabecular and papillary muscle preparations obtained from dog and rabbit hearts using conventional microelectrode techniques. The repolarization reserve was attenuated using the IKr blocker Dofetilide and the late Na^+ channel activator Veratrine. Transmembrane (IKs) were measured using the whole-cell configuration of the patch-clamp technique at 37°C.

Results and discussion: This study is the first to evaluate the combined effect of Hesperetin with IKr -blocking antiarrhythmic drugs on ventricle APD. Hesperetin 10 µM alone has no notable effect on APD, however, applying 10µM hesperetin after attenuating the repolarization reserve by using Dofetilide 100nM and Veratrine 50 µg caused significant prolongation of the steady APD (from 466±18 ms to 512±23 ms (n=10)). In agreement with action potential duration data, a moderate but statistically significant effect of 10 and 30 µM was observed in the magnitude of transmembrane IKs.

Conclusion: Hesperetin alone has no or negligible effect on action potential duration, therefore the risk of arrhythmia is low for healthy people. However; if the repolarization reserve has been attenuated due to some pathological conditions such as heart failure or some variable abnormalities such as adverse effects, genetic mutations and polymorphisms, electrophysiological remodeling, and changes in serum ion concentrations; a high amount of orange juice consumption might lead to ventricular arrhythmia due to the inhibition of Iks and prolongation in the action potential duration.

Keywords: hesperetin , APD, IKs, repolarization reserve

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CARDIOVASCULAR RESPONSE OF RATS TO BEHAVIOURAL STRESS MEASURED IN PHENOTYPER

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Psychosocial factors play a significant role in developing hypertension, and underlying causal connections are relatively complex. Therefore, defined experiments in animal models are necessary. In animals, the effects of psychosocial factors are usually studied through conditioned, pain-evoked tests. We took an alternative approach and measured the cardiovascular and behavioural parameters remotely and applied the weak stimuli randomly at different times of the day. Our aim was to test the cardiovascular response in telemetrically measured rats to weak, repeated and unconditional stress stimuli in rats kept in phenotypers. Adult male Wistar rats (n = 8; 444 ± 12 g) were included in the experiment. Relative humidity (50 – 70 %), air temperature (20 – 22 °C), and light regime (12L/12D; ZT0 = 8:00, beginning of the light phase) were controlled in the experimental room. We measured blood pressure (BP) and heart rate (HR) by telemetry (DSI, USA), a continuous 5 min recording with a sampling frequency of 500 Hz every 15 minutes. From beat-to-beat measurements, we analysed the number of normal-to-normal beats > 9 ms (NN9), the percentage of NN9 (pNN9) and the standard deviation of RR intervals (SDNN), which are presumably related to parasympathetic regulation. Before testing, the animals were tested and housed in PhenoTyper (Noldus, NL) for two days (habituation to the environment). After habituation, we exposed rats for 5 min to acoustic stress (scream typical for fights or pain; 0.2 – 20 kHz; http://ratbehavior.org) during both the light (ZT4.5 and 6.5) and dark (16.5 and 18.5) phases of the day. In ZT5.5 and 17.5, we exposed the animals to 5 min of air-jet stress. We compared the data by paired T-test or two-factor ANOVA (factors: stimulus vs phase or time vs phase).

We found higher HR ($p < 0.001$) and BP ($p < 0.001$) in rats during the dark compared to the light phase of the day. In contrast, markers of parasympathetic activity were significantly higher during the light compared to the light phase of the day (NN9, $p < 0.001$; pNN9, $p < 0.001$; SDNN, $p < 0.05$). The type of stimulus had a significant effect on the cardiovascular response. NN9, pNN9 and SDNN responded to behavioural stimuli less than HR and BP. We observed habituation and a high interindividual response to acoustic stress and a more pronounced and uniform response to air-jet stress. The phase of the day significantly affected the cardiovascular response to applied stressors.

In conclusion, the cardiovascular response to stress stimulus exhibited significant light/dark and interindividual variability. Repeated acoustic stress led to habituation, the response was attenuated compared to air-jet stress, and we did not observe cross-reactivity between these different stimuli. Combining PhenoTyper and telemetry, we can reveal specific cardiovascular responses typical for different behavioural strategies.

Keywords: heart rate, blood pressure, telemetry, behavioural stress, PhenoTyper

Funding:APVV-17-0178 and VEGA 1/0492/19

THE ELECTROPHYSIOLOGICAL EFFECTS OF CANNABIDIOL ON ACTION POTENTIAL AND TRANSMEMBRANE POTASSIUM CURRENTS IN DOG AND RABBIT CARDIAC PREPARATIONS

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Cannabis use is associated with known cardiovascular side effects such as cardiac arrhythmias or even sudden cardiac death. The mechanisms behind these adverse effects are unknown. The aim of the present work was to study the cellular cardiac electrophysiological effects of cannabidiol (CBD) on action potentials and several transmembrane potassium currents, such as the rapid (IKr) and slow (IKs) delayed rectifier, the transient outward (Ito) and inward rectifier (IK1) potassium currents in rabbit and dog cardiac preparations. CBD increased action potential duration (APD) significantly in both rabbit (from 211.7 ± 11.2. to 224.6 ± 11.4 ms, n = 8) and dog (from 215.2 ± 9.0 to 231.7 ± 4.7 ms, n = 6) ventricular papillary muscle at 5 μM concentration. CBD decreased IKr, IKs and Ito (only in dog) significantly with corresponding estimated IC50 values of 4.9, 3.1 and 5 μM, respectively, without changing IK1. Although the IC50 value of CBD was found to be higher than literary Cmax values after CBD smoking and oral intake, our results raise the possibility that potassium channel inhibition by lengthening cardiac repolarization might have a role in the possible proarrhythmic side effects of cannabinoids in situations where CBD metabolism and/or the repolarization reserve is impaired.

Keywords: cannabidiol, electrophysiology, action potential, potassium currents, rabbit, dog

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GROWTH-RELATED ACTIVITIES AT THE PLASMALEMMA IN NEONATAL CARDIAC MYOCYTES

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Cytoarchitecture of cardiac myocytes develops extensively during the early postnatal days when embryonal myocytes react to increased demand on pumping power of the myocardium by increasing the cell volume but also by optimizing the excitation-contraction machinery. The need for a better understanding of these processes is driven by the missing understanding of the diseased and adapting adult myocardium. We studied the morphology of cardiomyocytes in heart ventricles of 3- to 9-day old rats by transmission electron microscopy. The hearts were chemically fixed and samples of ventricles were processed for electron microscopy. A few days after birth, the rat myocardium comprises a relatively heterogeneous population of cardiomyocytes differing in the degree of myotube maturation. On day 4 postpartum, the myotube volume was rich in cytosol and mitochondria, with few and weak myofibrils in a single layer between nuclei and lateral plasmalemma. Cytoplasmic loci of newly formed myofibrils were present under plasmalemma together with tubular or vesicular profiles (proto-tubules) originating from plasmalemma and distinct from intracellular vesicles originating from the Golgi or endoplasmic reticulum. The plasmalemmal evaginations assumed a ring shape with a narrowing rim perimeter that closed and merged and thus produced intracellular vesicles of plasmalemma encapsulating extracellular fluid. These vesicles kept position near the z-disc where they originated and thus could give rise to proto-tubules in transversal direction by growing and merging together. The lateral plasmalemmal protrusions curved along the outer surface. At a distance of a few hundred nanometers, the protrusions merged at their tips back to the plasmalemma and thus encapsulated the extracellular fluid into flat vesicles. These vesicles were oriented under an angle relative to the plane of the lateral plasmalemma and thus could give rise to longitudinally oriented proto-tubules. Proto-tubules of either origin were often structurally and functionally coupled with the terminal cisternae of the sarcoplasmic reticulum with ryanodine receptors, thus forming the dyads. In the cytoplasm at the axilla of proto-tubules and plasmalemma, a dark material similar to z-disc accumulated and made the basis for attachment of actin myofilaments, the latter developing to the first sarcomeres, orientated parallel between the plasmalemma and the myofibril next to the nucleus. The plasmalemmal protrusions at the apical but also at the lateral sides of myotubes contacted with protrusions of neighboring myocytes, and created or extended intercalated discs with desmosomes and attached actin filaments. The morphological analysis revealed the sequence of steps leading to structural maturation of cardiac myocytes and gave clear evidence for proportional growth of myocyte structures. These findings also indicate the reason behind the appearance of the embryonic phenotype in myocytes of diseased myocardium.

Keywords: neonatal myocardium, cardiac myocytes, plasmalemma, development, morphology

Funding:VEGA 2/0091/19

INVESTIGATION OF THE EFFECT OF THE NOVEL MYOSIN ACTIVATOR DANICAMTIV ON THE CONTRACTILITY AND Ca^{2+} TRANSIENTS OF ISOLATED LEFT VENTRICULAR CARDIOMYOCYTES

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Introduction: Advanced stage systolic heart failure (HFrEF) remains a disease with a very poor prognosis. Its treatment is based on improving cardiac systolic dysfunction and myocardial contractility. Our current research has focused on a new pharmacological agent, danicamtiv directly influencing the cross-bridge cycle.

Aim: To study the effects of danicamtiv on the contractility and Ca^{2+} transients in isolated left ventricular cardiomyocytes.

Methods: The effects of danicamtiv were investigated on freshly isolated left ventricular canine cardiomyocytes. Cells were loaded with Fura-2 AM calcium sensitive fluorescent dye before the experiments. Different concentrations of danicamtiv (10 nM - 2 µM) were applied in the experiments. Cell contraction was induced by field stimulation (0.5-0.1 Hz), then a set-up capable of recording the shortening of the sarcomere length as well as the changes in intracellular Ca2+ concentration simultaneously was used.

Results: In the presence of 0.5 Hz stimulation and 2 µM danicamtiv, both the contraction duration (2.0±0.7 s vs. 0.8±0.2 s, mean±SEM, $P<0.05$) and the systolic ejection time were prolonged (1.6±0.6 s vs. 0.6±0.1 s, $P<0.05$), while the kinetics of contraction and relaxation were both decelerated (0.21±0.19 µm/s vs. 0.94±0.49 µm/s and 0.22±0.18 µm/s vs. 1.30±0.75 µm/s, $P<0.05$, respectively) (n=18). Treatment with 2 µM danicamtiv showed a positive ionotropic effect: a shortening could be observed in diastolic (1.59±0.13 µm vs. 1.90±0.03 µm $P<0.05$, n=18), as well as in systolic sarcomere lengths (1.46±0.08 µm vs. 1.68±0.0 µm $P<0.05$, n=18). The lowest effective danicamtiv concentration was 0.01 µM. Danicamtiv treatment was not associated with an increase in intracellular Ca2+ concentration, regardless of the frequency of stimulation.

Conclusion: Our results suggest that the positive inotropic effect of danicamtiv is accompanied by a significant reduction in resting sarcomere length of isolated cardiomyocytes and deceleration of relaxation kinetics, which may impair diastolic function. All these may limit the clinical efficacy of this novel myosin activator agent.

Keywords: positive inotropy, HFrEF, danicamtiv, myosin activator

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EFFECT OF ALDOSTERONE ANTAGONIST, SPIRONOLACTONE, ON NON-DIPPING BLOOD PRESSURE RHYTHM IN HYPERTENSIVE Ren-2 TRANSGENIC RATS

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The increased activity of the renin-angiotensin-aldosterone system is an important regulatory mechanism of blood pressure (BP) and leads to hypertension with disturbed 24-hour BP rhythm. Angiotensin II receptor type 1 blockers reduced BP and restored the non-dipping BP profile. The effects of angiotensin II on the 24-hour BP rhythm are mediated directly through vasoconstriction and indirectly through aldosterone synthesis. We aimed to analyse the potency of an aldosterone mineralocorticoid receptor blocker, spironolactone (SPIRO 30 mg/kg/day), on BP and its inverse 24-hour rhythm in male hypertensive Ren-2 transgenic rats (TGR mRen27, control group n = 6, SPIRO group n = 5, 12 weeks old) for 20 days. After 10 days of SPIRO treatment, we increased salt intake (1% in drinking water). BP was continuously measured by telemetry. Spectral power (significance of 24-hour rhythm) and acrophase (the time of rhythm peak) of the 24-hour rhythm of BP were analysed by Chronos-Fit software, and statistical analysis was done by GraphPad Prism software. Administration of SPIRO significantly ($p = 0.015$) reduced BP (light: 179 ± 4 mmHg, dark: 189 ± 3 mmHg) in TGR after 9 days in comparison with control (light: 192 ± 2 mmHg, dark: 208 ± 3 mmHg) in phase-dependent manner ($p < 0.001$). High salt intake increased BP ($p < 0.05$) in the light (control: 228 ± 6 mmHg, SPIRO: 211 ± 9 mmHg) and dark phase (control: 225 ± 7 mmHg, SPIRO: 209 ± 7 mmHg) of the day. SPIRO had no protective effect on the salt-induced increase of BP. SPIRO did not affect the spectral power of the 24-hour BP rhythm (74.5 ± 10.4 vs 67.4 ± 5.8) but tended to decrease the power of BP rhythm ($p = 0.061$) in combination with salt (80.8 ± 10.3 vs 54.0 ± 15.8). High salt intake reduced the spectral power of BP in the control (80.8 ± 10.3 vs 6.8 ± 1.8; $p < 0.001$) and in the SPIRO (54.0 ± 15.8 vs 8.9 ± 1.9; $p < 0.001$) groups. SPIRO neither affected BP acrophase (4.9 ± 0.3 h vs 5.2 ± 0.5 h) nor in combination with salt (5.0 ± 0.3 h vs 4.4 ± 0.3 h). However, increased salt intake shifted BP acrophase in both, control (5.0 ± 0.3 h vs 21.0 ± 0.5 h; $p = 0.001$) and SPIRO (4.4 ± 0.3 h vs 25.3 ± 3.8 h; $p < 0.001$) group. In conclusion, SPIRO decreased BP in TGR rats. However, SPIRO did not have a protective effect on BP in combination with high salt intake and did not restore disrupted BP rhythm. Inverse BP rhythm in TGR rats with the up-regulated renin-angiotensin-aldosterone system is not determined by the biological effects of aldosterone, but probably other angiotensin II-dependent post-receptor mechanisms.

Keywords: blood pressure, spironolactone, TGR rats, high salt

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ASSESSMENT OF THE FRACTION OF T-TUBULAR MEMBRANE IN CARDIOMYOCYTES: A NEW AND REVERSIBLE APPROACH

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The adequate excitation-contraction coupling in cardiomyocytes requires well developed t-tubular system (t-tubules) that allows fast transmission of excitation to the depth of the cell, near the stores of Ca^{2+}. A remodeling of t-tubules is associated with various cardiac pathologies including ischemia or heart failure. To understand the impact of t-tubules on the function of both healthy and diseased cardiomyocytes, several methodological approaches have been developed and applied. Unfortunately, these approaches produce diverse data and the widely used detubulation technique causes an irreversible impairment of the tested cells which disables their repeated use and also paired comparison of the data obtained with and without t-tubules.

We propose an alternative method that is fully reversible, allowing repetitive estimation of t-tubular characteristics on the same cell. Short-term perfusion (tens of seconds) of the cell measured with a low-conductive solution (isotonic sucrose solution, 0.3 M) substantially increases the electrical resistance of the t-tubular lumen and electrically separates the surface and t-tubular cell membrane. Analysis of the recorded membrane current activated by a sub-threshold voltage step (20 ms step from -80 to -75 or -70 mV) reveals two exponential current components. The numerical values of the parameters obtained from the current approximation by a sum of two exponential functions make it possible to calculate the t-tubular and surface membrane capacitances (Ct and Cs) and thus the fraction ft of the t-tubular membrane capacitance (ft=Ct / (Ct +Cs)) which can also be regarded as an indicator of the fraction of tubular membrane area.

Using the new technique during our whole-cell patch-clamp measurements in enzymatically isolated rat ventricular and atrial cardiomyocytes, we were able to estimate ft in these cells 0.337 ± 0.017 in ventricular and 0.144 ± 0.015 in atrial cells). Repetitive measurements in ventricular myocytes during the first and second sucrose perfusion resulted in ft of 0.345 ± 0.021 and 0.347 ± 0.023, respectively. The new method enabled to detection of a significant partial detubulation of ventricular cardiomyocytes using 15-min exposure to 150 μM imipramine and its following wash-out (a decrease of the total cell membrane capacitance by 30.5% and ft by 49.6% in the treated cells).

We conclude that our newly proposed method is fast, simple, and fully reversible, enabling us to properly and repetitively estimate an essential parameter of the t-tubular system ft. Hence, it may be used in studies analyzing properties of the cardiac t-tubular system including its transient changes, induced for example by a transient hypoosmolality.

Keywords: t-tubulus, isotonic sucrose solution, surface membrane capacitance, t-tubular membrane capacitance

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EFFECT OF OMACOR AGAINST THE INCREASED INCIDENCE OF MALIGNANT CARDIAC ARRHYTHMIAS TRIGGERED BY LIGHT POLLUTION

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Objective
Light pollution disturbs circadian rhythm and increases susceptibility to arrhythmias. Our aim was to determine whether rats exposed to continuous light will have altered myocardial gene transcripts/protein expression implicated in arrhythmogenesis as well as to assess Omacor supplementation on these changes.
Design and method
We used in the experiment male and female spontaneously hypertensive (SHR) and normotensive Wistar rats (WR) housed under standard 12 h/12 h light/dark cycles or exposed to 6-weeks continuous 300 lux light for 24 h. Half the rats were treated with 200 mg/100 g b.w. Omacor.
Results
Continuous light resulted in higher male rat vulnerability to malignant ventricular fibrillation (VF). It was associated with myocardial connexin-43 (Cx43) down-regulation and worsen intercellular electrical coupling, due to increased pro-inflammatory NF-kB and iNOS transcripts and decreased sarcoplasmic reticulum Ca^{2+}ATPase transcripts. Omacor supplementation increased the electrical threshold to induce the VF linked with amelioration of myocardial Cx43 mRNA and Cx43 protein levels and the suppression of NF-kB and iNOS. It can be concluded that rat exposure to continuous light outcomes deleterious cardiac alterations jeopardizing intercellular Cx43 channel-mediated electrical communication, thereby increasing the risk of malignant arrhythmias.
Conclusions
The adverse effects were attenuated by treatment with Omacor, thus supporting its potential benefit and the relevance of monitoring the omega-3 index in human populations at risk.

Keywords: Omacor; light pollution; rats; cardiac arrhythmias; connexin-43; NF-κB; iNOS

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ENDURANCE TRAINING INDUCED CELLULAR ELECTROPHYSIOLOGICAL REMODELING IN NEWLY DEVELOPED ANIMAL ATHLETE'S HEART MODELS

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Introduction: The benefits of regular training for a healthy life are undoubted. However, based on accumulating evidence, long-term heavy training beyond an optimal dose may also have cardiac electrophysiological adverse effects under certain circumstances. Structural and functional changes, including repolarization abnormalities because of vigorous training, may underlie the development of malignant arrhythmias.

Aims: To develop animal models with significant translational value for human athlete's heart and to investigate potential cellular electrophysiological causes of cardiac arrhythmias because of long-term heavy endurance training in in vitro studies.

Methods: Twenty-four dogs and twenty-six guinea pigs were randomized into sedentary ('Sed') and exercised ('Ex') groups (n=12-12; n=13-13). The latter groups underwent an intensive several week-long endurance training program on special treadmill system. Characteristics of athlete's heart were validated by ECG and echocardiography. After heart removal, the degree of interstitial fibrosis was quantified in semi-quantitative histopathological study and left ventricular myocytes were enzymatically isolated via retrograde perfusion. Transmembrane ionic currents were recorded using whole-cell configuration of patch-clamp technique and action potential duration (APD) was measured by perforated patch-clamp technique. Immunocytochemistry measurements were performed to determine the density of transmembrane ion channel subunits.

Results: Based on the ECG and ECHO results, the vigorous training program resulted in significant cardiac adaptation in both species. In addition, it caused mild ventricular fibrosis. The repolarization reflected as 90 percent of APD (APD90) was significantly lengthened in the left ventricular myocytes isolated from the 'Ex' dogs compared to the 'Sed' group (472.8±29.6 ms; n=29 vs. 369.3±31.4 ms; n=24 p=0.023), however, no statistically significant difference was detected between the 'Sed' and 'Ex' groups in case of guinea pigs. The amplitude of the transient outward potassium current (Ito), which is not expressed in the guinea pig heart, was significantly smaller in the 'Ex' dogs ('Ex' vs. 'Sed' 7.6±0.6 pA/pF, n=54 vs. 10.2±1.0 pA/pF, n=42, $p<0.05$). Under the currently used protocols, no differences were detected in the magnitude of other ionic currents between the groups. The HCN4 protein expression was significantly higher in isolated ventricular myocytes obtained from 'Ex' dogs.

Conclusion: Besides long-term heavy training induced in vivo observed changes, cellular electrophysiological remodeling, as well as, decreased Ito magnitude, therefore, prolonged action potential duration, and increased expression profile of HCN4 protein, moreover, enhanced level of fibrosis together may ay lead to increased susceptibility of life-threatening arrhythmias in a vulnerable period. Further studies are warranted to clarify this hypothesis in more detail.

Keywords: athlete's heart, patch-clamp, Ito-current, electrophysiology

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NEUROGENIC REGULATION OF SPLANCHNIC ARTERIES IN RATS TREATED WITH HIGH-FAT DIET IN COMBINATION WITH HIGH-FRUCTOSE INTAKE

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The aim was of this study was to examine the effects of obesitogenic diet on the mechanisms of neurogenic regulation in arteries of rat splanchnic area. Six-week-old Wistar-Kyoto rats were treated for 8 weeks with control diet (10 % fat), high-fat diet (HFD; 45 % fat), or combination of HFD with 10 % solution of fructose. Contractile and relaxant responses of isolated rat arteries (mesenteric artery, aorta) with preserved and removed perivascular adipose tissue to selected vasoactive stimuli were recorded isometrically by the force-displacement transducer. The results demonstrated that in young rats the eight-week-lasting HFD might lead to body fat accumulation and early excitation of cardiovascular sympathetic nervous system, as shown by increased heart rate and enhanced arterial contractile responses induced by endogenous noradrenaline released from perivascular sympathetic nerves. The addition of high-fructose intake deteriorated this state by impairment of arterial relaxation and resulted in mild elevation of systolic blood pressure; however, it did not cause the increase in sympathetic contractions observed after treatment with HFD alone, indicating the attenuating effect of high-fructose treatment on arterial sympathoadrenergic responses. It might be presumed that during electrical stimulation of perivascular nerves in isolated arterial preparations, the increased sensoric nerve activity might inhibit the resultant sympathetic contraction, an effect which could be responsible for the elimination of the enhancement of arterial contractile responses to electrical nerve stimulation when HFD was combined with high-fructose intake. The diet-induced alterations in isolated arteries were observed only in the presence of perivascular adipose tissue, indicating that this structure is important in initiation of early vascular changes during the development of metabolic syndrome.

Keywords: arteries; neurogenic regulation; high-fat diet; high-fructose intake; perivascular adipose tissue

Funding:The study was funded by the grants VEGA No. 2/0156/21 and 2/0153/21.

CARDIAC CONNEXIN-43 HEMICHANNELS AND PANNEXIN-1 CHANNELS: POTENTIAL NOVEL ANTIARRHYTHMIC TARGETS

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Background: Cardiac connexin-43 (Cx43) creates dodecameric gap junction channels (GJCs) at the cardiomyocyte contacts in intercalated disc as well as hexameric hemi-channels (HCs) at the peri-junctional plasma membrane and at sarcolemmal caveolae/rafts compartments. GJCs are fundamental for the direct cardiac cell-to-cell transmission of electrical and molecular signals which ensures synchronous myocardial contraction. In turn, disorders of GJCs and/or their redistribution to the lateral sides of the cardiomyocytes promote development of cardiac arrhythmias.

Recent findings: The HCs and structurally similar heptameric pannexin1 (Panx1) channels are active in stressful conditions, like inflammation or redox disorders. These channels are essential for paracrine and autocrine communication through the release of ions and signalling molecules to the extracellular environment, or for uptake from it. The HCs and Panx1 channel-opening profoundly affects intracellular ionic homeostasis and redox status and facilitates via purinergic signalling pro-inflammatory and pro-fibrotic processes. These conditions promote cardiac arrhythmogenesis due to the impairment of the GJCs and selective ion channel function. Crosstalk between GJCs and HCs/Panx1 channels could be crucial in the development of arrhythmogenic substrates, including fibrosis. Despite the knowledge gap in the regulation of these channels, current evidence indicates that HCs and Panx1 channel activation can enhance the risk of cardiac arrhythmias.

Meaning: It is extremely challenging to target HCs and Panx1 channels by inhibitory agents to hamper development of cardiac rhythm disorders. Progress in this field may contribute to novel therapeutic approaches for patients prone to develop atrial or ventricular fibrillation.

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APPLICATION OF MOLECULAR HYDROGEN IN THE CARDIAC SURGERY-ASSOCIATED ACUTE KIDNEY INJURY

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Cardiac surgery-associated acute kidney injury (CS-AKI) is a major complication associated with increased morbidity and mortality. It is assumed that oxidative stress may be involved in the mechanisms that influence CS-AKI. To protect injured tissues and prevent further damage of organs during cardiac surgery, the administration of antioxidant reagents for scavenging ROS has been widely applied. Molecular hydrogen (H_2) is a novel agent that has been previously shown to scavenge ROS. We evaluated the potential effect of H_2 application on the kidney in an in vivo model of simulated heart transplantation.

Prestice Black-Pied pigs were used for the simulation of heart transplantation by occluding venae cavae and pulmonary veins, cross-clamping of ascending aorta, and connection to extracorporeal circulation (ECC). Cold crystalloid cardioplegia was administered for 3 hours. After the time of cold arrest, the coronary arteries were rinsed with Plasmalyte solution and the aortic clamp was released. This was followed by rewarming the heart. After 60 minutes of reperfusion, the pigs were detached from ECC and the experiment was terminated. We used 2 experimental groups: T - pigs after transplantation, TH - pigs after transplantation treated with H_2 that was applied in gaseous form (3% H_2) during inhalation of anesthesia, as well as during oxygenation of blood in ECC. H_2 present in the arterial blood of pigs was measured using a needle-type Hydrogen Sensor. In this experiment, levels of creatinine, urea, and phosphorus in the plasma were detected. We focused also on the renal Na,K-ATPase activity, a key enzyme in maintaining intracellular sodium homeostasis. By methods of enzyme kinetics, depending on increasing concentrations of the energy substrate ATP and cofactor Na+ we have gained new insights into the functional properties of this enzyme in CS-AKI, as well as the potential effect of H_2 in these conditions. When activating the Na,K-ATPase with increasing concentrations of ATP, and/or cofactor Na+, its activity was lower in the whole concentration range in TH group vs. T. Evaluation of kinetic parameters revealed a significant decrease of the maximum velocity (Vmax) in TH group in comparison to T group (by 23,3% for ATP kinetic and by 19,7% for Na^+ kinetic) suggesting decreased sodium reabsorption. Unchanged Km values in both kinetics indicate that the cofactor affinity as well as the energy utilization by renal Na,K-ATPase remained unaffected after H_2 administration.

This experiment showed that the administration of H_2 had a protective effect on the kidneys of pigs after CS-AKI, especially in terms of normalization of urea, phosphate, and creatinine to control levels before cardiac surgery.

Keywords: molecular hydrogen, kidney, Na,K-ATPase, simulated heart transplantation

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FORMATION OF DYADS DURING POSTNATAL CARDIAC DEVELOPMENT IN RATS

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A fast increase of cytosolic Ca^{2+} concentration is necessary for the efficient contraction of cardiac myocytes. In adult mammals, this is achieved mostly by calcium-induced calcium release from the intracellular Ca^{2+} store (sarcoplasmic reticulum; SR) at specialized subcellular structures – dyads. Here, the sarcolemmal tubules come into contact with terminal cisternae of the SR containing ryanodine receptor calcium release channels (RyR2). The whole structure is held together, and the distance between the outer and inner membrane is maintained by the structural protein Junctophilin-2 (JP2). In newborn mammals, however, cardiac myocytes are not fully differentiated and still depend on Ca^{2+} influx from the extracellular space for their contraction. Transformation of excitation-contraction coupling (ECC) from the neonatal to the adult form involves structural and functional changes that are not well understood yet.

We studied the localization of the key proteins of cardiac excitation-contraction coupling - the sarcolemmal voltage-dependent calcium channels (Cav1.2) and the RyR2 and JP2 of SR in cardiomyocytes of developing rats on days 3 to 20 post-partum and of adult rat hearts. We have used confocal microscopy with multiplex immunofluorescent labelling and fluorescently conjugated wheat-germ agglutinin membrane probe, and transmission electron microscopy to identify the co-localization of specific antibodies and the development of dyads.

Localization of RyR2 channels followed sarcomeric distribution from the early days on. In contrast, Cav1.2 and JP2 were first localized predominantly at the surface sarcolemma, but later predominantly at the newly formed tubular sarcolemma, appearing in parallel to the growth of myocyte volume. This process was accompanied by an increased extent of co-localization of the three proteins, which occurred almost solely at the cell surface up to 7-8 days post-partum, but translocated to the cell interior during maturation. Fully developed dyads were observed by electron microscopy on day 4 only at the lateral sarcolemma, while on day 9 they were observed at both the lateral and tubular sarcolemma.

These findings indicate concerted development of dyads together with co-localization of their key proteins and proliferation of the sarcolemmal tubular system during the post-natal differentiation and growth of cardiomyocytes.

Keywords: EC coupling, dyads, cardiomyocytes

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SYMPATHOADRENERGIC CONTRACTIONS IN MESENTERIC ARTERIES FROM ZUCKER DIABETIC FATTY RATS: FOCUS ON PERIVASCULAR ADIPOSE TISSUE

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Obesity is associated with increased sympathetic nervous system activation, possibly contributing to higher cardiovascular risk. The aim was to study the sympathoadrenergic contractions in mesenteric arteries isolated from obese rats, and to assess the modulatory effect of mesenteric perivascular adipose tissue (PVAT). Experiments were performed on male 38-week-old Zucker lean (ZL) and Zucker diabetic fatty (ZDF) rats. Paired rings of isolated rat superior mesenteric arteries with preserved and removed PVAT were prepared and connected to a force-displacement transducer for recording of isometric tension. Contractile responses were elicited by increasing doses of exogenous noradrenaline and by endogenous noradrenaline released during electrical stimulation of perivascular adrenergic nerves. In PVAT-removed arterial preparations, ZDF rats had significantly enhanced contractile responses to sympathoneural stimulation comparing to ZL rats. Interestingly, preserving PVAT on arterial surface caused considerable anticontractile effect leading to significant reduction in adrenergic contractions of mesenteric arteries from ZDF rats. No vasomodulatory effect of PVAT was detected in mesenteric arteries from lean Zucker rats. The results of this study show that in obese Zucker rats, characterized by higher vascular sympathetic tone, the mesenteric arteries are specifically regulated by anticontractile effect of PVAT, leading to higher perfusion of mesenteric vascular bed. This could be associated with hyperphagia and increased nutrient-induced mesenteric vasodilatation in this rat strain.

Keywords: Zucker rat; mesenteric artery; adrenergic contraction; sympathetic nervous system; perivascular adipose tissue

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AUTHORS INDEX

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