ADVANCES IN CARDIOVASCULAR RESEARCH

From the bench to the patient's bed

International symposium
September 2 - 5, 2015

Dedicated to the 75th anniversary of Prof. Ján Slezák

Smolenice Castle - Congress center of the Slovak Academy of Sciences
Bratislava, Slovakia

Program & Book of Abstracts

Edited by T. Ravingerová and J. Slezák

VEDA
Publishing House of the Slovak Academy of Sciences
September 2015, Bratislava, Slovak Republic

ISBN 978-80-224-1452-4
ISBN 978-80-224-1453-1 (e-book)

Welcome address

Dear Colleagues and Friends,

It is our great pleasure to invite you to attend our traditional international symposium "Advances in Cardiovascular Research", which will be held in Smolenice Castle, the Congress Centre of the Slovak Academy of Sciences.
The Symposium will offer an opportunity to bring together renowned scientists; clinicians, as well as basic scientists from all areas of cardiovascular research, the field, which has become extremely important, challenging and rewarding over the last decades.
We hope to create an exciting, enjoyable and friendly atmosphere, which will be facilitated by your participation.
The Symposium will provide an excellent opportunity for an exchange of experience and new ideas and interaction between the experienced scientists and young investigators, which are of particular importance for young scientists. This forum will contribute to our understanding of the complexity of molecular processes and cellular pathways leading to major cardiovascular pathologies, as well as to understanding of the processes of reparation leading to cell survival. Moreover, novel approaches to the management of cardiovascular diseases will be discussed as well.

We hope that despite the tight scientific schedule, there will be sufficient space for fruitful and stimulating discussions and chances to enjoy the beautiful nature of the Small Carpathian Mountains and cultural spirit of Slovakia.

In addition to our program, this year we decided to commemorate the 10^{th} jubilee of the "Advances in Cardiovascular Research" meetings by a special event: some distinguished participants of the meeting will plant Linden trees, as a new tradition.

Looking forward to meeting you in Smolenice Castle

Ján Slezák, Táňa Ravingerová

MEETING ORGANIZERS

Ján Slezák (Honorary President),
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GENERAL INFORMATION

Venue and date

Congress Center of SAS – Smolenice Castle, Slovak Republic
September 2nd – 5th, 2015

Registration

Wednesday, September 2nd, 2015, 15.00 – 17.00

Language

The official language – English

Accommodation

On the premises of the Castle and in Smolenice village

Information for presenters

Oral presentations – 20 min including discussion;
♣ - Young investigators Oral and Poster Competition. Oral presentations: 10 min including discussion.

Posters should be mounted after the registration ACCORDING TO THEIR NUMBER IN THE LIST OF POSTERS. The authors should be present during the Moderated Poster session. Poster size – 80 x 120 cm (vertical).

PROGRAM OVERVIEW

Wednesday, September 2nd

15:00 – 17:00	Arrival to Smolenice, registration, accommodation
17:00 – 18:30	Opening of the meeting. **Session I**.
19:00	*Welcome Party & Dinner*

Thursday, September 3rd

08:00 – 09:00	Breakfast
09:00 – 10:20	**Session II**. PART I.
10:20 – 10:50	*COFFEE BREAK and PHOTO*
10:50 – 13:00	**Session II**. PART II.
13:00 – 14:00	*LUNCH*
14:00 – 15:40	**Session III**. PART I.
15:40 – 16:00	*COFFEE BREAK*
16:00 – 17:10	**Session III**. PART II.
17:10 – 19:00	Moderated Poster session. (Cheese & Wine)
19:00	*Dinner*

Friday, September 4th

07:30 – 08:30	Breakfast
08:30 – 09:40	**Session III**. continued. PART III.
09:45 – 11:25	**Session IV**. PART I.
11:25 – 11:40	*COFFEE BREAK*
11:40 – 13:00	**Session IV**. PART II.
13:00 – 14:00	*LUNCH*
14:00 – 15:00	*Linden trees* planting
15:00	Special Session dedicated to 75th anniversary of Prof. Ján Slezák Greetings
16:30 – 20:00	Wine tasting – Garden Party
	Announcement of the Winners of Young Investigator Oral and Poster Competitions. Awards giving ceremony.

Saturday, September 5th

08:00 – 09:00	Breakfast
09:00 – 11:50	**Session V**.
11:50 – 12:10	Plenary session of IACS-ES.
12:15	Closing remarks
12:30 – 13:30	*LUNCH*
14:00	Departure

WEDNESDAY, September 2nd

15:00 – 17:00	**Arrival to Smolenice, registration.**
17:00 – 17:20	**Opening of the meeting. Welcome greetings**
17:30 - 18:30	**Session I. Novel strategies to combat cardiovascular diseases** ***Chairs: J. Slezák (Slovak Republic)*** ***T. Ravingerová (Slovak Republic)***
17:30 - 17:50	**N. S. Dhalla (Winnipeg, Canada)** CO_2 Bath as a Novel Therapy for Peripheral Vascular Disease
17:50 - 18:10	**B. Ošťádal (Prague, Czech Republic)** Exercise induced cardioprotection
18:10 - 18:30	**M. Karmazyn (London, Ontario, Canada)** Attenuation of Myocardial Remodelling, Hypertrophy and Heart Failure by Natural Products
19:00	**Welcome Party & Dinner**

THURSDAY, September 3rd

09:00 – 13:00	**Session II. Excitation-contraction coupling in the heart under physiological and pathological conditions; pharmacological interventions**
09:00 – 10:20	***PART I. Electrophysiological aspects of heart function*** ***Chairs: A. Varró (Hungary), N. Tribulová (Slovak Republic)***
09:00 – 09:20	**A. Varró (Szeged, Hungary)** NCX inhibition and Ca handling
09:20 – 09:40	**I. Baczkó (Szeged, Hungary)** Comparison of the cardiac electrophysiological and antiarrhythmic effects of chronic amiodarone and desethylamiodarone administration in dogs

09:40 – 10:00	**F. Kolář (Prague, Czech Republic)** Determinants of antiarrhythmic protection in chronically hypoxic hearts
10:00 – 10:10	**♣ T. Rajtik, A. Szobi, M. Lichy, Z.V. Varga, G. Doka, P. Musil, E. Goncalvesova, M. Hulman, P. Leszek, M. Kusmyerczyk, J. Kyselovic, P. Ferdinandy, A. Adameova (Bratislava, Slovak Republic; Budapest, Hungary; Warszawa, Poland)** Role of CaMKIIδ in end-stage human heart failure
10:10 – 10:20	**♣ K. Frimmel, J. Križák, M. Breierová, B. Lipták, J. Navarová, V. Knezl, I. Bernátová, Ľ. Okruhlicová (Bratislava, Slovak Republic)** Effect of carotenoids on cx40 and cx43 expression in left ventricle of normotensive rats during inflammation
10:20 – 10:50	**Coffee break, PHOTO**
10:50 – 13:00	***PART II: Contractile function of the heart: can it be restored? Reparative processes versus cardiac cell modifications leading to loss of the functional tissue.*** ***Chairs: P. K. Singal (Canada), F. Kolář (Czech Republic)***
10:50 – 11:10	**P. K. Singal (Winnipeg, Canada)** Innate signalling in heart failure
11:10 – 11:30	**A. Adameová (Bratislava, Slovak Republic)** New insights into cardiac contractility in pathologically-modified heart
11:30 – 11:50	**M. Czubryt (Winnipeg, Canada)** Targeting Scleraxis: Cardiac Fibrosis in the Crosshairs
11:50– 12:10	**N. Tribulová (Bratislava, Slovak Republic)** Ultrastructural alterations reflecting disorders in $Ca2^{+}$ handling and cell-to-cell coupling precede acute heart failure
12:10 – 12:30	**D. Djuric (Belgrade, Serbia)** Homocysteine and thiolactone metabolites: progress in cardiovascular research
12:30 – 12:50	**J. Groenendyk (Edmonton, Alberta, Canada)** ER stress in cardiac fibrosis and heart failure
12:50 – 13:00	**♣ A. Szobi, E. Goncalvesova, T. Rajtik, M. Lichy, ZV. Varga, P. Leszek, M. Kusmierczyk, M. Hulman, G. Doka, P. Musil, J. Kyselovic, P. Ferdinandy, A. Adameova (Bratislava, Slovak Republic; Budapest, Hungary; Warszawa, Poland)** Relevance of necroptosis in pathologies of the cardiovascular system.

13:00 – 14:00	**Lunch**
14:00 – 17:10	**Session III. Constitutive and inducible forms of cardioprotection. Novel drug therapy and new approaches to management of cardiovascular diseases**
14:00 – 15:40	***PART I.***
	Chairs: B. Ošťádal (Czech Republic) ***G. N. Pierce (Canada)***
14:00 – 14:20	**A. Lazou (Thessaloniki, Greece)** Exploring newer cardioprotective strategies: PPARs in perspective
14:20 – 14:40	**T. Ravingerová (Bratislava, Slovak Republic)** Mending the "broken" heart: from molecular mechanisms to the bedside
14:40 – 15:00	**M.-S. Suleiman (Bristol, UK)** Is Propofol Cardioplegia Protective in Patients Undergoing Coronary Artery Bypass Grafting or Aortic Valve Replacement?
15:00 – 15:20	**R. Hatala, P. Hlivák (Bratislava, Slovak Republic)** Can we cure atrial fibrillation? A critical reappraisal from cell to bedside.
15:20 – 15:30	**♣ D. Iacobazzi, H Lin, M Caputo, M Ghorbel, R Tulloh & M.-S. Suleiman (Bristol, UK)** Developmental changes in PDE5A expression and strategies for cardioprotection.
15:30 – 15:40	**♣ V. Ledvényiová-Farkašová, S. Čarnická, M. Murárikova, L. Griecsová, I. Gablovský, F. Kolář, T. Ravingerová (Bratislava, Slovak Republic)** Remote ischemic preconditioning in six-months old male and female rats and its impact on the hearts exposed to ischemia/reperfusion injury: intrinsic mechanisms behind
15:40 – 16:00	**Coffee break**
16:00 – 17:10	***PART II.***
	Chairs: D. Djuric (Serbia), O. Pecháňová (Slovak Republic)
16:00 – 16:20	**D. Muntean (Timisoara, Romania)** Novel Pharmacological Modulators of the Cardiac ATP-sensitive Potassium Chanels
16:20 – 16:40	**O. Pecháňová (Bratislava, Slovak Republic)** Effects of nanoparticle-loaded aliskiren in the cardiovascular system of hypertensive rats

16:40 – 17:00	**V. Jakovlevic (Kragujevac, Serbia)** Oxidative stress in exercise: from basic science to applied clinical investigations
17:00 – 17:10	**♣ M. Zálešák (Bratislava, Slovak Republic)** Influence of ischemic preconditioning and simulated hyperglycemia on heart resistance to ischemia/reperfusion injury
17:15 – 19:00	**Moderated Poster session. Cheese and wine.** ***PART I*: Posters 1-17** ***Chairs: D. Muntean (Romania), M. Czubryt (Canada), Miroslav Ferko (Slovak Republic),*** ***PART II*: Posters 18-34** ***Chairs: M. Karmazyn (Canada), I. Baczko (Hungary), J. Neckar (Czech Republic)***
19:00	**Dinner**
	FRIDAY, September 4th
08:30 – 09:40	**Sesssion III. cont.** ***PART III.*** ***Chairs: N.S. Dhalla (Canada), P. Ferdinandy (Hungary)***
08:30 – 08:50	**P. Ferdinandy (Budapest, Hungary)** Why do we still not have cardioprotective drugs? Importance of the "omics" approach
08:50 – 09:10	**E. Gonçalvesová (Bratislava, Slovak Republic)** Long-term ventricular assist devices - a challenge for basic research
09:10 – 09:30	**M. Barteková (Bratislava, Slovak Republic)** Mechanisms of oxytocin action in the heart
09:30 – 09:40	**♣ V.L. Mascetti, RA Pedersen (Cambridge, UK)** Human-Mouse Chimerism Validates In Vitro Derived Human Pluripotent Stem Cells and Their Cardiovascular Progeny
09:45 – 13:00	**Session IV. Vessels and their contribution to cardiovascular diseases: are they not neglected in assessment of cardiovascular mortality?**

09:45 – 11:25	***PART I.***
	Chairs: A. Srivastava (Canada), I. Bernátová (Slovak Republic)
09:45 – 10:05	**N. Maulik (Farmington, Connecticut, USA)** New Molecular Targets of VEGF Signaling in Cardiovascular Disease
10:05 – 10:25	**A. Srivastava (Montreal, Quebec, Canada)** Role of Egr-1 in vascular biology.
10:25 – 10:45	**L. H. Kurahara (Fukuoka, Japan)** TRP channels in vascular disorders
10:45 – 11:05	**G. Bkaily (Sherbrooke, Quebec, Canada)** Reactive oxygen species and nuclear calcium homeostasis of human vascular smooth muscle cells
11:05 – 11:25	**I. Bernátová (Bratislava, Slovak Republic)** Endothelial dysfunction and oxidative stress in hypertension: Chicken-and-egg problem
11:25 – 11:40	**Coffee break**
11:40 – 13:00	***PART II.***
	Chairs: G. Bkaily (Canada), M.-S. Suleiman (UK)
11:40 – 12:00	**M. Anand-Srivastava (Montreal, Quebec, Canada)** Role of Natriuretic peptide receptor C in the regulation of blood pressure
12:00 – 12:20	**D. Jacques (Sherbrooke, Quebec, Canada)** Endocardial endothelium and cardiac hypertrophic factors
12:20 – 12:40	**S. Ramachandran, C.C. Kartha (Trivandrum, India)** Cyclophilin A - a serological marker for vascular disease in type 2 diabetes
12:40 – 13:00	**J. Beltowski (Lublin, Poland)** Effect of high-fat diet on hydrogen sulfide production in perivascular adipose tissue – implications for cardiovascular diseases
13:00 – 14:00	**Lunch**
14:00	*Lindentrees* **planting** ☺
15:00	**Special Session dedicated to 75th anniversary of Prof. Ján Slezák** **Greetings**
	Wine tasting – Garden Party

19:00	**Farewell Dinner.** **Announcement of the Winners of Young Investigator Oral and Poster Competitions.** ***Supported by the Educational grant for the IACS Awards Program provided by Mitsubishi Rayon Cleansui Co. Ltd., Tokyo, Japan***
	SATURDAY, September 5th
09:00 – 11:50	**Session V. Non-cardiac pathologies leading to heart damage and dysfunction** ***Chairs: G. N. Pierce (Canada), J. Slezák (Slovak Republic)***
09:00 – 09:20	**J. Slezák (Bratislava, Slovak Republic)** Possibilities of preventing cardiovascular injury caused by therapeutic radiation of the mediastinum
09:20 – 09:40	**M. Barančík (Bratislava, Slovak Republic)** The role of matrix metalloproteinases in chronic responses of rat hearts to irradiation and doxorubicin treatment
09:40 – 10:00	**G. N. Pierce (Winnipeg, Canada)** The influence of chlamydia pneumoniae infection on cardiovascular disease
10:00 – 10:20	**J. Török, A. Zemančíková (Bratislava, Slovak Republic)** Visceral perivascular adipose tissue regulates arterial smooth muscle responsiveness
10:20 – 10:40	**Coffee break**
10:50 – 11:10	**G. Wallukat, W. Schulze (Berlin, Germany)** Pathogenic role of functional autoantibodies against G-protein couple receptors in cardio-vascular diseases: Use of aptamers as a new therapeutic option
11:10 – 11:30	**K. Kjeldsen (Copenhagen, Denmark)** Potassium shifts during exercise as trigger of sudden cardiac death
11:30 – 11:50	**Hari S. Sharma (Amsterdam, The Netherlands)** Calcium Homeostasis and Cardiovascular Dysfunction: Relevance to the Vitamin D
11:50 – 12:10	**Plenary Session of IACS-ES**
12:15	**Closing remarks**
12:30 – 13:30	**Lunch**
14:00	**Departure**

Poster presentations

1. **A. Barta, M. Cebová, J. Klimentová, S.Vranková, Z. Matúšková, R. Reháková, M. Kovácsová, V. Závišová, M. Koneracká, P. Kopčanský, O. Pecháňová** (Bratislava, Slovakia): Structural changes in myocardium and aorta after aliskiren-iduced inhibition of renin-angiotensin-aldosterone system.
2. **J. Dienová, K. Frimmel, Ľ. Okruhlicová** (Bratislava, Slovakia): Endotoxin- and omega-3 fatty acids-induced changes of endothelial occludin expression in heart.
3. **O. M. Duicu, A. Sturza, A. Anechitei, M. Dănilă, L. Noveanu, D. M. Muntean** (Timisoara, Romania): Methylene Blue Modulates Mitochondrial Function in Diabetic Rat Hearts.
4. **M. Ferko, M. Jašová, I. Kancirová, I. Waczulíková, S. Čarnická, M. Muráriková, J. Kucharská, O. Uličná, O. Vančová , A. Chytilová , T. Ravingerová, A. Ziegelhöffer †** (Bratislava, Slovakia; Prague, Czech Republic): Short-term adaptation of the heart induced by pathological stimuli: The role of free radicals.
5. ♣ **T. Benova, C. Viczenczova, V. Knezl, J. Radosinska, B. Szeiffova Bacova, J. Navarova, M. Zeman, N. Tribulova** (Bratislava, Slovakia): Benefit of melatonin and omega-3 fatty acids intake in rats exposed to sucrose diet.
6. **M. Hlavackova, G. Borchert, K. Holzerova, J. Zurmanova, J. Neckar, F. Novak, O. Novakova, T. Ravingerová, F. Kolar** (Prague, Czech Republic; Bratislava, Slovakia): Involvement of PKCepsilon and BKCa Channels in cardioprotection induced by adaptation to chronic continuous hypoxia.
7. ♣ **L. Griecsová, V. Farkašová, I. Gablovský, I. Bernátová, Z. Tatarková, T. Ravingerová** (Bratislava, Martin, Slovakia): Maturation-related changes in response to ischemia-reperfusion injury and adaptation in male rat hearts: Study of potential molecular mechanisms.
8. **J. Hrdlička, J. Neckář, S. Čarnická, F. Papoušek, J. Vašinová, P. Alánová, F. Kolář** (Prague, Czech Republic; Bratislava, Slovakia): Effect of continuous normobaric hypoxia and moderate exercise training on postinfarction heart failure in rats.
9. ♣ **M. Jašová, I. Kancirová, M. Muráriková, S. Čarnická, I. Waczulíková, A. A. Chytilová, Ziegelhöffer †, M. Ferko** (Prague, Czech Republic; Bratislava, Slovakia): Remote ischemic preconditioning induces changes in the structural properties of heart mitochondria.

10. ♣ **I. Kancirová, M. Jašová, M. Muráriková, S. Čárnická, Z. Sumbalová, O. Uličná, T. Ravingerová, A. Ziegelhöffer †, M. Ferko** (Bratislava, Slovakia): Energy metabolism in the remote ischemic preconditioned and diabetic rat heart.

11. **B. Kaločayová, E. Sekereš, L. Mézešová, V. Jendruchová, J. Vlkovičová, N. Vrbjar** (Bratislava, Slovakia): Effect of experimental Diabetes mellitus type 1 on the cardiac Na,K-ATPase in female rats.

12. ♣ **A. Sturza, O. Duicu, A. Vaduva, L. Noveanu, A. Privistirescu, M. Danilă, M. Munteanu, R. Timar, D. Muntean** (Timisoara, Romania): Calcitriol modulates vascular expression of receptor for advanced glycation end products and monoamine oxidase in experimental diabetes.

13. **M. Cebová, J. Klimentová, A. Barta, Z. Matúšková, R. Reháková, O. Pecháňová** (Bratislava, Slovakia): The effect of aronia melanocarpa on cardiovascular system in L-NAME-induced hypertension.

14. **E. R. Diez, N.J. Prado, T. Benova, B. Szeiffova Bacova, C. Viczenczova, N. Tribulova, R. M. Miatello** (Mendoza, Argentina; Bratisava, Slovakia): Wine grape pomace protects against reperfusion arrhythmias in dysmetabolic rats.

15. ♣ **M. Kluknavsky, P. Balis, A. Puzserova, I. Bernatova** (Bratislava, Slovakia): (-)-Epicatechin reduced blood pressure, motor activity and improved vascular reactivity in young spontaneously hypertensive male rats.

16. ♣ **B. Kaprinay, R. Sotníkova, B. Lipták, V. Knezl, J. Navarová, I. Bernátova, K. Frimmel, J. Križák, Ľ. Okruhlicová** (Bratislava, Slovakia): Detrimental effect of LPS-induced inflammation on the rat heart and vessels function.

17. **L. Mézešová, V. Jendruchová, J. Vlkovičová, Ľ. Okruhlicová, K. Frimmel, J. Navarová, Z. Brnoliaková, N. Vrbjar** (Bratislava, Slovakia): Effect of LPS-induced inflammation on renal Na,K-ATPase in male rats.

18. ♣ **J. Krizak, E. Breierová, K. Frimmel, J. Navarová, R. Sotníková, Ľ. Okruhlicová** (Bratislava, Slovakia): Can carotenoids affect aortic endothelial occludin expression during inflammation?

19. ♣ **B. Kura, R. C. Kukreja, Ch. Yin, A. K. Bagchi, N. Bernardes, P. K. Singal, M. Fülöp, A. Šagátová, J. Slezák** (Bratislava, Slovakia; Richmond, Virginia, USA; Winnipeg, Canada): Expression of miRNAs and TNF-α in irradiated rat myocardium and potential targets for mitigation of injury.

20. ♣ **K. Frimmel, B. Kura, Ľ. Okruhlicová, J. Slezák** (Bratislava, Slovakia): Changes in the expression of connexins 40 and 43 of the rat aortic tissue 6 weeks after mediastinum irradiation.

21. ♣ **C. Viczenczová, B. Szeiffová Bačová, B. Kura B, T. Beňová, J. Slezák, N. Tribulová** (Bratislava, Slovakia): Effect of treatment with aspirin and atorvastatin on myocardial connexin-43 after irradiation of rat heart.

22. **B. Kura, M. Zálešák, J. Graban, T. Ravingerová, D. Pancza, N. Tribulová, J. Slezák** (Bratislava, Slovakia): Protective effect of molecular hydrogen on the heart in situations of increased production of oxygen free radicals: radiation and reperfusion injury.

23. ♣ **C. Norris, W. Kafienah, R. Ascione** (Bristol, UK): Investigating the regenerative potential of human pericardial adipose tissue-derived stem cells.

24. **J. Neckar, D. Kasparova, J. Novotny, J. Zurmanova, Slavka Carnicka, F. Kolar** (Prague, Czech Republic; Bratislava, Slovakia)**:** Myocardial mRNA levels of antioxidant enzymes in rats adapted to protective and non-protective regimens of chronic hypoxia.

25. **P. Novák, V. Marková, V. Sulimenko, N. Tribulová, T. Soukup** (Prague, Czech Republic; Bratislava, Slovakia): Expression of calcium binding proteins in skeletal and heart muscles of rats with altered thyroid status.

26. **H. Rauchova, M. Vokurkova, S. Pavelka, M. Behuliak, N. Tribulova, T. Soukup** (Prague, Czech Republic; Bratislava, Slovakia): Six week lasting supplementation with n-3 polyunsaturated fatty acids does not significantly affect changes of lipid metabolism induced in male inbred strain Lewis rats by altered thyroid status.

27. ♣ **S. Satta, S. J. George, R. Ascione** (Bristol, UK): Cellular recruitment and matrix remodelling of biological vascular grafts following transplantation in a porcine carotid model.

28. **J. Radosinska, E. Giannakos, E. Vardali, M. Bartekova, M. Fogarassyova, M. Barancik** (Bratislava, Slovakia; Tessaloniki, Greece): The role of MMPs in relation to heart failure.

29. ♣ **N. Sulaiman, S. Satta, S. J. George, M.-S. Suleiman, R. Ascione** (Bristol, UK; Kuala Lumpur, Malaysia): Effect of decellularization protocol of human saphenous veins on cytotoxicity and matrix component.

30. ♣ **B. Szeiffová Bačová, C. Viczenczová, T. Beňová, J. Radošinská, P. Seč, M. Čertík, N. Tribulová** (Bratislava, Slovakia; Berlin, Germany): Omega-3 fatty acids moderate susceptibility of the heart to lethal arrhythmias in aged male and female spontaneously hypertensive rats.

31. **M. Zálešák, J. Graban, B. Kura, D. Pancza, T. Ravingerová, J. Slezák** (Bratislava, Slovakia): Molecular hydrogen reduces ischemia/reperfusion injury in the isolated rat heart subjected to postconditioning: novel cardioprotective intervention.

32. ♣ **K. Zidlikova, Z. Kulhova, N. Tribulova, T. Benova, I. Ellinger, M. Zeman** (Bratislava, Slovakia; Vienna, Austria): Structural changes of microcirculation in the brain after melatonin treatment in control and spontaneously hypertensive rats.

33. **A. Chytilova, Z. Drahota, V. Ledvényiová-Farkašová, R. Weissova, M. Kalous, M. Pravenec, O. Novakova, J. Neckar** (Prague, Czech Republic; Bratislava, Slovakia): Chronic continuous hypoxia increases the reserve activity of cytochrome-c oxidase in hearts of spontaneously hypertensive rats.

34. **Z. Matuskova, S. Vrankova, A. Barta, J. Murinova, J. Klimentova, M. Kovacsova, R. Rehakova, M. Cebova, I. Riecansky, O. Pechanova** (Bratislava, Slovakia): Social-isolation rearing and changes of nitric oxide synthase activity in brain and cardiovascular system.

ABSTRACTS OF ORAL PRESENTATIONS

NEW INSIGHTS ON EXCITATION-CONTRACTION COUPLING: A ROLE OF POSTTRANSLATIONAL MODIFICATIONS OF CAMKII

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Ca^{2+}/calmodulin-dependent protein kinase, and particularly its delta isoform (CaMKIIδ), has emerged as an interesting protein regulating various processes within cardiac myocytes. It can be activated by the increased levels of Ca^{2+}, and by posttranslational modification through phosphorylation and oxidation ($p\text{-}Thr^{287}$ and $oxMet^{281/282}$, respectively) indicating the capability of a sustained overactivaton in the presence of Ca^{2+} deregulation and oxidative stress. Although great research has been undertaken in this field, it is still not very clear whether and how the posttranslationally-modified forms of CaMKIIδ contribute to its entire activity and finally to disturbances in cardiac excitation-contraction coupling.

Acute ischemic attacks and chronic coronary artery disease leading into heart failure are characterized by worsening of cardiac pump function which in turn is dependent on a regular control of contractile myofilaments and proper Ca^{2+} cycling. As CaMKIIδ activates proteins involved in both these mechanisms, it seems to be likely that this protein kinase may underscore contractile dysfunction in these pathologies. In the present work, the posttranslational modifications of CaMKIIδ and their consequences on the cycle of contraction and relaxation, regulated by cardiac myosin-binding protein-C (cMyBP-C), and on sarcoplasmic reticulum Ca^{2+} storage/uptake will be presented. In addition, a role of the active forms of CaMKIIδ on arrhythmias development will be discussed.

Supported by VEGA 1/0638/12.

THE ROLE OF MATRIX METALLOPROTEINASES IN CHRONIC RESPONSES OF RAT HEARTS TO IRRADIATION AND DOXORUBICIN TREATMENT

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Matrix metalloproteinases (MMPs) are enzymes that play an important role in degradation and remodeling of extracellular matrix. These proteins are also suggested to play an important role in pathogenesis of several diseases. MMP-2 has been shown as a mediator of the acute mechanical dysfunction of the heart after ischemia/reperfusion and increased circulating MMPs were found to be closely associated with the development of heart failure.

The current study investigated the involvement of MMPs during development of chronic effects induced by mediastinal irradiation (MI) or doxorubicin (DOX) treatment and their potential role in pathology of radiation- and/or DOX-induced cardiovascular toxicity in rats. Determined were the alterations in levels/activation of myocardial and circulating MMPs. Moreover, the effects of potential protective substances on MMPs were studied.

In the study were used male Wistar rats exposed to heart and lung irradiation (single dose of 20 Gy) or treatment with DOX (cumulative dose 15mg/kg BW). Samples of heart tissue or plasma were used for further investigations.

We found that chronic effects of both MI and DOX treatment were associated with significant up-regulation of circulating 72 kDa MMP-2 activities. The effects of DOX were also linked to a stimulation of plasma MMP-9. By the study of effects of potential protective substances we found that application of acetylsalicylic acid or atorvastatin markedly reduced the effects of MI on circulating MMP-2. MI had different effects on modulation of MMP-2 activities in the right (RV) and the left (LV) ventricle. In RV, the activities of 72 kDa MMP-2 decreased after MI. On the other hand, in LV led MI to up-regulation of 72 kDa MMP-2. Application of DOX led in LV, similarly to the efects of MI, to MMP-2 activation at eight weeks after the end of DOX treatment. The effects of MI or DOX on MMP-2 activities were not connected with significant modulation of protein levels of this enzyme.

The observed data suggest that the activation of MMP-2 may have a negative impact on the progression of pathological changes induced in consequence of mediastinal irradiation and/or doxorubicin treatment.

Supported by grants: VEGA-SR 2/0108/15, 2/0021/15, APVV-0241-11, APVV-0348-12.

COMPARISON OF THE CARDIAC ELECTROPHYSIOLOGICAL AND ANTIARRHYTHMIC EFFECTS OF CHRONIC AMIODARONE AND DESETHYLAMIODARONE ADMINISTRATION IN DOGS

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Amiodarone (AMIO) remains one of the most effective antiarrhytmic agents with lower proarrhythmic risk compared to other currently used antiarrhythmic compound. However, AMIO exhibits serious extracardiac adverse effects which limit its clinical use. It has been suggested that desethylamiodarone (DEA), the active metabolite of AMIO may possess cardiac electrophysiological effects similar to the parent compound. We hypothesized that chronic DEA treatment would exert similar electrophysiological and antiarrhythmic effects compared to chronic AMIO application, with reduced AMIO related adverse effects.

In conscious Beagle dogs, RR, QT, QT_c intervals were measured from ECGs, short-term variability of the QT interval (STV_{QT}) was calculated. Action potential (AP) (V_{max}, APD_{90}) parameters and cardiac ionic currents were measured by conventional microelectrode and patch-clamp techniques, following 4-week oral DEA (30 mg/kg/day) and AMIO (45 mg/kg/day) administration. AMIO and DEA tissue levels were also measured. For the atrial fibrillation studies, dogs were subjected to right atrial and right ventricular pacemaker implantation and radiofrequency catheter ablation of the AV node, followed by a 4-6 week atrial tachypacing at 400/min. Baseline ECG measurements were taken by inducing AF with 10-second long 800/min burst stimulation 25 times and AF episodes were recorded. Animals were then orally administered either 50 mg/kg/day AMIO or 25 mg/kg/day DEA for 4 weeks, followed by another series of AF induction.

Significantly increased RR (by 13.3%; 39.4%) and prolonged QT (by 14.5%; 23.1%) and QTc (by 9.6%; 11.3%;) intervals were measured following both 4-week DEA and AMIO administration compared to baseline. No differences were observed in STV_{QT}. V_{max} was significantly reduced by 21.0% and 14.4%, moreover APD_{90} was slightly but significantly prolonged by 6.0% and 10.0% as a result of DEA or AMIO application compared to control. Decreased I_{to} current in the AMIO group, significantly reduced I_{Kr} current and a decreasing trend in I_{KACh} current in both DEA and AMIO groups were observed. The length of AF episodes was significantly reduced by both AMIO (log_{10} duration of episodes 0.8±0.36 vs. 3.4±0.52 at baseline) and DEA administration (1.1±0.41 vs. 3.4±0.24 at baseline). Left atrial tissue levels for DEA was higher in the AMIO treated group compared to the DEA treated group (11.9±0.44 vs. 5.4±0.84 μg/tissue g) with the presence of high AMIO levels in the AMIO treated group (35.2±1.96 μg/tissue g). AMIO treatment resulted in more than 4 times higher lung (+441%) and 5 times higher liver (+553%) DEA levels with the presence of high AMIO levels as well in all investigated tissue types.

In conclusion, these results suggest that it may be possible to substitute chronic AMIO treatment with chronic DEA application that could represent a similarly effective but significantly safer therapeutic option for the management of arrhythmias, including atrial fibrillation.

Supported by the Hungarian Scientific Research Fund (OTKA K 109610), and TÁMOP-4.2.2.B-15/1/KONV-2015-0006.

MECHANISMS OF OXYTOCIN ACTION IN THE HEART

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Oxytocin is a neurohypophysal hormone that exerts its main physiological effects in the reproduction system, e.g. uterokinetic effects during delivery or milk releasing effects during nursing. On the other hand, oxytocin receptors have been found to be present also in the heart suggesting its role in the control of cardiovascular system (CVS) function. Additionally, oxytocin belongs to the hormones which levels are markedly elevated during intensive stress. CVS belongs to the systems which are often negatively influenced by chronic stressors. However, the role of oxytocin in the heart tissue in stress situations as well as its role in the heart in general is not fully clarified. Therefore the aims of the present studies were: i, to explore the effect of prolonged treatment of rats with oxytocin on heart function and resistance to ischemia/reperfusion (I/R) injury; ii, to explore molecular mechanisms involved in oxytocin action in the heart tissue and iii, to find out how blockade of oxytocin receptors during repeated stress influence molecular pathways in the heart tissue.

Male Wistar rats were treated with oxytocin or vehicle via subcutaneously implanted osmotic minipumps for 2 weeks. At the end of the treatment, hearts from the rats were isolated and Langendorff perfused to test their resistance to I/R injury maintained by 25 min global ischemia and consequent 120 min reperfusion. In the second part of the study rats were exposed to repeated immobilization (2 hours daily, lasting 2 weeks) and simultaneously treated (or non-treated) with atosiban, an oxytocin and vasopressin receptor antagonist. Levels of selected proteins in the heart tissue were analysed.

The results showed enhanced tolerance of rat hearts to I/R in consequence of oxytocin treatment. This was associated with enhanced phosphorylation of Akt kinase and p-38 MAPK in heart tissue as well as increased levels of phospho-HSP-27 and ANP. Additionally, exposition of rats to stress paradigm led to increased phosphorylation of Akt, increased expression of Bcl-2 and decreased level of cleaved caspase-3. However, blockade of oxytocin receptors failed to modify these changes. On the other hand, treatment with atosiban reversed increase of HSP-90 and its substrate p53 that were elevated due to stress exposition.

In conclusion, our results showed positive effects of oxytocin in I/R injury of rat hearts and a potential role of Akt kinase phosphorylation in oxytocin action in the heart. On the other hand, the role of oxytocin in the heart during prolonged stress still remains the matter of doubt, however, our data suggest certain regulatory role of oxytocin in stress-induced changes of HSP-90 and related proteins.

Supported by VEGA SR 2/0140/12 and 2/0128/14

EFFECT OF HIGH-FAT DIET ON HYDROGEN SULFIDE PRODUCTION IN PERIVASCULAR ADIPOSE TISSUE – IMPLICATIONS FOR CARDIOVASCULAR DISEASES

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Perivascular adipose tissue (PVAT) surrounds most of the large and medium-sized arteries. "Healthy" PVAT secretes mediators which dilate the vessel as well as inhibit smooth muscle cell proliferation and inflammatory reaction. Recent studies suggest that endogenous hydrogen sulfide (H_2S), synthesized from L-cysteine by cystathionine gamma-lyase (CSE), contributes to PVAT-dependent vasorelaxation. High-fat diet is the well-known risk factor of cardiovascular diseases such as arterial hypertension and atherosclerosis. We examined the effect of high-fat diet on H_2S system in perivascular adipose tissue. Male Wistar rats were fed standard or high-fat diet for either 1 or 3 months. H_2S production by periaortic adipose tissue (PAT) was measured ex vivo by the polarographic sensor. In addition, phenylephrine-induced contractility of isolated aortic rings with intact or removed adipose tissue was examined. High-fat diet (HFD) had a time-dependent effect on H_2S production by PAT. Both H_2S production and anticontractile effect of PAT on aortic rings were augmented in rats fed HFD for 1 month but reduced in animals fed HFD for 3 months in comparison to the control group. CSE expression and activity in PAT was similar in rats fed standard or high-fat diet for 1 month but decreased significantly after 3 months of high-fat feeding. By measuring H_2S production in the absence as well as in the presence of stigmatellin which inhibits mitochondrial H_2S oxidation, we demonstrated that in rats receiving HFD for 1 month H_2S synthesis is normal but its metabolism is impaired. Markers of mitochondria density in PAT such as the amount of mtDNA, citrate synthase and cytochrome c were lower in HFD-fed than in lean animals, as was the expression of transcription factors involved in mitochondrial biogenesis, PGC-1, NRF-1 and Tfam. Effects of obesity on H_2S production, anticontractile properties of PAAT and mitochondria density/biogenesis were reversed by cannabinoid CB_1 receptor antagonist, rimonabant, which had, however, no effect in rats fed standard chow. In addition, endogenous cannabinoid system was stimulated in PAT of HFD-fed rats as evidenced by higher concentration of 2-arachidonyl-glycerol and the expression of CB_1 receptor. In conclusion, high-fat feeding has a time-dependent effect on H_2S system in perivascular adipose tissue with stimulation or inhibition after short- and long-term HFD, respectively. Up-regulation of H_2S after short-term HFD feeding may be a compensatory mechanism to maintain vascular homeostasis despite endothelial dysfunction. In contrast, inhibition of H_2S system induced by long-term HFD may contribute to the increase in vascular resistance and atherogenesis.

ENDOTHELIAL DYSFUNCTION AND OXIDATIVE STRESS IN HYPERTENSION: CHICKEN-AND-EGG PROBLEM

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Endothelium produces various substances collectively termed endothelium-derived factors. There is complex cross-talk among the individual endothelium-derived factors with the aim to maintain appropriate endothelial function and its dysregulation results in alteration of normal physiological processes carried out by the endothelium i.e. in endothelial dysfunction (ED). ED was found in human primary hypertension as well as in various animal models of arterial hypertension which have been developed in rodents to study the mechanisms involved in development of high blood pressure (BP). However the issue if ED is a cause or consequence of high BP is still open. The most frequently used genetic model of hypertension is a model of spontaneously hypertensive rats (SHR). This model produces inconsistent results regarding the role of ED in the development of hypertension as the findings of ED in SHR depend on many factors (age, sex, artery type, methods used for determination of ED). In many studies ED in SHR was observed later than increased BP, suggesting that ED is rather secondary to BP. Similarly, our findings observed in borderline hypertensive rats (BHR) showed that increase of BP precedes development of ED. Moreover, no differences in TEAC, SOD, CAT and GPx in blood were observed in BHR vs. WKY. Furthermore, no signs of oxidative damage to blood lipids were found in young BHR and SHR, thus oxidative stress in blood does not seem to be causatively related to development of hypertension in these models of (pre)hypertension. However, despite activated antioxidant defence system in SHR, positive correlation between TEAC and lipid peroxidation in blood suggests slow progress of oxidative damage that seems to be rather the consequence than the cause of hypertension. On the other hand, both oxidative stress and ED were observed in various pharmacological and diet-induced models of hypertension. As oxidative stress may result from a broad spectrum of genetic and environmental factors, it is very difficult to distinguish between primary and secondary ED in some experimental models of hypertension because the processes of endothelial function damage and elevation of BP are usually simultaneous. In conclusion, on the basis of current state-of-the-art, ED can be both a cause and consequence of hypertension, depending on the experimental model used.

Supported by the APVV-523-10 and VEGA 2/0084/14.

REACTIVE OXYGEN SPECIES AND NUCLEAR CALCIUM HOMEOSTASIS OF HUMAN VASCULAR SMOOTH MUSCLE.

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The reactive oxygen species (ROS) are mainly produced by the NADPH oxidases (NOXs) in the cardiovascular tissue. Several circulating factors affect the function of the cardiovascular system at least in part via activation of NOXs which produces superoxide. This later is known to modulate intracellular calcium homeostasis. Given that several cardiovascular active factors also possess receptors at the nuclear envelop membranes' and since the nucleus has intranuclear structures that contribute to modulation of nuclear calcium homeostasis, it is likely that these nuclear membranes' receptors such as those for ET-1 and AngII may regulate ionic nuclear hemostasis via generation of nuclear ROS. Our results showed that as the sarcolemma membrane, nuclear membranes' possess different types of NOXs and their activation by nuclear membranes' GPCRs modulate nuclear calcium homeostasis as well as nuclear structures such as nuclear T-tubules. Thus, nuclear membranes' GPCRs via activation of nuclear membranes NOXs contribute to generation of local nuclear ROS. This later contributes to modulation of nuclear calcium homeostasis which is known to affect excitation-contraction coupling as well as excitation gene- expression and remodeling of vascular smooth muscle cells such as in aging.

Granted by the Canadian Institutes of Health Research, National Sciences and Engineering Research Council of Canada and Heart and Stroke Foundation of Canada.

TARGETING SCLERAXIS: CARDIAC FIBROSIS IN THE CROSS-HAIRS

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In cardiac fibrosis, resident fibroblasts undergo phenotype conversion to myofibroblasts and significantly increase their production of extracellular matrix (ECM) components such as collagen. Fibrosis adversely affects cardiac systolic and diastolic function as well as normal sinus rhythm, contributing to patient mortality, yet at present there are no direct pharmacologic interventions available. Our work has demonstrated that the transcription factor scleraxis controls cardiac fibroblast phenotype as well as ECM production. Over-expression of scleraxis induces the conversion of cardiac fibroblasts to myofibroblasts as exemplified by increased expression of ECM proteins as well as up-regulation of the α-smooth muscle actin gene via direct promoter transactivation, resulting in increased contractility – a hallmark of myofibroblasts. In contrast, scleraxis knockdown dramatically reduces ECM gene expression and attenuates the TGFβ/Smad3 signaling axis, the primary driver of myofibroblast activation and fibrosis. Scleraxis null mice exhibited a loss of nearly half of the cardiac ECM, a ~50% loss of cardiac fibroblasts and attenuation of TGFβ/Smad3 signaling, demonstrating a central requirement of scleraxis in ECM synthesis. Our data supports a model of impaired epithelial-to-mesenchymal transition of cardiac fibroblast precursors during development, with direct transcriptional regulation of various mesenchymal marker genes by scleraxis. Scleraxis null mice thus possess fewer cardiac fibroblasts as well as exhibit reduced ECM production by these cells. Alternative loss-of-function approaches targeting scleraxis, such as inhibition of post-translational modification, confirm scleraxis' sufficient and required role in ECM production. Given its central role in governing ECM synthesis by and phenotype conversion of cardiac fibroblasts, scleraxis presents a viable target for novel anti-fibrosis therapies.

Supported by the Canadian Institutes of Health Research (grant MOP-136862).

CO_2-BATH AS A NOVEL THERAPY FOR PERIPHERAL VASCULAR DISEASE

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Peripheral artery disease (PAD) is a major health problem whereby narrowed arteries reduce blood flow to the ischemic limbs. We investigated the effects of CO_2-enriched water bath (CEWB) therapy on blood flow in the ischemic hind limb. The femoral artery was occluded in rats to induce PAD and the animals were treated with or without CEWB at 37°C for 4 weeks (20 min/day; 5 days/week) starting one week after artery occlusion. CEWB was prepared by using Carbothera (Mitsubishi Rayon Engineering, Tokyo). Peaks, mean and minimal blood flows, as measured by Pulse Wave Doppler Ultrasound technique, were not detected in the untreated ischemic hind limb of animals due to arterial ligation. However, blood flow values were about 50% of the control upon treatment with CEWB; 67% of the ligated animals showed positive blood flow by CO_2 treatment. Morphological examination of the treated ischemic skeletal muscle revealed a 3-fold increase in small artery numbers indicating the formation of new blood vessels. Although plasma triglycerides decreased and plasma NO concentration increased in ischemic animals, CEWB treatment produced no effects on these parameters. It is suggested that beneficial action of CO_2 therapy on blood flow to hind limb may be due to the development of angiogenesis in the ischemic skeletal muscle.

Infrastructure support for this study was provided by the St. Boniface Hospital Foundation.

HOMOCYSTEINE AND THIOLACTONE METABOLITES: PROGRESS IN CARDIOVASCULAR RESEARCH

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This presentation deals with the effects of homocysteine (Hcy) isoforms on rat cardiodynamics, cardiac oxygen consumption, oxidative stress, NMDA receptors and cardiac acetylcholinesterase (AChE) activity. Cardiodynamics and oxidative stress were estimated following administration of any Hcy isoform (with thiolactone ring which is considered to increase toxicity), i.e. DL-Hcy, DL-Hcy TLHC or L-Hcy TLHC (all applied in 10 μmol/L) in isolated rat heart (Wistar, male, groups n =6, age 8 weeks, b.w. 180–200 g, CPP 70 cmH_2O). Any Hcy isoform induced decrease of cardiac contractility as well as decrease in coronary flow, while only L-Hcy TLHC significantly affected O_2^- production but not TBARS, nitrites, superoxide anion, or hydrogen peroxide in coronary perfusate. Interestingly, 60 min following acute i.p. administration of DL-Hcy or DL-Hcy TLHC (both applied in a dose of 8 mmol/kg b.m i.p.), the plasma values of antioxidant enzymes (catalase, glutathione peroxidase, superoxide dismutase) mainly increased, and index of lipid peroxidation decreased, probably as an compensatory response to Hcy prooxidant effects. Rate of oxygen consumption was measured at rat cardiac tissue homogenate, before and after administration of any Hcy isoform, and it was found that DL-Hcy, DL-Hcy TLHC or L-Hcy TLHC (all applied in 10 μmol/L) induced decrease in oxygen consumption. AChE activity of the rat cardiac tissue homogenate was estimated 60 min after DL-Hcy or DL-Hcy TLHC (both applied in a dose of 8 mmol/kg b.m i.p.), and it was found that DL-Hcy or DL-Hcy TLHC decreased AChE activity vs. control, but there was no difference between estimated enzyme activities in both groups. Other study was performed to determine the role of NMDA receptors in cardiac function and oxidative stress stimulated by DL-Hcy TLHC (10 μmol/L) in isolated rat heart. It has been concluded that both, stimulation or inhibition of NMDA receptors (by 50 μmol/L MK-801) induced cardiac depression. Finally, homocysteine and homocysteine-thiolactone induced cardiac dysfuntion; there was interplay with oxygen consumption, oxidative stress, AChE and NMDA receptors. It seems that stimulation of NMDA receptors and decreased activity of AChE induced by Hcy isoforms could be involved in arrhythmia development.

WHY DO WE STILL NOT HAVE CARDIOPROTECTIVE DRUGS? IMPORTANCE OF THE "OMICS" APPROACH.

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Ischemic heart disease including myocardial infarction develops on the basis of several risk-factors and co-morbidities such as obesity, diabetes, hypertension, and hypercholesterolemia. Ischemic heart disease is the leading cause of mortality worldwide, therefore, identification of novel drug targets for cardioprotection is of great importance. Ischemic preconditioning, postconditioning, and remote conditioning trigger endogenous cardioprotective mechanisms that render the heart more resistant to lethal ischemic-reperfusion injury. However, major cardiovascular risk factors such as hyperlipidemia, diabetes, and their routine medications interfere with these cardioprotective mechanisms thereby limiting the efficacy of cardioprotection. Ischemia/reperfusion injury and cardioprotection by conditioning have been shown to affect global myocardial gene expression profile at the transcript level, including both mRNA and micorRNA. Cardiovascular risk factors and their medications have been also shown to affect global cardiac gene expression profile. Instead of hypothesis driven research on single mechanisms of cardioprotection, further understanding and the comprehensive analysis of the cardioprotective gene and protein expression fingerprint by the "omics" approach in normal, protected, and comorbid conditions may lead to identification of novel molecular targets for cardioprotection and better translation to the clinical arena.

♣ EFFECT OF CAROTENOIDS ON Cx40 AND Cx43 EXPRESSION IN LEFT VENTRICLE OF NORMOTENSIVE RATS DURING INFLAMMATION

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Connexins (Cxs) are proteins of gap junctions (GJs), which facilitate electrical cell-cell coupling in heart. Cx43-GJs are present mainly in working cardiomyocytes of ventricles, while Cx40-GJs in conductive myocytes and atrium. Cx40 is also expressed in vascular endothelium, modulating cellular homeostasis and myoendothelial communication. Changes in Cx isoforms expression observed in heart during pathophysiological conditions emphasize importance of Cxs as therapeutic targets in cardioprotection. Bacterial inflammation is one of risk factors for cardiovascular diseases. Application of natural substances represents important alternative in prevention and supportive treatment of heart diseases. Therefore, aim of our pilot study was to examine the effect of LPS (bacterial endotoxin) on expression of Cx43 and Cx40 in left ventricle (LV) and to investigate anti-inflammatory and anti-oxidative effects of natural carotenoids (Car) in yeast biomass (Rhodotorula glutinis) on expression of both Cx isoforms in LV. Inflammation was induced by a single dose of LPS (E.coli, 1 mg/kg, i.p.) in adult Wistar rats. Rats were fed with Car (10mg/kg/day) for 10 days. We measured selected inflammatory markers (NFkB, TNF-α, MDA, and activities of NOS and NAGA) and enzymes activities (GlP, DPPIV, AlP). LPS reduced Cx40 expression, while Cx43 expression was unchanged when compared with controls. Yeast biomass administration to LPS rats caused upregulation of Cx40 expression and did not change Cx43 expression compared with LPS group. Administration of yeast biomass to control rats had no effect on expression of Cxs. LPS elevated levels of all measured inflammatory markers in plasma and LV tissue, indicating the presence of inflammation. LPS locally decreased GlP activity and caused rarefaction of capillaries of arterial bed as well. Yeast carotenoids reduced inflammatory markers and protected GlP activity and capillary bed against injury in LPS rats. Our pilot work demonstrated LPS-induced different expression of both studied Cx isoforms. It indicates their various roles in modulation of heart function during short-term inflammation. Yeast carotenoids protected only Cx40 isoform expression. The results may contribute to progress in molecular medicine.

Supported by VEGA 2/0065/13.

RECOVERY AFTER LVAD IMPLANTATION - A CHALLENGE FOR BASIC AND CLINICAL RESEARCH

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Left ventricular assist devices (LVADs) are increasingly used to bridge end-stage heart failure patients to heart transplantation or implanted as a lifetime therapy. Implantation of LVAD introduce a bypass from left ventricle to aorta and leads to ventricular undloading.

Heart failure is associated with remodeling a process that consist of adverse cellular, structural and functional changes in the myocardium. Clinically it results in progressive enlargement of the ventricle, reduction in contractility and increasing in intracardial pressures. Remodeling was until recently considered as one way, progressive and irreversible. But in up to 5% of patients after LVADs implantation complete or partial reversal as a consequence of profound and long-term myocardial unloading have been observed. Investigations have described an impact of unloading on cardiomyocyte hypertrophy, fibrosis, microvascular changes, adrenergic pathways and on myocardial function or electrophysiologic properties. In has been also shown that reverse structural remodeling does not always equate with clinical and functional recovery.

LVADs have established utility in increasing cardiac output and reversing end-organ damage. Consequences of this intervention for myocardial structure and function are increasing studied and attract a lot of interests both clinicians and basic researchers. LVADs implantation provide very attractive clinical model for investigation of pathways leading to myocardial structural and functional recovery. Key clinical features of LVAD usage as well main results of the research on myocardial reverse remodeling and recovery will be presented.

INCREASED EXPRESSION OF CALRETICULIN IN THE HEART: CARDIAC FIBROSIS AND HEART FAILURE.

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One detrimental aspect of cardiac failure is an increase in fibrosis with surplus deposition of extracellular matrix proteins. This can reduce cardiac function but the underlying mechanism of why this happens is still unclear. Increased abundance of calreticulin in adult heart has been associated with dilated cardiomyopathy and heart failure. Here, we discovered that increased expression of calreticulin in the adult mouse heart leads to severe cardiac fibrosis. To investigate the mechanism behind calreticulin-dependent increase in cardiac fibrosis, we utilized microarray hybridization and monitored global gene expression in calreticulin transgenic hearts with impaired ER homeostasis. We observed significantly enhanced expression of TGF-ß1, a pleiotropic cytokine, as well as fibrillar collagens when compared with control hearts. Validation of protein expression showed that TGF-ß1 expression and secretion into the circulatory system was significantly increased as well as receptor-regulated Smad2/3 expression, also activated in calreticulin transgenic hearts. Several pro-inflammatory factors and markers of fibrosis, including NFκB p65, and pro-inflammatory cytokines, TNFα, IL-1ß, and IL-6, were noticeably up-regulated. The expression and localization of periostin, a ligand for integrins that supports cellular adhesion and migration, was increased in calreticulin transgenic hearts. Furthermore, ER stress was increased as measured by XBP1 splicing analysis (IRE1 activity), due to the overexpression of calreticulin in the heart. However, cardiac fibrosis triggered by calreticulin overexpression was effectively reduced by administration of tauroursodeoxycholic acid (TUDCA), possibly due to TUDCA's inhibitory effects on ER stress. We concluded that the mechanism leading to cardiac fibrosis in adult hearts overexpressing calreticulin may involve impaired ER homeostasis triggering activation of ER stress coping responses, activation the TGF-ß1/Smad2/3 signaling pathway which may lead to cardiac fibrosis with this pathogenesis suppressed by TUDCA treatment.

Supported by grants from Canadian Institutes of Health Research.

CAN WE CURE ATRIAL FIBRILLATION? A CRITICAL REAPPRAISAL FROM CELL TO BEDSIDE.

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Atrial fibrillation (AFIB) is the most common sustained cardiac arrhythmia in the adult population with an overall prevalence exceeding 3% . In order to cure any disease we have to know it's exact cause (-s) and be able to definitely eliminate it / them. In complex diseases like AFIB indefinite remission essentially equates with cure. Catheter ablation can cure almost 100% of supraventricular tachycardias with well defined reentrant / ectopic arrhythmia substrate. However, substrate of AFIB is both pathophysiologically and anatomically heterogenous and complex. In the last 2 decades it was demonstrated that abnormal electrical activity emerging from the distal portions of the pulmonary veins and their junction with left atrium play crucial role in triggering and sustaining AFIB. It was demonstrated that atrial fibrosis promoted mainly by the renin-angiotensin-aldosterone system stimulating fibroblasts is the decisive pathologic process behind atrial proarrhythmia. By means of MRI of the atria atria it was demonstrated that the more is AFIB advanced (becoming permanent) the more fibrotic are the atria. AFIB Pulmonary vein isolation (PVI) by means of catheter ablation using radiofrequency current or cryoenergy became the therapy of choice to eliminate AFIB. It's short and mid-term results are impressive with success rates close to 90%. Our data have shown that the overall arrhythmia burden time can be dramatically reduced by a factor of 20-50. Nevertheless, the long term effect of this therapy remains in many patients below the expectations revealed by clinical trials - after 5 years only about 50% of treated patients remain arrhythmia free. In patients where PVI is not sufficient to eliminate AFIB, additional lines and lesions in the left and/or right atrium and/or coronary sinus are deployed in order to eradicate the arrythmogenic substrate. Thus , it is paradoxical that by creating massive areas of atrial fibrosis by catheter ablation we aim to cure AFIB which is by itself in causal relation to atrial fibrosis. The explanation of this contradiction remains unclear. Different character of the fibrosis is discussed as one explanation: Spontaneous atrial fibrotic disease is characterized by expanding extracellular space being occupied by fibroblast creating progressive dispersion and isolation of atrial myocytes with resulting abnormal electrical behavior. On the other hand, post-ablation fibrotic lesions tend to be homogenous and transmural with no residual surviving cells. However, we still do not know enough about the long term impact of such iatrogenic fibrotic remodeling of the atria and its impact on atrial function. Thus, once again in the rather short history of interventional cardiac electrophysiology we continue our cognitive process of "learning by burning" (and some times by freezing).

♣ DEVELOPMENTAL CHANGES IN PDE5A EXPRESSION AND STRATEGIES FOR CARDIOPROTECTION

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Myocardial ischaemia-induced perioperative myocardial damage is a key determinant of postoperative morbidity and mortality following surgical repair in paediatric patients undergoing open heart surgery. In fact ischaemic arrest duration and subsequent reperfusion injury are closely linked to postoperative morbidity during paediatric cardiac surgery. Although there is strong evidence to suggest that the resistance of developing mammalian hearts, to the damaging effects of cardiac insults (e.g. hypoxia and ischaemia) is greater than adult hearts, there are reports showing that the immature myocardium is more, not less, vulnerable to injury from cardiac insults than the adult heart. One aspect of experimental studies that may have contributed to this controversy is the selected developmental age. The recovery of developing rat heart after ischaemia and reperfusion follows a bell-shaped relationship with 14-day old hearts showing the highest resistance whilst adult hearts tended to be most vulnerable. The mechanisms underlying this profile are not currently known. However, we found that there is a link to the level of survival signalling (pERK). ERK is also linked to cardioprotection by the PDE5A inhibitor Sildenafil. In this study we investigated the developmental changes in PDE5A expression in rat and pigs and correlated this with recovery after ischemia and reperfusion.

In this preliminary work, we found that rat cardiac PDE5A protein and gene expression from different age groups appeared to increase and then decrease during postnatal development in rat heart, with higher expression at 14 days old. Furthermore, this age group benefits the highest cardio-protective effect of Sildenafil after reperfusion injury, compared with other age groups.

Changes in PDE5A expression during postnatal development is also evident in our preliminary data on pigs with younger age groups having relatively more protein expression. This indicates that Sildenafil is likely to confer significant protection in neonatal pigs compared to adult. This is currently being investigated at our centre.

ENDOCARDIAL ENDOTHELIUM AND CARDIAC HYPERTROPHIC FACTORS.

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Our insights into cardiac function have evolved from an autoregulatory muscle pump, with coronary perfusion and neurohormonal control to a pluricellular, multifunctional organ, in which the endocardial endothelial cells (EECs) seem to play an important role. Even if major differences exist between vascular endothelial cells (VECs) and EECs, all endothelial cells (ECs) including EECs release a variety of auto- and paracrine factors such as $NO^{\cdot}$, endothelin-1 (ET-1) and angiotensin II (Ang II). All these factors are known to affect cardiomyocytes contractile performance and growth. As VECs are a barrier between the superfusing blood and vascular smooth muscle cells (VSMCs), EECs are also a barrier between superfusing blood and the cardiomyocytes. This barrier is in direct contact with circulating factors that are known to induce cardiomyocytes hypertrophy such as ET-1, Ang II, NPY and Insulin. Although the mechanisms of these cardiac hypertrophic factors are known at the level of cardiomyocytes, there is no information on whether they also affect the size of EECs as well as its secretory capacity. Our results showed that effectively EECs undergo hypertrophy as cardiomyocytes. This remodeling of EECs seems to affect its secretion capacity which directly contributes to the overall cardiac hypertrophy. Thus, it is highly important to take into consideration the excitation-secretion coupling of EECs in order to better understand the physiological and pathological states of the heart.

Granted by the Canadian Institutes of Health Research and the Heart and Stroke Foundation of Canada.

OXIDATIVE STRESS IN EXERCISE: FROM BASIC SCIENCE TO APPLIED CLINICAL INVESTIGATIONS

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Cellular damage by reactive oxygen species (ROS) is a significant causal factor involved in heart diseases, especially during myocardial ischemia-reperfusion. L-arginine/NO system and its main metabolic product, nitric oxide (NO) is bioregulatory sistem who plays important role in cardiovascular homeostasis. The bioactivity of endothelium-derived NO is impaired in cardiovascular diseases, caused in part by the increased vascular production of ROS. Regarding this data, we tried to connect our basic exeperiments in isolated heart with some clinical events where oxidative stress is involved. One part of our basic investigation was to examine the acute effects of nandrolone decanoate (ND) on oxidative stress in isolated rat heart. The hearts of male Wistar albino were excised and perfused according to the Langendorff technique at gradually increasing coronary perfusion pressures (40-120 cmH2O). The hearts were perfused with ND at doses of 1, 10 and 100 μM. Oxidative stress markers, including the index of lipid peroxidation (thiobarbituric acid reactive substances (TBARS)), nitric oxide (nitrites; NO2 -), the superoxide anion radical (O2 -) and hydrogen peroxide (H2O2) were measured in the coronary venous effluent. Our results showed that acute effects of ND do not promote the production of reactive oxygen species (ROS). Our finding pointed out that the highest concentration of ND may even possess some anti-oxidative potential, which should be examined further. Furthermore, the aim of second experimental research was to assess exercise-induced changes in mechanics of hearts isolated from rats, as well as time-course of those changes. Nine weeks of moderate exercise induced slight depression of coronary function (decrease of dp/dt max, dp/dt min, SLVP and DLVP), while 3 additional weeks of moderate training improved hearts function, but not to the extent that the strenuous training program did. The results of our study add evidence about beneficial effects ofregular moderate exercise on heart, and furthermore, show that exercising frequently, if the intensity stays within moderate range, may not have detrimental effects on cardiodynamics. On the other hand, the aim of our clinical studies were to examine parameters of oxidative stress in sternous exercise, The results of our studies in top athletes suggested that the type of sport and training activity (anaerobic compared to aerobic) affects oxidative stress, as well as on its dynamic in sternous exercise. Furthermore, programmed physical activity increases SOD activity as a first line in antioxidant defense, while excess H_2O_2 production deplets CAT activity. Finally, correleation of measured parameters were age specific.

ATTENUATION OF MYOCARDIAL REMODELLING, HYPERTROPHY AND HEART FAILURE BY NATURAL PRODUCTS.

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The incidence of heart failure has been increasing dramatically over the past two decades as life span increases coupled with increased survival after acute myocardial infarction. Mortality rates have remained high and there is substantial agreement that novel, and effective, therapies must be identified in order to bring this syndrome under better control. One potential approach is to identify naturally occurring products which may be of substantial benefit, especially when used as adjunctive therapy with existing medications. Indeed, the use of natural products for the treatment of heart disease has been increasing tremendously over the past number of years. For example it has been reported that there are currently more than 15 million complementary and alternative medicine users in the United States alone. Herbal supplements rank at the top and are particularly common in patients with already prescribed conventional medications. Among these is ginseng whose medicinal properties have been recognized in Asian societies for thousands of years. Recent evidence suggests that alterations in the gastrointestinal microbiota can also affect cardiovascular health. In this regard, probiotics have been used extensively for improving digestive health but recent evidence suggests that administration of probiotics can reduce cardiovascular risk by reducing plasma cholesterol levels. Both ginseng and probiotics can also improve cardiac function in response to pathology. For example, work from the author's laboratory has found that both ginseng as well as probiotics can reduce post-infarction remodelling, hypertrophy and improve left ventricular function. In addition both can abrogate hypertrophy of cultured ventricular myocytes in response to various pro-hypertrophic factors including the α_1 adrenoceptor agonist phenylephrine as well as endothelin-1 and angiotensin 2. The exact underlying mechanisms for the beneficial effects of these substances are still under investigation but in the case of ginseng these appear to be due to attenuation of calcineurin activation in the heart. The mechanistic bases for the effects of probiotics are more difficult to define but these likely involve modulation of gut-derived factors including the inhibition of the leptin to adiponectin ratio. As probiotics can also directly attenuate cardiomyocyte hypertrophy it is likely that a probiotic-derived antihypertrophic factor or factors may also be responsible. Taken together, our studies and those of others suggest that natural products may be effective for the treatment of heart failure, especially in combination with existing medications.

Work from the author's laboratory is supported by the Canadian Institutes of Health Research.

POTASSIUM AS TRIGGER AS TRIGGER OF SUDDEN CARDIAC DEATH.

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Worldwide around 3 million people suffer sudden cardiac death annually often due to arrhythmia emerging from a complex interplay between substrate and triggers. Disturbed potassium(K)-homeostasis among heart cells is such a trigger. Long-term K-homeostasis depends on renal potassium excretion. However, skeletal muscles play an important role in short-term K-homeostasis because they contain the largest pool of potassium in the body and possess a huge capacity for potassium exchange due to the large content of K-pumps and K-channels.

Hypokalemia is relatively common in patients with cardiovascular diseases due to the use of non-sparring diuretics, insufficient potassium intake, and shift of K into stores due to increased K-uptake stimulated by catecholamines, β-adrenoceptor agonists and insulin. Importantly, there is protection against further reduction in plasma-K (pK) in severe hypokalemia. In moderate hypokalemia the reduction in pK is blunted but still severe. Thus, in control rats terbutalin reduced pK by 0.7 mmol/l ($p = 0.01$), in moderate hypokalemia the decrease was reduced by 0.3 mmol/l for each 1 mmol/l decrease in pK ($n = 8$, $R^2 = 0.82$, $p = 0.002$) and in severe hypokalemia (pK 1,7 mmol/l) terbutain induced no further reduction in pK. Normalisation of pK by KCl infusion immediately abolished protection against terbutalin induced pK reduction[1].

Hyperkalemia is less common but as the use of aldosteron antagonists for heart failure has increased, a fear for hyperkalemia has developed especially if renal function is reduced. Importantly, there is protection against further exercise-induced increase in pK in hyperkalemia. Thus, when renal failure patients exercised, pK increased less before haemodialysis. The pK increase was linearly correlated with pK before exercise ($\beta = -0.21$, $R^2 = 0.23$, $p = 0.001$).

Still, pK shifts may trigger cardiac arrhythmia through modulation of cardiac repolarization. Thus, the decrease in pK during recovery was a determinant of QT hysteresis. QT hysteresis was linearly correlated with the decrease in pK during recovery ($\beta = -28$ msec/(mmol/l), $R^2 = 0.36$, $p = 0.006$) and low pK was associated with relatively longer QT interval[2].

In conclusion: The protection against β adrenoceptor agonist induced reduction in pK in hypokalemia avoids the development of deleterious reduction in pK. The protection against exercise induced increase in pK avoids development of deleterious increase in pK. Thus, it seems advisable to maintain pK in the middle or upper normal range.

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DETERMINANTS OF ANTIARRHYTHMIC PROTECTION IN CHRONICALLY HYPOXIC HEARTS

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Lethal ventricular arrhythmias induced by acute myocardial infarction remain the leading cause of cardiovascular mortality. Adaptation to chronic hypoxia limits the extent of irreversible myocardial injury and may also potentially reduce the incidence and severity of arrhythmias associated with ischemia/reperfusion (I/R) insult. However, available data are often contradictory. Antiarrhythmic effects depend on the specific model/regimen of chronic hypoxia and are markedly affected by experimental conditions and other factors that can be responsible for confusing results. For example, intermittent hypoxia protects against both ischemic and reperfusion arrhythmias without tachyphylaxis, while the antiarrhythmic effect of continuous hypoxia is only transient, fading with prolonging the hypoxic exposure. Quantitatively and qualitatively different results can be obtained when the impact of I/R injury is tested in whole animal model and in isolated perfused hearts. Moreover, the susceptibility to I/R-induced arrhythmias is subject to significant diurnal and seasonal variation, which can affect the outcome of chronic hypoxia. Besides age- and sex-dependent effects, other factors including housing conditions, diet composition etc. also play an important role in determining the overall score of antiarrhythmic protection and should be taken into account when interpreting data from individual studies.

Supported by the Czech Science Foundation 303/12/1162 and APVV-SK-CZ-2013-0075.

NOVEL FUNCTION OF TRPM7 IN VASCULAR ENDOTHELIUM REMODELING

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Vascular endothelial cells are activated by a variety of pathological stimulation, such as oxidative stress, mechanical stretch, and elevated autocrine-paracrine mediators, thereby undergoing proliferation, differentiation (to fibroblasts), and production of cytokines and extracellular matrix molecules. During endothelium remodeling processes, a number of Ca^{2+} dependent signaling pathways and molecules are activated, which are often closely associated with the pathophysiology of vasculature such as arthero-/arterio-sclerosis and essential and pulmonary hypertensions.

We focused on stretch- and swelling-activated cation channel TRPM7 (transient receptor potential melastatin subfamily member 7), which is highly expressed in human umbilical vein endothelial cells (HUVEC) cells. TRPM7-siRNA knockdown and -selective antagonist (FTY-720) experiments suggested that TRPM7 is essential for a TGF-β mediated endothelial-mesenchymal transition (EMT) signaling cascade. The FTY-720 is an active ingredient extracted from Cordyceps sinensis, which is also known as the Chinese caterpillar fungus and used in traditional Chinese medicine to treat fibrotic diseases. Extracts of Cordyceps sinensis showed the same efficacy as FTY-720 did, significantly suppressing EMT of and stress fiber formation in TGF-β2 treated HUVEC cells.

The above results suggest that TRPM7 may contribute to EMT processes in vascular endothelium remodeling, and could thus be a novel therapeutic target for some fibrotic disorders in the cardiovascular system.

EXPLORING NEWER CARDIOPROTECTIVE STRATEGIES: PPARs IN PERSPECTIVE

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Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear transcriptional activators that belong to the superfamily of nuclear receptors. PPARs serve as cellular sensors of fatty acids and fatty acid derivatives and they are considered as major transcriptional regulators of lipid metabolism and energy homeostasis. Agonists of PPARα (fibrates) and PPARγ (thiazolidinediones) are widely used as hypolipidemic and anti-diabetic drugs, respectively. However, it has become evident that the therapeutic effects of PPAR ligands reach far beyond their use as lipid lowering agents or insulin sensitizers. PPARs have been also implicated as regulators of cellular inflammatory and redox responses. Accummulating evidence indicates that their activation leads to cardioprotection after ischemia/reperfusion (I/R) and prevents cardiac remodelling in a variety of experimental models. However, the exact role of PPAR isoforms in cardiac pathophysiology has not been fully defined yet. In this context, we recently used a genetic heart failure model characterized by an acute inflammatory reaction, extensive cardiomyocyte death, fibrosis and calcification. Treatment with the PPARβ/δ ligand protected against cardiomyocyte death, cardiac remodeling and development of inflammation in the myocardium leading to the amelioration of cardiac tissue damage. Collectively, the data suggest that PPAR ligands could prove to be useful in the management of cardiac disorders.

Supported by the European Union and Greek national funds of the National Strategic Reference Framework (NSRF) - Program "Cooperation" (grant 09SYN-21-965).

♣ REMOTE ISCHEMIC PRECONDITIONING IN SIX-MONTHS OLD MALE AND FEMALE RATS AND ITS IMPACT ON HEARTS EXPOSED TO ISCHEMIA/REPERFUSION INJURY: INTRINSIC MECHANISMS BEHIND

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Remote ischemic preconditioning (RIP) represents a form of innate cardioprotection conferred by short episodes of ischemia applied in a distant organ/tissue. Much attention is paid to identification of afferent signals emitted from the remote ischemic area and to association between signals and beneficial responses in the heart. Efficiency of RIP in increasing myocardial resistance against ischemia-reperfusion (I/R) injury has been shown in 3-months old male rats with RIP applied either directly prior to I/R or 24 h before ischemia. However, sex-dependent effects of RIP have not yet been explored. This study investigated the effect of RIP on cardiac tolerance to I/R in older adult male and female Wistar rats.

Rats of 6-months age were anesthetized and RIP was performed in the right hind limb. Its protocol consisted of three cycles of 5-min non-invasive limb occlusion followed by 5-min reperfusion. Subsequently, hearts were excised, Langendorff-perfused and exposed to 30-min global I and 2-h R for the evaluation of reperfusion-induced ventricular arrhythmias, infarct size and recovery of contractile function.

Hearts of female rats exhibited significantly improved recovery of contractile function (LVDP, LVEDP) as well as enhanced resistance to ventricular tachyarrhythmias compared to male hearts. Interestingly, RIP had no effect on parameters of myocardial I/R injury compared to controls irrespective of sex.

RIP induced prior to sustained myocardial ischemia (“first window”) in mature rats is not able to confer cardioprotection. However, RIP applied 24 h before heart ischemia (“second window”) may act as a more powerful stimulus for increasing myocardial tolerance to prolonged ischemia. Moreover, cardioprotective effects of RIP show age dependency, since in younger adult males, RIP represents an effective approach for enhancing myocardial resistance against I/R injury.

Supported by grants: VEGA 2/0201/15, APVV-0102-11, APVV-SK-CZ-2013-0075.

ROLE OF NATRIURETIC PEPTIDE RECEPTOR C IN THE REGULATION OF BLOOD PRESSURE

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C-$ANP_{4-23,}$ a ring deleted analog of atrial natriuretic peptide (ANP) that specifically interacts with natriuretic peptide receptor-C (NPR-C) has been shown to decrease the enhanced expression of Giα proteins, implicated in the pathogenesis of hypertension. In the present study, we investigated whether in vivo treatment of spontaneously hypertensive rats (SHR) with C-ANP_{4-23} could attenuate the development of high blood pressure (BP) and explore the underlying mechanism/s responsible for this response. The BP started increasing in SHR at 4 weeks and increased to about 190 mmHg at 8 weeks. However, intraperitoneal injection of C-ANP_{4-23} at the concentration of 2 or 10 nmole/kg body weight) to prehypertensive SHR attenuated the development of high BP and at 8 weeks it was decreased by about 20 mmHg and 50 mmHg respectively but not in WKY rats. The C-ANP_{4-23} treatment also decreased the enhanced levels of Giα proteins in heart and aorta as determined by Western blotting. In addition, the enhanced levels of superoxide anion (O_2^-), peroxynitrite, NADPH oxidase activity and the enhanced expression of NOX-4, $P47^{phox,}$ nitrotyrosine, and decreased levels of eNOS were attenuated by C-ANP_{4-23} treatment, however, the altered levels of NPR-A/NPR-C were not affected by this treatment. ***Conclusions:*** These results indicate that NPR-C activation by C-ANP_{4-23} attenuates the development of high BP in SHR through the inhibition of enhanced levels of Giα proteins and nitroxidative stress and not through eNOS /cGMP pathway and suggest that NPR-C ligand may have the potential to be used as therapeutic agent in the treatment of cardiovascular complications including hypertension.

Supported by grant from CIHR.

♣ HUMAN-MOUSE CHIMERISM VALIDATES IN VITRO DERIVED HUMAN PLURIPOTENT STEM CELLS AND THEIR CARDIOVASCULAR PROGENY

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Pluripotent stem cells are defined by their capacity to differentiate into all three tissue layers that comprise the body. Chimeras, generated by stem cell transplantation to the embryo, represent the gold standard assessment of stem cell pluripotency to date. However, the ability of human pluripotent stem cells (hPSCs) to resume normal development in vivo by participating in embryonic organ formation remains in question. A resolution of this key problem is critical to secure the utility of hPSCs for stem cell science and for regenerative medicine applications.

Our experimental objective was to determine the capacity of human pluripotent stem cells (hPSCs) and their cardiovascular progeny to participate in early mouse embryo development. Early post implantation mouse embryos were dissected and hPSCs or their in vitro derived cardiovascular progeny were transplanted prior to culture. Subsequent, in vitro analysis of lineage-specific contribution by confocal fluorescence microscopy was used assess functional integration of the stem cells and their progeny.

hPSCs transplanted into post-implantation stage mouse embryos were able to integrate and contribute to each of the three tissue layers; endoderm, ectoderm and mesoderm. Both hPSCs and in vitro derived cardiovascular progeny formed interspecies chimeras with high efficiency, whereby they colonised the embryo in a manner predicted from classical developmental fate mapping. Thus we show that hPSCs and their progeny have the capacity to participate in normal development when transplanted into gastrula stage mouse embryos, providing for the first time proof of their full pluripotent status.

In sum, hPSCs and their cardiovascular derivatives can incorporate into mouse embryos in a stage- and location-specific (synchronous, orthotopic) manner. This novel approach enables the study of cell fate decisions and plasticity of tissue specific progenitors during normal development. Moreover, our results nullify the existence of a barrier to interspecies developmental competency. This faithful recapitulation of tissue-specific fate post-transplantation is the ultimate functional validation for the inherent pluripotency of hPSCs and thus for their therapeutic relevance.

Supported by US National Institutes of Health; UK Medical Research Council; British Heart Foundation; and by the Cambridge NIHR Biomedical Research Centre.

NEW MOLECULAR TARGETS OF VEGF SIGNALING IN CARDIOVASCULAR DISEASE

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Therapeutic angiogenesis is a promising approach to the treatment of ischemic injury. Angiogenesis is a global highlight in the medical field. It offers enormous potentials for therapeutic intervention of many disorders in human. Many angiogenesis related factors are involved in the development of vessels during vasculogenesis, as well as the induction of new vessels in response to physiological or pathological stimuli. VEGF modulates the complex process of angiogenesis and other various aspects of endothelial cell function through either of its two tyrosine kinase receptors, VEGFR1/Flt-1 or VEGFR2/Flk1/KDR via its target protein MAPKinase 2. VEGF mediated angiogenesis signaling is widely accepted however relatively little is known regarding VEGF mediated downstream signaling through Flt-1 and/or Flk-1. The use of Affymetrix gene chip technology in Flk-$1^{+/-}$ knockout mice allowed us first time to identify several target genes downstream of VEGF/Flk-1 signaling in ischemic preconditioned myocardium. By Affymetrix gene chip analysis we demonstrated first time down regulation of Pellino-1(Peli1) after ischemic insult to the Flk-$1^{+/-}$. Our study showed that overexpression of Peli1 by adeno-Peli1 gene therapy (Ad-Peli1) (gain-of-function) significantly increased angiogenic effect, increased ejection fraction and reduces ventricular remodeling in myocardial infarction (MI) model. Western blot analysis 24 h after MI revealed increased phosphorylation of Akt (3.4fold), eNOS (1.9 fold) and MAP Kinase 2 (1.7 fold) with Ad-Peli1 treatment compared to Ad-LacZ. Immunohistochemical analysis for fibrosis with picrosirius red staining exhibited a decrease in collagen deposition in Ad-Peli1MI group as compared to Ad-LacZMI. Vascular density and connexin-43, a major ventricular gap junction protein was found to be increased in Ad-Peli1MI group compared to Ad-LacZMI. Collectively, our study documents Peli1 as a promising molecule in the treatment of myocardial infarction, which could potentially lead to new therapeutic target.

MODULATION OF MITOCHONDRIAL FUNCTION BY NOVEL SYNTHETIC BENZOPYRAN ANALOGUES

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A substantial body of evidence indicates that modulation of mitochondrial function is protective in the setting of ischemia/reperfusion injury. The aim of the present study was to characterize the effects of novel synthetic benzopyran analogues, derived from a BMS-191095, a selective mK_{ATP} opener, on mitochondrial respiration and reactive oxygen species (ROS) production in isolated rat heart mitochondria. Mitochondrial respiratory function was assessed by high-resolution respirometry and H_2O_2 production was measured by the Amplex Red fluorescence assay. Four compounds, namely KL-1487, KL-1492, KL-1495, and KL-1507, applied in increasing concentrations (50, 75, 100 and 150 microM, respectively) were investigated. When added in the last two concentrations, all compounds significantly increased state 2 and 4 respiratory rates, effect that was not abolished by 5-hydroxydecanoate (5-HD, 100 μM), the classic mK_{ATP} inhibitor. The highest concentration also elicited an important decrease of the oxidative phosphorylation in a K^+ independent manner. Both concentrations of 100 and 150 microM for KL-1487, KL-1492, and KL-1495, and the concentration of 150 microM for KL-1507, respectively mitigated the mitochondrial H_2O_2 release. In isolated rat heart mitochondria, the novel benzopyran analogues act as protonophoric uncouplers of oxidative phosphorylation and decrease the generation of reactive oxygen species in a dose-dependent manner.

Research financed by a grant of the Romanian Ministry of National Education, CNCS – UEFISCDI, project number PN-II-ID-PCE-2012-4-0512.

EXERCISE-INDUCED CARDIOPROTECTION

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Ischemic heart disease remains a major cause of death worldwide. Therefore, development of pragmatic countermeasure to reduce myocardial injury is vital. In this regard, a plethora of evidence indicates that regular exercise can protect the heart during an ischemia/reperfusion (IR) insult. It has been observed that both short-term (i.e. 1-5 days) and long-term (i.e. weeks to months) endurance exercise provides cardioprotection in rats. Several studies indicate that endurance training improves myocardial tolerance to ischemia reperfusion injury in both young and old animals of both sexes. Specifically, studies reveal that exercise training protects the heart against arrhythmias, oxidative injury, mitochondrial damage and cell death. The first phase of protection is acquired rapidly following an acute exercise bout (i.e. 0.5 h after exercise). However, the initial protection is rapidly lost within 3 h post exercise. The second or late phase of exercise-induced protection is achieved within 24 h after the exercise and persists for at least 9 days (following a 5-day exercise) and is far more robust than the early protection. In this connection it is necessary to mention that sustained exercise results in ventricular hypertrophy characterized by myocyte enlargement. The development of hypertrophy is regulated in sex-specific manner: female mice exhibit an increased hypertrophic response and show an increased exercise capacity compared with male mice. The mechanisms responsible for exercise-induce protection against IR injury remain a debated issue. Potential intrinsic changes include increased glycolytic flux, altered nitric oxide signaling, increased levels of heat shock proteins, amplified myocardial cyclooxygenase-2 activity, elevated endoplasmic reticulum stress proteins, enhanced function of sarcolemmal and/or mitochondrial ATP-sensitive potassium channels, and increased cytosolic antioxidant capacity. Moreover, recent study provides evidence that endurance exercise protects cardiac mitochondria from IR-induced damage. While the last decades of work have lead to comprehensive understanding of the basic structural and functional characteristics of exercise-induced cardiac adaptation, many unanswered questions remain: little is understood about the optimal duration and intensity of exercise as well as the relationship between the positive and possible negative consequences of adaptation.

EFFECTS OF NANOPARTICLE-LOADED ALISKIREN IN THE CARDIOVASCULAR SYSTEM OF HYPERTENSIVE RATS.

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Aliskiren, the most recent inhibitor of renin, has been shown to exert cardioprotective and anti-atherosclerotic effects independent on blood pressure (BP) lowering activity. However, clinical use of aliskiren is limited by its short lifetime. Therefore, we aimed to study the effect of nanoparticle-loaded aliskiren, with gradually realized drug, on BP and nitric oxide production in the heart and aorta. 12-week-old male SHRs were divided into the untreated group, group treated with powdered aliskiren (25mg/kg per day) or nanoparticle-loaded aliskiren (25mg/kg per day) and group treated with nanoparticles only for 3 weeks. BP was measured by tail-cuff plethysmography. NOS activity was determined by conversion of ^{3}[H]Arginine to ^{3}[H] Citrulline. At the end of experiment, BP was lower in both powdered and nanoparticle-loaded aliskiren groups with more pronounced effect in the second one, which correlated well with increased NOS activity in the heart. Both aliskiren groups reduced myocardial hypertrophy in comparison to untreated SHR. There was no change in NOS activity in the aorta. Interestingly, nanoparticle-loaded aliskiren decreased eNOS expression in both heart and aorta. Nanoparticles only decreased eNOS expression in the tissues even in comparison to the nanoparticle-loaded aliskiren group. In conclusion, despite the diminished effect of polymeric nanoparticles on the tissues, aliskiren realised gradually was able to increase NOS activity in the heart which could contribute to blood pressure and hypertrophy reduction. Thus, aliskiren may represent an effective, novel approach to the treatment of hypertension, however, more suitable and biocompatable polymeric nanoparticles are needed.

Supported by VEGA 2/0195/15, 2/0144/14, 2/0165/15; APVV-14-0932, APVV-0742-10.

THE INFLUENCE OF CHLAMYDIA PNEUMONIAE INFECTION ON CARDIOVASCULAR DISEASE

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Infectious disease and inflammation are thought to be involved in a variety of chronic diseases including heart disease. Chlamydia Pneumoniae is typically thought to be an infectious agent for lung disease. However, it has also been associated with atherosclerotic heart disease. The talk presented in this meeting will address the questions of a direct involvement of Chlamydia Pneumoniae in cardiovascular disease. How does a lung infection cause atherosclerosis? Can Chlamydial infection actually induce atherosclerosis directly? If so, what is its mechanism of action? What factors modulate the atherogenic response in the vasculature to this infectious agent? Data will be presented using both animal models of infection and cell culture approaches to answer these important clinical questions. Ultimately, we believe that infections like Chlamydia Pneumoniae can directly induce atherogenesis but the environment plays a critical role in modulating this response.

Supported by CIHR and St. Boniface Hospital Foundation.

♣ ROLE OF CaMKIIδ IN END-STAGE HUMAN HEART FAILURE

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Background: End-stage human heart failure (HF) is characterized by Ca^{2+} and reactive oxygen species (ROS) overload which may in turn promote autophosphorylation and oxidation of Ca^{2+}/calmodulin-dependent protein kinase IIδ (p-Thr^{286}-CaMKIIδ and oxMet$^{281/282}$-CaMKIIδ). CaMKIIδ dysregulation has been suggested to induce contractile dysfunction and arrhythmias. Important proteins regulating Ca^{2+} cycling (phospholamban – PLN and SERCA2a) and sarcomere function (cardiac myosin binding protein-C - cMyBP-C) are phosphorylated by CaMKIIδ, whereas protein phosphatase 1 and 2A (PP1B, PP2A) counteract these effects; therefore, the regulatory axis involving the aforementioned proteins controlling mechanical and electrical activity of cardiomyocytes may play a significant role in the pathogenesis of HF.

Methods: In the samples of left ventricles from healthy controls and patients with terminal stage HF of ischemic (I) and non-ischemic (NI) origin (dilated - DCM, restrictive – RCM and hypertrophic - HCM), the expression of proteins was analyzed by SDS-PAGE/Western blotting.

Results: Expression of p-Thr^{286}-CaMKIIδ was upregulated in all NI HF groups, while in the I HF was unchanged. Oxidized form, oxMet$^{281/282}$-CaMKIIδ, wasn't changed in I HF group similarly as in the case of phosphorylated form; however, in DCM NI HF group the expression was decreased. Nevertheless, these posttranslational levels of CaMKIIδ did not correlate with QT interval duration. In all types of HF, the content of CaMKIIδ-phosphorylated proteins such as p-Thr^{17}-PLN and p-Ser^{282}-cMyBP-C was significantly downregulated. The expression of total form of SERCA2a wasn't changed compared to controls. Content of PP2A did not differ among the groups while PP1B was significantly upregulated in I HF and DCM group of NI HF.

Conclusions: Posttranslational modifications of CaMKIIδ via oxidation and phosphorylation differ between I and NI HF. Phosphorylation of its downstream targets regulating contractile machinery and Ca^{2+} cycling was down-regulated in all failing hearts. Independently, of phosphorylation status of those proteins, there was no difference in the expression of SERCA2a between control groups and I or NI HF groups, respectively. Levels of CaMKIIδ are unlikely to be associated with pro-arrhythmogenic QT interval in end-stage HF.

Supported by grants: VEGA1/0638/12.

CYCLOPHILIN A: A SEROLOGIOCAL MARKER FOR VASCULAR DISEASE IN TYPE 2 DIABETES MELLITUS

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Serological markers of chronic diseases such as diabetes and their associated complications have become increasingly important for monitoring disease activity. Secretory Cyclophilin A (CyPA) in response to inflammatory stimuli such as hypoxia, infection and oxidative stress may mediate intercellular communication acting as an autocrine/paracrine factor. Cyclophilin A belongs to the peptidyl prolyl cis-trans isomerase family which comprises of a class of proteins that play a central role in multiple biological processes, such as protein folding, trafficking and assembly, as well as intracellular calcium handling, chemotaxis, and cell-cycle progression. This immunophilin has been implicated in the development of several cardiovascular diseases, such as vascular stenosis, hypertension, atherosclerosis, cardiac hypertrophy, arrhythmias, ischemic and non-ischemic cardiomyopathy and heart failure. Extracellular CyPA stimulates proinflammatory signals in endothelial cells (EC) and vascular smooth muscle cells (VSMC).

A mounting body of evidence from our laboratory suggests its involvement in key processes underlying progression of macrovascular disease in type 2 diabetes. A proteomic analysis of high glucose-primed monocytes, identified CyPA as a potential secretory marker of inflammation in type 2 diabetes. CyPA expression was reduced in circulating monocytes from patients with type 2 diabetes. The levels of CyPA in plasma samples of patients with diabetes and coronary artery disease were higher in comparison with its level in plasma obtained from healthy volunteers. Results of our studies suggest that CyPA secreted from monocytes could be an important pro-inflammatory stimulus for vascular inflammation in patients with diabetes. We argue for a promising role of secretory cyclophilin-A in the pathogenesis of vascular diseases and its clinical utility as a potential serological marker.

The study was supported by a grant from Indian Council of Medical Research.

MENDING THE "BROKEN" HEART: FROM MOLECULAR MECHANISMS TO THE BEDSIDE

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Although early revascularization of the ischemic myocardium is a main prerequisite of heart salvage, it is associated with the development of ischemia/reperfusion injury (IRI). The extent of IRI can be successfully reduced by ischemic preconditioning (PC). However, application of PC in humans is limited by technical requirements (chest opening) and short-term duration. On the other hand, some novel forms of PC, such as „remote" PC (RPC), in particular, its non-invasive modes are already being used in clinical conditions, especially in patients with acute myocardial infarction challenged with other confounders. Underlying mechanisms of RPC are not yet clear; however, involvement of nuclear receptors PPAR has been proposed besides other pathways. We have previously shown that upregulation of PPAR-α confers PC-like protection associated with activation of pro-survival cascades.

The study aimed to assess the efficiency of RPC applied on hind limb of anesthetized 3-months old rats using 3 cycles of 5-min pressure cuff inflation (200 mmHg,)/5-min deflation with or without PPAR-α antagonist MK886 (MK, 3 mg/kg i.p., given prior to RPC) on the size of infarction (IS), recovery of function (LVDP) and occurrence of malignant ventricular tachyarrhythmias (VT) in Langendorff-perfused hearts exposed to 30-min global I/120-min R. In parallel groups, LV tissue was sampled for the examination of PPAR-α gene expression and protein levels of PKCε.

RPC significantly increased mRNA levels of PPAR-α and post-IR protein expression of PKCε. This intervention did not affect baseline heart function, however, RPC significantly reduced IS, incidence and severity of VT and improved recovery of LVDP not only in the normal rat hearts, but also in the hypertrophic hearts of hypertensive (SHR) rats, albeit with a lower degree of efficiency. Moreover, most of cardioprotective effects as well as RPC-induced up-regulation of PPAR-α and increased levels of PKCε were suppressed by application of MK. The results confirm positive effect of non-invasive RPC against IRI in healthy and diseased heart. Up-regulation of PPAR-α may be involved as one of potential cardioprotective mechanisms of RPC in conjunction with activation of PKCε.

Grants VEGA 2/0201/15, APVV-0102-11, APVV-SK-CZ-2013-0075.

CALCIUM HOMEOSTASIS AND CARDIOVASCULAR DYSFUNCTION: RELEVANCE TO THE VITAMIN D

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Vitamin D is essential for the preservation of serum calcium and phosphate levels and its insufficiency could be associated with cardiovascular dysfunction. Recent studies have shown that diminished levels of 25(OH)D and 1,25-dihydroxyvitamin D are associated with prevalent myocardial dysfunction, deaths due to heart failure and sudden cardiac deaths. The rapid calcium release and reuptake at the sarcoplasmic reticulum are processes that critically determine normal systolic and diastolic myocardial function. Increasing evidence points to a molecular disturbance of Ca2+ homeostasis in ischemic and failing myocardium. We investigated the expression of mRNAs for Ca2+ binding proteins related to the sarcoplasmic reticulum in a porcine model of myocardial stunning. In 22 anaesthetised pigs, stunning was achieved by one or two cycles of 10 min left anterior descending coronary artery occlusion and reperfusion. Hearts were excised at various timepoints of the protocol. Total RNA was extracted from stunned (experimental) as well as normally perfused (control) myocardium. Northern blot analysis revealed that mRNAs encoding Ca(2+)-ATPase, phospholamban and calsequestrin increased as compared to the control at 90 min of the second reperfusion whereas, calmodulin and alpha, beta myosin heavy chain mRNA levels remained unchanged. Nuclear run-on assays confirmed increased transcription for calcium regulatory proteins. The results suggest specific repair mechanisms both at transcriptional and translational level indicating the role of calcium regulatory proteins related to the sarcoplasmic reticulum in ischemic myocardium. Interventional supplementation of vitamin D and calcium are warranted to elucidate whether vitamin D supplementation is useful for treatment and/or prevention of myocardial diseases.

STROMAL INTERACTION MOLECULE-1 MEDIATES ANGIOTENSIN-II-INDUCED EXPRESSION OF EARLY GROWTH RESPONSE PROTEIN-1 IN VASCULAR SMOOTH MUSCLE CELLS

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The early growth response protein 1 (Egr-1) is a zinc finger transcription factor that has been suggested to regulate the expression of genes linked with inflammation and cell cycle regulation. An upregulation of Egr-1 expression has been reported in models of atherosclerosis and intimal hyperplasia and various vasoactive peptides and growth promoting stimuli have been shown to induce the expression of Egr-1 in VSMC. Angiotensin-II (Ang-II) is a key vasoactive peptide that has been implicated in the pathogenesis of vascular diseases. Ang-II elevates the intracellular level of calcium through activation of store operated calcium entry involving inositol-3-phosphate receptor (IP3R)-coupled depletion of endoplasmic reticular calcium and stromal interaction molecule 1 (STIM-1). However, an involvement of IP3R/STIM-1- induced alteration in calcium pathway in Ang-II-induced Egr-1 expression in VSMC remains unexplored. Therefore in the present studies we have examined the role of Ang-II-induced calcium release in Egr-1 expression in VSMC and investigated the contribution of STIM-1 in this process. Calcium chelation with BAPTA-AM as well as pharmacological blockade of IP3R with 2-aminoethoxydiphenyl borate (2-APB) decreased Ang-II-induced calcium release measured in cells loaded with Fura-2. Consistent with this, both BAPTA-AM and 2-APB attenuated Ang-II-induced enhanced expression of Egr-1 protein and mRNA levels. Furthermore, RNA interference targeting STIM-1 significantly abrogated STIM-1 protein and mRNA expression and resulted in an attenuation of Ang-II-induced Egr-1 expression. In summary, our data suggest that IP3R/ STIM-1-dependent calcium flux plays a key role in mediating Ang-II-induced expression of Egr-1 in VSMC.

Supported by a grant from CIHR.

INNATE SIGNALING AND CYTOKINE INTERACTIONS IN HEART FAILURE

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Heart failure subsequent to myocardial infarction is associated with an increase in tumor necrosis factor-α (TNF-α) and a decrease in interleukin-10 (IL-10). In isolated cardiomyocytes, IL-10 has been shown to antagonize the pro-apoptotic effect of TNF-α. Although the anti-apoptotic action of IL-10 in cardiomyocytes is now generally accepted, its molecular basis is not yet well understood. We studied the role of IL-10 and Toll-like Receptor 4 (TLR4) and its downstream signals in the survival of adult cardiomyocytes. In IL-10 stimulated cardiomyocytes, TLR4 expression was followed by the upregulation of myeloid differentiation primary gene 88 (MyD88). Its activation led to IRF3 dimerization and phosphorylation which augmented IL-1β translational activity. Degradation of Ikk suggested that Ikkβ is an activating kinase for IRF3-regulated NF-κB activation and its nuclear translocation. There was an activation of Bcl-xL which attenuated the proteolytic activity of Caspase3 and PARP cleavage. Inhibition of MyD88 modulated IL-10 induced expression of TLR4, IRF3-dependent IL-1β production and NFκB p65 phosphorylation and translocation. There was a significant decrease in Bcl-xL expression leading to PARP cleavage. These data suggest that anti-apoptotic function of IL-10 through TLR4 activation, requires MyD88 activation for the cardiomyocyte survival signal.

Supported by CIHR.

POSSIBILITIES OF PREVENTING CARDIOVASCULAR INJURY CAUSED BY THE THERAPEUTIC RADIATION OF THE MEDIASTINUM

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Chronic injury of the myocardium is increasingly recognized as an undesired side effect of irradiation after thoracic/mediastinal radiation therapy of malignancies. The beneficial effects of Aspirin, Atorvastatin, Sildenafil, Enbrel and molecular hydrogen on cardiac function and selected molecular markers of injury following 6 weeks of exposure to a single dose radiation (10 and 25 Gy), applied to the mediastinal area of normal adult Wistar rats were investigated. Ultrastructural manifestations of endothelial cell degeneration/regeneration, activated fibroblasts and monocytes were observed. Gene expression of PPARα was significantly lower in left ventricle (LV) of irradiated rats indicating a shift in substrate preferences from fatty acids to glucose. Expression of miRNA-1 was significantly decreased while microRNA-21 was increased nearly 10-fold in these hearts. miRNA-15b was downregulated by 42% and Bax protein decreased, indicating triggered adaptive mechanisms reflected in a reduction of size of myocardial infarction. Myocardial Cx43 was upregulated possibly via reduced miRNA-1. Activity of circulating 72kDa MMP-2 was significantly increased. Furthermore, irradiation caused a decrease in IL-10 and TNF- α as well. The IL-10/TNF-α ratio in the LV was significantly increased in the Enbrel treated group. Treatment of rats with Aspirin caused a significant increase in IL-10 in LV and irradiation blunted this increase significantly. Enbrel caused a significant decrease in the TNF-α level in the LV. Hydrogen downregulated proinflammatory cytokines including TNF- α. All drugs showed some protection against the negative impact of radiation on healthy tissue as demonstrated by changes in miRNA, IL-10 and TNF- α values. Our studies have also shown that hydrogen has antioxidant, anti-inflammatory, and antiapoptotic protective effects on cardiovascular system.

CONCLUSION: Characteristic changes in cytokines as well as miRNAs expression, may be reflected in limiting the extent of injury associated with the upregulation of proteins that are implicated in the protection of the heart. Protective effect of hydrogen administration remains to be elucidated.

Studies supported by grant: APVV-0241-11, VEGA 2/0021/15 and partially by grants from NIH R37L051045 to RC and CIHR to PKS.

IS PROPOFOL CARDIOPLEGIA PROTECTIVE IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS GRAFTING OR AORTIC VALVE REPLACEMENT?

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Propofol is a general anaesthetic widely used during cardiac surgery and in postoperative sedation. It has also been shown to protect hearts against cardiac insults in a variety of experimental models. . a free radical scavenger inhibition of plasma membrane calcium channels. However, this issue was controversial in 1980 & 1990s. This largely due to the fact that the drug was used in High doses, proposed mechanisms were vague, was not clear whether the effects were systemic or at the cellular level. In view of its standard use in cardiac surgery, it was argued that this could be used as an additive to cardioplegic solutions.

Work started in 1996 in Bristol has provided evidence showing that propofol is cardioprotective in normal and in diseased heart using different experimental models and clinically relevant concentration. Furthermore, this drug was found to protect the heart against ischaemia/reperfusion by inhibiting the mitochondrial permeability transition pore. The cardioproteive efficacy was also demonstrated in a pig model of normothermic blood cardioplegic arrest and cardiopulmaonry bypass.

A single-centre randomized controlled trial was designed to investigate the effects of propofol cardioplegia on blood and myocardial biomarkers of stress and injury in patients with isolated coronary artery bypass grafting or aortic valve replacement using cardiopulmonary bypass. This trial has been completed and the outcome is positive but inconclusive. Further work is planned to complete this trial.

♣ PRESENCE OF NECROPTOSIS IN END-STAGE HUMAN HEART FAILURE

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Background and aims: Until recently, necrosis was considered to be an unregulated form of cell death; however, with the discovery of necroptosis, a programmed cell death subtype dependent on RIP1 (receptor-interacting protein kinase 1), RIP3 and MLKL (mixed-lineage kinase domain-like protein) phosphorylation, examination of programmed necrosis in various cardiac pathologies was made possible. In this regard, chronic heart failure (HF), a progressive disease characterized by cardiomyocyte loss and contractile dysfunction, represents a potentially important pathology. Indeed, the nature of cell loss during HF is controversial and many studies have only focused on the potential role of apoptosis in failing hearts, while very limited research was done on necrotic processes. The aim of this work was therefore to investigate whether necroptosis could be relevant in human chronic HF.

Methods: Samples were obtained from left ventricles of healthy donors (C) as well as from explanted hearts of end-stage (NYHA III-IV) HF patients due ischemic (CAD) and dilated (DCM) cardiomyopathy. Pronecroptotic proteins RIP1, RIP3 and pThr^{357}-MLKL were analyzed by the means of Western blotting both in total tissue lysates as well as in subcellularly fractionated samples. In addition, selected apoptotic proteins were determined since some of them directly antagonize necroptosis initiation (caspase-8).

Results: All markers of necroptosis were increased in HF samples while apoptotic markers, such as Bax, caspase-3 and PARPp89, did not confirm apoptotic cell death. RIP1 and RIP3, both constitutively active in necroptosis induction, were increased in CAD as well as DCM. Caspase-8 expression was greatly downregulated in HF groups suggesting pronecroptotic conditions. Total MLKL expression was unchanged by HF; however, its phosphorylation at Thr^{357}, a terminal marker of necroptosis execution, was only present in HF samples while being more greatly increased in DCM. Analysis of subcellular localization of necroptotic proteins revealed their accumulation in cytoplasm and nucleus along with lipid rafts of membranes.

Conclusions: Obtained data show end-stage human HF due to ischemic or dilated cardiomyopathy is positive for markers of necroptosis activation and execution. These results implicate programmed necrosis in cell loss associated with human chronic HF.

Supported by grants VEGA1/0638/12 and FaF/19/2015.

VISCERAL PERIVASCULAR ADIPOSE TISSUE REGULATES ARTERIAL SMOOTH MUSCLE RESPONSIVENESS

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Perivascular adipose tissue (PVAT) functions as a paracrine and endocrine organ secreting a number of vasoactive compounds including adipokines, inflamatory cytokines, reactive oxygen species and number of vasoactive substances which influence vascular tone in a paracrine way. These substances regulate adipocyte metabolism and other cellular processes including vascular smooth muscle tone. However, little is known about whether PVAT – derived substances can influence neuronal responses in blood vessels. The aim of this study was to assess the effect of perivascular fat on contractile responses of conduit arteries (thoracic aorta, mesenteric artery) to vasoactive substances in normotensive and hypertensive rats. Moreover, this study was also conducted to investigate the effects of PVAT on sympathetic perivascular neurotransmission. Both normotensive WKY and SHR rat vessels with PVAT displayed weaker contractions to noradrenaline compared to their counterparts without PVAT, however, this anticontractile effect of PVAT was reduced in SHR. The anticontractile effect of PVAT is not a non-specific event, since not all agonists are uniformly affected even in the same arterial preparation. Anticontractile effect has been demonstrated in isolated segments of the vena cava The anticontractile effect of PVAT was mediated through the activation of potassium channels since it is abrogated by inhibiting potassium channels by 4-aminopyridine (1 mmol/l), a voltage-dependent potassium channel blocker. Inhibiting effect was greater in WKY than in SHR rats. Blocking other K+ channels with glybenclamide (10 μmol/l) had no effect. Electrical field stimulation (EFS) elicited frequency-dependent vasoconstriction of the mesenteric artery. In the absence of PVAT, neurogenic contractile responses were attenuated. The force generated by EFS was greater in absence compared with presence of 4-AP in WKY rats only, but not in SHR. Tyramine, an indirect sympathomimethic agent, produced a concentration dependent contraction that was dependent on the presence of PVAT in normal rat thoracic aorta. It suggests that tyramine can reveal functional adrenergic system in PVAT. Conclusion: PVAT regulates vascular smooth muscle tone through the release of adipocyte-derived relaxing factor .Our results confirm the existence of these anticontractile factor in conduit arteries and veins. This factor operates, at least in part, by activation of voltage-dependent potassium channels and its action is less effective in SHR than in WKY rats.A knowledge of mechanism of potasium channels activation by ADRF could bring a new pharmacological tools that can mimic the ADRF effect and thus can be beneficial against vascular dysfunction in hypertension and obesity.

The study was supported by grant VEGA No. 2/0202/15.

NCX INHIBITION AND Ca HANDLING

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The NCX is an important transport system in the heart and it is a major contributor to the maintenance of intracellular Ca2+ homeostasis. Although the sodium-calcium exchanger (NCX) is an electrogenic ion-transport system potentially carrying net outward and/or inward currents, its contribution to the cardiac action potential have not yet been directly studied due to the lack of specific inhibitors.

In this study we have investigated the effects of selective NCX inhibition on cellular Ca^{2+} handling, action potential and compared to those of computer simulations by applying the conventional microelectrode and whole cell configuration of the patch-clamp techniques.

ORM-10962, the highly selective NCX inhibitor blocked forward and reverse mode of NCX in dog ventricular myocytes with estimated EC50 values of 54 nM, and 68 nM, respectively without altering other important transmembrane ionic currents.

Inhibition of NCX by ORM-10962 elicited modest and variable changes on action potential waveforms depending on the relative strength of the forward or reverse mode of NCX function on different types of cardiac tissue preparations studied.

Also the inotropic response and the corresponding cellular Ca^{2+} transient of the myocytes varied according to the relative strength of forward and reverse mode of NCX function

The experimental results only partially agreed with in silico modeling results suggesting that our current understanding of NCX function is limited and further investigations are needed to clarify its pathophysiological role in arrhythmia initiation.

PATHOGENIC ROLE OF FUNCTIONAL AUTOANTIBODIES AGAINST G-PROTEIN COUPLE RECEPTORS IN CARDIO-VASCULAR DISEASES: USE OF APTAMERS AS A NEW THERAPEUTIC OPTION

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Autoantibodies (AABs) against G-protein coupled receptors are detectable in several cardiovascular diseases. These AABs are directed against epitopes localized on the first or second extracellular loop of the membrane spanning receptors and acting like the corresponding agonists. These epitopes differed from the binding site of the classical agonists which bind to a pore formed by the seven membrane spanning domains of the G-protein coupled receptor. Therefore, we assume that the AABs realize their agonist like effect by cross-linking of the receptor dimere and the stabilization of the active receptor conformation.
In contrast to the classical agonists, the functional AABs prevent the receptor desensitization normally seen by the permanent stimulation with the agonists. One of the investigated AABs is an antibody directed against the β1-adrenoceptor. These AABs were found in the sera of patients with idiopathic dilated cardiomyopathy (DCM). Several experimental and clinical observations indicate that this agonist-like AAB may play a role in the development and maintenance of DCM. This β1-adrenoceptor AAB activate the protein kinase A and the L-type Ca^{++} channel and prevent the receptor desensitization and may induce a harmful adrenergic overdrive which can cause a Ca^{++} overload and apoptosis. Therefore, we started several years ago a program to remove the AABs from the patient sera by immunoadsorption (IA). IA removed effectively the AABs and improved the hemodynamic function of the DCM patients. Moreover, the beneficial remove of the AABs by IA leads to a prolonged survival of the DCM patients. However, this therapeutic option is time and cost consuming. Therefore, we started recently a new therapeutic concept. We identified and characterized aptamers which were able to recognize and neutralize specifically AABs directed against G-protein coupled receptors. Aptamers are short DNA or RNA oligonucleotides which high specifically bind to peptides, proteins, and toxins but also to virus particles. In our animal experiments we were able to show that the aptamer infusion caused a long-lasting disappearance of the AABs in a rat and in a dog model.

♣ INFLUENCE OF ISCHEMIC PRECONDITIONING AND SIMULATED HYPERGLYCEMIA ON HEART RESISTANCE TO ISCHEMIA-REPERFUSION INJURY

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Aim of our study was to characterize the influence of simulated acute hyperglycemia (HG, 2-fold glucose increase in perfusion buffer) and ischemic preconditioning (IP), on heart resistance against ischemia-reperfusion (I/R) injury mediated by the PI3K/Akt pathway. Testing was performed in isolated rat hearts perfused according to Langendorff. IP was induced by two 5-min cycles of coronary occlusion intercepted with 5-min reperfusion and non-IP hearts were served as controls. Severity of I/R (30-min ischemia followed by 120-min reperfusion) injury was determined by evaluation of the infarct size (IS; in % of area at risk), and also by the amount of heart-type fatty acid binding protein (h-FABP, a marker of cardiac cell injury) released to the effluent from the hearts. Both, lower IS and amount of h-FABP released from the preconditioned hearts confirmed its beneficial effect on the heart resistance under standard conditions (NG, perfusion with normal glucose buffer, 11 mmol/l). Similarly, HG suppressed h-FABP release from the control hearts. In contrast, IP under HG conditions increased IS and a release of h-FABP as compared to respective controls. Prosurvival PI3K/Akt and apoptotic activity expressed as P-Akt/Akt and caspase-3/procaspase-3 (cas-3/procas-3), respectively, in cytosol were evaluated (WB) in further protocol of 30-min ischemia and 40-min reperfusion. Increase of P-Akt/Akt after long-term ischemia and its decrease after reperfusion, coupled with reduced cas-3/procas-3 in IP hearts under NG and HG-controls compared to NG-controls point out to a beneficial effect of PI3K/Akt pathway and its potential negative feed-back regulation which prevents persistent PI3K/Akt activity. However, HG reversed IP-mediated PI3K/Akt signaling and tended to elevate cas-3/procas-3 in IP hearts compared to non-IP controls. It seems that HG may alter PI3K/Akt signaling triggered by IP in the early stage and promote its continuous activation detrimental to the heart attenuating its protection against apoptosis.

Supported by grants: VEGA SR 1/0638/12, VEGA SR 2/0201/15, APVV-0102-11.

ABSTRACTS OF POSTER PRESENTATIONS

STRUCTURAL CHANGES IN MYOCARDIUM AND AORTA AFTER ALISKIREN-IDUCED INHIBITION OF RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM

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The renin-angiotensin aldosterone system (RAAS) is a hormonal cascade that functions in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume. Dysregulation of the RAAS plays an important role in the pathogenesis of cardiovascular and renal disorders. Development of agents that block the RAAS (e.g., beta blockers, ACE inhibitors, and angiotensin receptor blockers) began as a therapeutic strategy to treat hypertension. Aliskiren is the first orally active inhibitor of renin to be approved for clinical use as an antihypertensive agent. Its high molecular weight still results in a low bioavailability (2.7%). However, the absorbed aliskiren is scarcely metabolized and slowly excreted, with a consequently long half-life of 24 to 40 hours.

In our experiment, 12-week-old male SHRs were assigned to untreated group, group treated with powdered aliskiren (25mg/kg per day), group treated with nanoparticle-loaded aliskiren (25mg/kg per day) and group treated with polymeric nanoparticles only for 3 weeks by gavage. At the end of experiment, BP was lower in both powdered aliskiren and nanoparticle-loaded aliskiren groups with more pronounced effect in the second one. Moreover, nanoparticle-loaded aliskiren was able to decrease collagen content (by 11%) and CSA (by 25%) in comparison to the powdered aliskiren group, while it had no significant effect on the similar parameters in the heart. There were no significant changes in elastin content, WT and ID among aliskiren groups and control group. Polymeric nanoparticles, however, increased collagen and elastin contents and WT of the aorta. In conclusion, nanoparticle-loaded aliskiren seems to be promising drug in large vessels protection, more suitable polymeric nanoparticles, however, are needed for better tissue protection.

Supported by VEGA 2/0165/15, 2/0144/14, APVV-14-0932.

♣ UNDERLYING MECHANISMS OF CARDIOPROTECTIVE EFFECTS OF MELATONIN AND OMEGA-3 FATTY ACIDS INTAKE IN RATS EXPOSED TO SUCROSE DIET.

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Background and Purpose: Obesity is an increasingly prevalent metabolic disorder and one of the risk factors for cardiovascular diseases. Previously, we have shown adverse effects of hyperglycemia on cardiac gap junction connexin-43 (Cx43) channels that are crucial for synchronized heart function. On the other hand, the benefit of melatonin and omega-3 fatty acids (omega-3 FA) intake was demonstrated in diabetic and hypertensive rats. The aim of this study was to test our hypothesis that aged female rats fed with a high sucrose diet may develop metabolic disorders that affect cardiac Cx43 and PKC signaling and consequently the susceptibility of the heart to malignant arrhythmias. The response of these rats to omega-3 FA and melatonin treatment was examined as well.

Material and Methods: Experiment was performed on 9-month-old female Wistar rats. Animals were divided into four groups: 1) controls; 2) rats drinking 30% sucrose solution (HSD); 3) HSD supplemented with melatonin (40 μg/ml/day); 4) HSD supplemented with omega-3 FA (Omacor, 25g/kg of rat chow). Selected biometrical and biochemical parameters were registered and evaluated. Expression of Cx43, PKCε and PKCδ were determined using Western blot. Real-time PCR was used for mRNA analysis of Cx43 and miR-1, which suppresses Cx43 expression. Electrically induced sustained ventricular fibrillation (sVF) was examined using Langendorff-perfused heart.

Key Results: Significant increase of body weight, heart weight, left ventricular weight, adiposity, plasma triglycerides and cholesterol, but not blood glucose was registered in rats exposed to HSD. Melatonin and omega-3 FA did normalize these parameters. Comparing to control rats, the hearts of HSD rats were much prone to sVF, while melatonin and omega-3 FA exhibited clear antiarrhythmic effect. There were no differences in myocardial Cx43 mRNA levels among the groups. Significantly reduced expression of Cx43 protein and its functional phosphorylated forms in HSD rats were normalized by melatonin and omega-3 FA. Levels of miR-1 were higher in HSD rats vs. controls and suppressed after the treatment with melatonin. Moreover, omega-3 FA and melatonin normalized diminished expression of PKCε and enhanced the expression of PKCδ in HSD rats.

Conclusions: Findings indicate that high sucrose diet itself deteriorates myocardial signaling mediated by connexin-43 and PKC that may contribute to increased susceptibility of the heart to malignant arrhythmias. Our results indicate clear anti-arrhythmic effect of melatonin and omega-3 fatty acids after prolonged treatment. Underlying mechanisms are most likely due to up-regulation of Cx43 and PKC signaling. Findings support supplementation of melatonin and omega-3 fatty acids, in patients with cardiovascular diseases who often suffer from deficit of these compounds.

THE EFFECT OF ARONIA MELANOCARPA ON CARDIOVASCULAR SYSTEM IN L-NAME-INDUCED HYPERTENSION.

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Polyphenols are a class of natural products exhibiting multiple health benefits beyond their antioxidant potential. Aronia melanocarpa (black chokeberry) has attracted scientific interest due to its dense contents of polyphenols, especially anthocyanins. The aim of the present study was to analyze effects of non-alcoholic concentrate from aronia melanocarpa (AM) on blood pressure (BP), total NOS activity and cytokine level in the left ventricle of L-NAME-induced hypertensive rats. 12-week-old male WKY rats were assigned to control group, group treated with L-NAME (40mg/kg/day), group treated with AM concentrate (1ml/kg/day), and group treated with combination of L-NAME (40mg/kg/day) and AM concentrate (1ml/kg/day) in tap water. The experiment lasted 3 weeks. BP was measured by the tail-cuff-plethysmography. NOS activity was determined by conversion of ^{3}[H] Arginine to ^{3}[H] Citrulline in the left ventricle (LV). Cytokine levels were investigated using the Bio-Plex Pro Cytokine kit in the plasma. After 3 weeks of L-NAME treatment BP was increased by 28% than the control group. AM reduced BP by 21% in L-NAME + AM group in comparison to L-NAME group. Moreover, AM inhibited TNF α and IL-6 production in the plasma in L-NAME + AM group in comparison to L-NAME group. NOS activity of LV in L-NAME group was decreased by 40%, on the other hand AM was able to increase NOS activity on 90% of control level. The results of our study show that active substances from Aronia melanocarpa may have positive effect on blood pressure, cytokine level and NOS activity in L-NAME- induced hypertension.

Supported by VEGA 2/0165/15, 2/0144/14, APVV-14-0932.

CHRONIC CONTINUOUS HYPOXIA INCREASES THE RESERVE ACTIVITY OF CYTOCHROME-C OXIDASE IN HEARTS OF SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Mitochondria play an essential role in improved cardiac ischemic tolerance conferred by adaptation to chronic hypoxia. Cytochrome-c oxidase (COX) is the terminal enzyme of the mitochondrial respiratory chain and serves as an important regulatory site of oxidative phosphorylation. In the present study we analyzed COX activity (basic and reserve) in the left ventricle of normoxic and chronically hypoxic spontaneously hypertensive rats (SHR) compare with a novel conplastic strain SHR-mt^{BN} characterized by the selective replacement of the mitochondrial genome of SHR with that of more ischemic resistant strain Brown Norway.

Methods and results: Rats were adapted to continuous normobaric hypoxia (CNH; 10 % O_2) for 3 weeks. Animals kept at room air served as normoxic controls. Mitochondrial respiration and COX activity were measured at 30°C using Oxygraph-2k. The respiratory pattern and respiratory control index were similar in mitochondria isolated from left ventricles of normoxic and hypoxic rats. CNH did not affect mitochondrial quantity, as indicated by unchanged citrate synthase activities. Basic COX activity did not differ between the strains and was not affected by CNH. However, the reserve COX activity (measured in the presence of 0.02 % lauryl maltoside) was significantly increased after adaptation to CNH in both strains (by 45 % in SHR and 38 % in SHR-mt^{BN}).

Conclusion: Adaptation to CNH increased the reserve COX activity in the left ventricle of SHR independent of mitochondrial genome.

This work was supported by grants GAUK 1592614 and APVV-SK-CZ-2013-0075.

ENDOTOXIN- AND OMEGA-3 FATTY ACIDS-INDUCED CHANGES OF ENDOTHELIAL OCCLUDIN EXPRESSION IN HEART

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Endothelial hyperpermeability induced by various risk factors including bacterial endotoxin (LPS) represents a significant problem in development of atherosclerotic heart disease. However, mechanisms involved in regulation of endothelial permeability are not still completely understood. Tight junctions (TJs) are intercellular junctions, playing a key role in regulation of paracellular endothelial permeability. Occludin, one of TJ proteins, is responsible for TJ formation. Its abnormal expression might cause TJ dysfunction and/or absence respectively and contribute to endothelial barrier dysfunction. Omega-3 fatty acids (o-3 FA) are known for their protection of endothelial integrity and heart function. Therefore, the focus of our pilot study was to examine the effect of LPS and o-3 FA supplementation on occludin expression in tissue of left ventricle. LPS was administered to adult male Wistar rats in a single dose (1 mg/kg, i.p.). After that, rats were fed with o-3 FA (30 mg/kg/day) for 10 days. Left ventricle of heart was processed for 1) Western blot analysis of occludin and NFkB (p65) (transcription nuclear factor playing multiple roles e.g. in immune diseases, acute phase response, stress, injury), 2) immunofluorescent distribution of occludin and 3) electronmicroscopy of endothelium. Heterogeneous immunofluorescence of occludin was detected within vascular endothelium of the hearts of all experimental groups. LPS caused upregulation of total occludin expression, which was 20% higher when compared with healthy rats. It was associated with increased expression of its non-phosphorylated isoform. Electronmicroscopy revealed LPS-related subcellular alterations of endothelial cells indicating both endothelial dysfunction and angiogenesis. Locally observed widened inter-endothelial junctions correspond with disturbances of occludin expression and TJ dysfunction. On the other hand, high amount of pinocytic vesicules in endothelium indicates transcellular pathway activation. LPS also increased NFkB expression. O-3 FA supplementation of LPS rats resulted in downregulation of total occludin expression, which was 34% lower than in LPS rats. Expression of phosphorylated isoform of occludin was increased while expression of the non-phosphorylated one was decreased. O-3 FA had no effect on NFkB expression in heart of LPS rats. Our pilot results indicate involvement of occludin in protection of LPS-induced endothelial barrier dysfunction with o-3 FA.

Supported by VEGA 2/0065/13.

WINE GRAPE POMACE PROTECTS AGAINST REPERFUSION ARRHYTHMIAS IN DYSMETABOLIC RATS

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Severe reperfusion arrhythmias are associated with increased mortality after acute myocardial infarction. High fat and fructose diet (HFFD) increases arrhythmic risk and predispose to diseases such as diabetes, hypertension and dyslipidemia.

Wine grape pomace (WGP) is a left-over of seeds and skins generated in the winemaking process. It contains relatively high amounts of bioactive components such as dietary fibre and polyphenols. Food rich in polyphenols has been proposed to prevent cardiovascular disease.

We tested whether rats fed with a HFFD increased arrhythmias during the reperfusion, and if a WGP supplementation could counteract this effect.

Male Wistar rats were fed for 6 weeks with: control diet (C), HFFD (20 % fat and 20% fructose w/w), and HFFD supplemented with WGP in a dose of 1 g/Kg/day. Isolated hearts were perfused with Krebs-Henseleit solution and after 15 min stabilization, submitted to 10 min of regional ischemia followed by 5 min of reperfusion. Incidence and severity of arrhythmias were analyzed.

Metabolic syndrome was confirmed in HFFD and WGP groups. An increase in ventricular fibrillation incidence (5/6, $p<0.01$ vs C by Fisher Exact test) and duration (3min 36s, $P<0.05$ by Kruskal-Wallis) was seen in respect to both C (1/10 y 1min) and WGP (1/10 y 1 min). Other arrhythmias occurred in similar proportion in all groups.

The above results suggest that a HFFD facilitates the occurrence of sever arrhythmias during reperfusion and WGP prevents these events, particularly in respect to severity and length of the episodes.

METHYLENE BLUE MODULATES MITOCHONDRIAL FUNCTION IN DIABETIC RAT HEARTS

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Background: Mitochondrial dysfunction and reactive oxygen species (ROS) generation are critical events in the pathophysiology of type II of diabetes mellitus (DM), including the long-term complications of this devastating disease. Pharmacological agents able to prevent ROS production and/or to improve mitochondrial function are nowadays highly investigated therapeutic targets; among them an emerging compound is methylene blue (MB), a tricyclic phenothiazine with mild redox potential. We previously demonstrated that 0.1 μM MB significantly improved the bioenergetic profile of H9c2 cardiomyoblasts. The present study was purported to characterize the effects of 0.1 μM MB on oxidative phosphorylation, the sensitivity of the mitochondrial permeability transition pore (mPTP) to calcium overload, and ROS production in rat heart mitochondria (RHM) isolated from diabetic rats. **Methods.** Respiration of diabetic RHM was measured by high resolution respirometry. ROS production and the amount of total mitochondrial Ca^{2+} retained before opening of mPTP were measured spectrofluorimetrically. **Results.** In RHM respiring on both Complex I and II substrates a significant increase in all bioenergetic parameters was found in diabetic RHM treated with 0.1 μM MB. No change of sensitivity to Ca^{2+}-induced mPTP opening was found in the presence of MB. With respect to the oxidative stress, MB significantly increased mitochondrial ROS production in the presence of CI substrates (glutamate + malate), but decreased ROS production when RHM were energized with the CII substrate, succinate (+ rotenone).

Conclusions. Our data indicate that, in diabetic rat hearts, 0.1 μM methylene blue improved mitochondrial respiratory function, regardless the substrates used, and elicited a dichotomic, substrate-dependent effect on ROS production.

Research funded by the POSDRU grant no. 159/1.5/S/136893 titled: "Strategic partnership for the increase of the scientific research quality in medical universities through the award of doctoral and postdoctoral fellowships–DocMed.Net_2.0"

THE ROLE OF HEART MITOCHONDRIA IN THE PROCESS OF MYOCARDIAL PROTECTION IN PRESENCE OF ACUTE DIABETES MELLITUS AND REMOTE ISCHEMIC PRECONDITIONING

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Background: Diabetic myocard is constantly in a state of pseudohypoxy, which leads to impaired ability of diabetic heart mitochondria (MIT) to utilize oxygen. In this way, myocard in acute state of diabetes mellitus acquires characteristics similar to ischemic preconditioning and can stimulate process of endogenous protection leading to adaptation to the stimulus. Aim of the study was to investigate the effects of remote preconditioning (RIPC) and PC - induced acute diabetes mellitus (DIA), on the biophysical and biochemical properties of cardiac MIT and to elucidate the role of cardiac MIT in the process of enhanced myocardial protection in presence of DIA and RIPC.
Methods: Male Wistar rats (220±20g) were used in the experiment. DIA was induced by a single dose of streptozotocin, (65 mg/kg, i.p.). RIP was induced by 3 series of 5-min. ischemia and 5-min. reperfusion applied on the right hind limb. MIT were isolated by means of differential centrifugation. Mg^{2+}-dependent and 2, 4-dinitrophenol-stimulated ATPase activity was estimated by measuring of the P_i liberated from ATP splitting. MIT membrane fluidity was assessed spectrofluorometrically by means of 1, 6-diphenyl-1, 3, 5-hexatriene. Conjugated dienes in MIT membrane lipids were estimated spectrophotometrically at 230 nm. Content of oxidized isoforms of coenzyme Q (CoQ_{9ox} and CoQ_{10ox}) in the isolated MIT was estimated by means of HPLC.
Results: MIT from acute (8 days) DIA hearts exhibited significantly ($p<0.05$) elevated Mg^{2+}-ATPase activities without any considerable elevation of conjugated dienes formation. Functional remodeling of DIA heart MIT and also healthy heart subjected by RIP is associated with increase in the fluidity of MIT membranes. It was most expressed in the DIA group. Significant increased of membrane fluidity found in the DIA group is not supported by presence of RIP. MIT from RIP treated hearts seemed to exhibit relatively high level of CoQ9ox. However, it appears that the RIP prevents the ischemia/reperfusion-associated increase in CoQ9ox oxidation. Investigation of CoQ10ox formation in MIT from control and RIP-treated hearts yielded less marked results.
Conclusion: Diabetes mellitus induced changes in physical parameters as well as in activities of some enzymatic components of MIT membranes. They are involved to compensation and myocardial adaptation processes and lead to endogenous protective mechanisms. RIPC induced endogenous protective mechanisms by increasing the membrane fluidity, which can be associated with enhanced mitochondrial Mg2+-ATPase activity. It is not observed the additive fluidization effect of combinations DIA and RIP. Changes in functional properties of MIT membranes associated with diabetic- and RIP-induced remodeling are involved in increased ischemia-tolerance of the myocardium.

Grants: VEGA 2/0133/15; 2/0201/15; APVV-0102-11, APVV-SK-CZ-2013-0075

♣ CHANGES IN THE EXPRESSION OF CONNEXINS 40 AND 43 OF THE RAT AORTIC TISSUE 6 WEEKS AFTER MEDIASTINUM IRRADIATION.

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One of the methods used in the treatment of cancer patients is radiotherapy. The irradiation of patients in the thoracic region may lead later to the development of cardiovascular disease and heart failure. In the thoracic region, in addition to heart and its vessels, may also be damaged aortic tissue and its function. Important for the proper functioning of the aorta is a direct intercellular communication mediated by Gap Junctions (GJs). GJs are formed by connexins (Cx). Their role is transport of low-molecular substances, ions and signals between adjacent cells. Among the most expressed connexin in the aorta is CX40 (in the endothelium) and Cx43 (in the media). The aim of this study was to assess the effect of ionizing radiation on changes of Cx40 and Cx43 expression which may indicate changes in the aortic function. We also tried to reverse any changes induced by irradiation with substances that affect blood vessel function. Therefore we studied the effect of Tadalafil, which throught cGMP-specific phosphodiesterase type-5 inhibition affects the relaxation of the aorta.

In the six-week lasting experiment were used male Wistar rats 3 months old that were divided into three groups Control (C), Irradiated (Ir, single dose of 25 Gy), and irradiated group treated with Tadalafil (IrT, in a dose 0,25 mg/Kg/day). Blood pressure, body weights, Cx`s expression, NFkB (as inflammation marker) and occludin (as marker of endothelial integrity, protein of tight junctions) in whole aortic tissue were assessed.

Six weeks after irradiation the body weights in Ir group and IrT were 20% less than in C. Systolic blood pressure was without any significant change. The expression of NFkB did not change in all affected groups compared with C. In Ir group decreased expression of Cx40 (15%) and increased expression of Cx43 (17%) were found. Treatment with Tadalafil in IrT group normalized Cx40 and Cx43 expression to C levels. Expression of occludin after irradiation increased 42%, however, treatment with Tadalafil (IrT group) significantly decreased its expression under the control levels.

Our results suggest that the Cx43 and Cx40 in the aortic tissue are included in the tissue radiation damage. The normalized Cx40 and Cx43 expression in treated (Tadalafil) irradiated rats may indicate an improvement of the aortic function.

This work was supported by grants APVV-0241-11, VEGA 2/0021/15 and VEGA 2/0065/13.

♣ MATURATION-RELATED CHANGES IN RESPONSE TO ISCHEMIA-REPERFUSION INJURY AND ADAPTATION IN MALE RAT HEARTS:STUDY OF POTENTIAL MOLECULAR MECHANISMS.

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Reduced tolerance to ischemia/reperfusion (IR) injury has been shown in elder human and animal hearts, however, the onset of this unfavorable phenotype and cellular mechanisms behind remain unknown. Moreover, aging may interfere with the mechanisms of innate myocardial protection (preconditioning, PC) and cause defects in protective cell signaling.

We studied the changes in myocardial function, response to ischemia and selected proteins involved in "pro-survival" pathways in the hearts from juvenile (1.5 months), younger adult (3 months) and mature adult (6 months) male Wistar rats. In Langendorff-perfused hearts exposed to 30-min I/120-min R without or with prior PC (one cycle of 5-min I/5-min R), we measured infarct size (IS, TTC staining), susceptibility to reperfusion arrhythmias and recovery of contractile function (left ventricular developed pressure, LVDP; left ventricular end-diastolic pressure, LVEDP). In parallel groups, LV tissue was sampled at baseline (BL) and after IR for the detection of protein levels (WB) of Akt kinase, phosphorylated (activated) Akt (p-Akt), endothelial NO synthase (eNOS) and protein kinase Cε (PKCε) as components of "pro-survival" cascades.

Maturation did not affect heart function, however, it impaired cardiac response to lethal IR injury (increased IS) and promoted arrhythmogenesis. PC reduced occurrence of arrhythmias, IS and improved LVDP and LVEDP recovery in younger animals, while its efficacy was attenuated in the mature adults. Loss of PC protection was associated with age-dependent reduced Akt phosphorylation and levels of eNOS and PKCε in the hearts of mature animals compared with the younger ones, as well as with a failure of PC to upregulate these proteins.

Aging-related alterations in myocardial response to ischemia may be caused by dysfunction of proteins involved in protective cell signaling that may occur already during the process of maturation.

Grants VEGA SR 2/0201/15, APVV-0102-11, APVV-0523-10.

INVOLVEMENT OF PKCepsilon AND BKCa CHANNELS IN CARDIOPROTECTION INDUCED BY ADAPTATION TO CHRONIC CONTINUOUS HYPOXIA

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Chronic continuous hypoxia (CCH) renders the heart more tolerant to acute ischemia/reperfusion injury. The aim of this study was to analyze the involvement of protein kinase C-epsilon (PKCepsilon) and mitochondrial large-conductance Ca^{2+}-activated potassium channels (BKCa) in cardioprotection induced by adaptation to CCH. Adult male Wistar rats were exposed to CCH (10% O_2, 3 weeks) or kept under normoxic conditions. Ventricular cardiomyocytes isolated from CCH rats exhibited better survival and lower lactate dehydrogenase (LDH) release caused by simulated ischemia/reperfusion than cells from normoxic group. Adaptation to CCH increased myocardial PKCepsilon at protein and mRNA levels. The application of the PKCepsilon inhibitory peptide (KP-1633) blunted the CCH-induced salutary effects on cell viability and LDH release, while the control peptide KP-1723 had no effect. While CCH did not affect protein abundance of the BKCa channel regulatory beta1-subunit, it markedly decreased its glycosylation level. The cytoprotective effects of CNH were attenuated by the BKCa channel blocker paxilline, while the opener NS1619 reduced cell injury in the normoxic group but not in the CCH group. This study indicates that PKCepsilon and activation of the mitochondrial BKCa channel likely contribute to CCH-induced cardioprotection.

Supported by grant APVV-SK-CZ-2013-0075.

EFFECT OF CONTINUOUS NORMOBARIC HYPOXIA AND MODERATE EXERCISE TRAINING ON POSTINFARCTION HEART FAILURE IN RATS

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Background: Adaptation to chronic hypoxia and exercise training are known to protect the heart against acute ischemia/reperfusion injury. Much less is known about potential therapeutic effect of these interventions on myocardial infarction (MI). The aim of this study was to find out whether chronic hypoxia or moderate exercise training can attenuate the progression of postinfarction heart failure.

Methods and results: MI was induced in two-month-old male rats by coronary artery occlusion. Seven days after surgery, the MI rats were randomly assigned to three groups: i) sedentary controls kept at room air, ii) exposed to continuous normobaric hypoxia (12% O_2, 3 wks) or iii) trained on a treadmill (15 m/min, 60 min/day, 5 days/wk, 3 wks). Echocardiography examination of the left ventricle (LV) was performed 3 days before MI and 7, 14 and 28 days after MI. MI resulted in a gradual increase in systolic and diastolic diameter (LVDs, LVDd) and a decrease in relative posterior wall thickness (RWT) compared to sham-operated animals. Fractional shortening (FS) decreased from 42,8 % before MI to 15,1 % on day 28 post-MI. Chronic hypoxia attenuated ventricular dilatation without significantly affecting FS. Moderate exercise training had no effect on LV geometry and function.

Conclusion: Our data suggest that prolonged exposure to continuous hypoxia has certain potential to attenuate the progression of unfavourable changes in ventricular geometry induced by MI in rats.

This work was supported by grants GAUK 798813 and APVV-SK-CZ-2013-0075.

♣ STRUCTURAL PROPERTIES OF HEART MITOCHONDRIA: CHANGES INDUCED BY ACUTE *DIABETES MELLITUS*, PHARMACOLOGICAL AND REMOTE ISCHEMIC PRECONDITIONING

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Background: Heart mitochondria are the main target for the adaptation changes induced by acute *diabetes mellitus* (DM), pharmacological preconditioning with mitochondrial K_{ATP} opener - diazoxide (DZX) and remote ischemic preconditioning (RIP). Their structural and functional alterations is an important factor leading to preservation of energy function in myocard which is manifested by increased tolerance to acute ischemia. Aim of this work was contribute to elucidation of processes by which heart mitochondria are involved in different types of preconditioning.

Methods: Male Wistar rats (9-11 weeks) were divided into healthy (n=49) and diabetic group (n=49). RIP was induced by three 5-min occlusions of the right hind limb. Acute DM was induced by a single dose of streptozotocin (65 $mg.kg^{-1}$, i.p.). Isolated heart mitochondria were exposed to increasing concentrations of 200 µl DZX (0-7 $\mu mol.l^{-1}$). Mitochondrial Mg^{2+}-ATPase activity was assessed by ATP splitting. Mitochondrial membrane fluidity was estimated by fluorescence anisotropy with the aid of 1,6-diphenyl-1,3,5-hexatriene.

Results: Exposition of healthy heart mitochondria to DZX (5; 6 and 7 $\mu mol.l^{-1}$) and DM both induced an increase ($p<0.05$) in Mg^{2+}-ATPase activity. However, exposition of diabetic heart mitochondria to the same concentrations of DZX induced only a moderate further elevation of Mg^{2+}-ATPase activity. A similar moderate increase of membrane fluidity was also observed in diabetic and the DZX-threated heart mitochondria while RIP induced a significant ($p<0.05$) increase in this parameter.

Conclusion: Increase in Mg^{2+}-ATPase activity and membrane fluidity of mitochondria observed in the experimental models employed seem to belong to basic mechanisms which participate in an increase of cardiac ischemic tolerance. Stimulation of MIT Mg^{2+}-ATPase (ATP synthase) plays an essential but not exclusive role in cardioprotective effect provided by both DZX and DM.

Grants: VEGA 2/0133/15, 2/0201/15, APVV 0102-11, APVV-SK-CZ-2013-0075.

EFFECT OF EXPERIMENTAL DIABETES MELLITUS TYPE 1 ON THE CARDIAC Na,K-ATPase IN FEMALE RATS

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Na,K-ATPase, also known as sodium pump, is the enzyme responsible for active transport of sodium and potassium ions across plasma membrane against their concentration gradients. From previous studies it is known that diabetes mellitus type 1 (DM1) is connected with variety of complications in many organs. In this study we investigated the effect of various duration of DM1 on properties of the cardiac Na,K-ATPase derived from normal and STZ-diabetic female rats. Diabetes lasting eight days (short lasting model) and eight weeks (long lasting model) which was induced by intraperitoneal single dose of streptozotocin (65 $mg \cdot kg^{-1}$) was followed by significant changes in body weight gain and blood glucose. In the above stages of DM1 development the sodium binding properties of the cardiac Na,K-ATPase were studied using enzyme kinetic measurements as a tool. When we activated the enzyme with increasing concentration of cofactor Na^+ (2-100 $mmol.l^{-1}$) we observed that short lasting DM1 caused decrease of cardiac Na,K-ATPase activity throughout the investigated concentration range of sodium. On the other hand, in eight weeks lasting diabetes Na,K-ATPase was followed by significant increase of activity in female rats with DM1 in comparison to control group. Kinetic parameters in experimental group with long lasting diabetes showed significantly higher value of V_{max} and accompanied with lower K_{Na} value. We can conclude that females subjected to long lasting DM1 revealed adaptation to hyperglycemia as a consequence of improved affinity to sodium as indicated by lowered K_{Na} value. This may result in better transport of the redundant sodium out of the cardiac cells.

The study was supported by Slovak Grant Agency: VEGA-2/0141/13.

♣ MITOCHONDRIAL BIOENERGETICS IN THE REMOTE ISCHEMIC PRECONDITIONED RAT HEART

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Background: It was previously reported that mitohondria by opening their K_{ATP} and by preventing formation of mitochondrial transition pores (mPTP) represent end-effectors of remote ischemic preconditioning (RPC) signaling pathways. However, the effect of RPC itself on mitochondria is not yet fully understood.

Methods: RPC procedure consisted of 3 episodes of 5 min ischemia followed by 5 min reperfusion of descending branches of the right hind limb femoral artery. Not-preconditioned (controls) and preconditioned isolated rat hearts were subsequently tested to ischemia-reperfusion injury (T-IRI): 30 min global ischemia followed by 40 min postischemic reperfusion according to Langendorff. After each phase of T-IRI mitochondria were isolated and parameters of oxidative phosphorylation, activity of ATP synthase and content of oxidized isoforms of coenzyme Q ($CoQ_{9\text{-}ox}$ and $CoQ_{10\text{-}ox}$) were determined.

Results: Results of high resolution respirometry showed that ischemic-reperfusion injury significantly decreased state 3 respiration by 48.5% in control and 38.9% in RPC group. There was no significant change in state 2 respiration. The heart mitochondria without RPC exhibited a significant decrease in ATP synthase activity after reperfusion phase of T-IRI. RPC did not prevent the ATP-synthase activity decline. Moreover, in RPC group an increase of $CoQ_{9\text{-}ox}$ and $CoQ_{10\text{-}ox}$ content induced by reperfusion was less pronounced compared to control group.

Conclusion: Our results revealed that RPC probably does not alter mitochondrial respiration but its cardioprotective effects can be attributed at least in part to the reduction of the free radical formation after ischemic-reperfusion injury. We assume that the observed immediate effect of RPC will be more developed after 24 hours post stimulus of RPC - the second window of RPC-induced cardioprotection.

Grants: VEGA 2/0133/15, APVV 0102-11

♣ DETRIMENTAL EFFECT OF LPS-INDUCED INFLAMMATION ON THE RAT HEART AND VESSEL FUNCTION

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Although it is not completely proven that inflammation causes cardiovascular disease, inflammation is common for heart disease and is thought to be a sign of atherogenic response. Inflammatory processes induce negative changes in the vascular endothelium with significant impact on the vascular and heart function. The aim of the work was to examine whether inflammation induced by a single dose of LPS (lipopolysaccharide E.coli) could lead to the functional injury of the heart and vessels.

Experiments were performed in Wistar male rats. Inflammation was induced by injection of LPS in the dose of 1mg/kg intraperitoneally (LPS group). Healthy rats of the same age served as controls (C group). After 10 days, the animals were killed by the heart excision in the thiopental anesthesia. Function of the heart was studied by Langendorff method with constant perfusion pressure. We measured left ventricular pressure (LVP), heart rate (HR) and coronary blood flow (CF). The endothelium-dependent relaxation of isolated aortic rings by acetylcholine was evaluated under isometric conditions. Following biochemical parameters were also studied in the liver and plasma: levels of tumor necrosis factor-α (TNFα) and thiobarbituric acid reactive substances (TBARS), and N-acetyl-D-glucosamine (NAGA)-activity. Values of these parameters were significantly increased in LPS group, demonstrating the presence of inflammation. In experiments on isolated rat hearts we observed significant decrease in heart work and also in maximal velocity of contraction and relaxation. Moreover, the time to onset of the electric stimulation-induced fibrillation was shortened. Decreased endothelium-dependent relaxation responses to acetylcholine were observed in aortas from LPS rats, reflecting impairment of endothelial functions.

In summary, inflammation induced by a single dose of LPS caused negative changes leading to injury of the rat heart and blood vessel function.

Supported with VEGA 2/0065/13 and 2/0054/15

♣ (-)-EPICATECHIN REDUCED BLOOD PRESSURE, MOTOR ACTIVITY AND IMPROVED VASCULAR REACTIVITY IN YOUNG SPONTANEOUSLY HYPERTENSIVE MALE RATS.

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(-)-Epicatechin (Epi) is the most abundant flavan-3-ol present in cocoa seeds and cocoa-derived food. Epidemiological and experimental studies have shown that the intake of cocoa products or pure Epi decreases blood pressure (BP) and formation of reactive oxygen species in humans and animal models of hypertension. This work investigates the effects of chronic Epi administration (2 weeks, 100 mg/kg/day, *ad libitum* in water) on the BP development, endothelial function in the aorta, nitric oxide synthase activity (NOS) (brain stem, cerebellum, left heart ventricle, aorta), superoxide anion (O_2^-) production (left heart ventricle, aorta) as well as motor activity and anxiety level in the open field test (OF) in 5-week-old spontaneously hypertensive male rats (SHR). We also studied Epi effect on gene expression of three main NOS isoforms (neuronal - nNOS, inducible - iNOS and endothelial - eNOS) and p22phox subunit of nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase) in the tissues of brain stem, cerebellum and the left heart ventricle (LHV). There was a significantly lower BP (138±2 vs. 169±5 mm Hg) and improved NO-dependent vasorelaxation in Epi group vs. control group. Epi elevated significantly NOS activity (8.2±0.7 vs. 4.9±0.9 pmol/mg/min ; 11±0.5±0.8 vs. 5,8±0.2 pmol/mg/min) and reduced O_2^- production in the LHV and aorta (947±115 vs. 1629±200 cpm/mg ; 1070±194 vs. 2522±706 cpm/mg) vs. control group. Interestingly, no changes in eNOS, nNOS, iNOS and p22phox expression were observed in the heart. On the other hand Epi significantly increased expression of nNOS in the brain stem and cerebellum as well eNOS and p22phox in the cerebellum. Epi also significantly reduced total distance travelled (34.8±3.5 m vs. 21.8±2.5 m), distance travelled and time spent in the central zone of OF (1.2±0.4 m vs. 3.6±1 m ; 8.4±3.2 vs.23.1±8 sec) and elevated total immobility and time spent in corners vs. controls (399.5±23.8 sec vs. 308.8±39.2 sec ; 423.8±19.5 sec vs. 324.5±46.5 sec).

In conclusion, Epi reduced development of hypertension, increased NO production and reduced O_2^- production in the cardiovascular system, increased nNOS expression in the brain and decreased motor activity of young SHR males. Our results showed that 2-week Epi administration partially prevented development of both cardiovascular and behavioural disorders in young SHR by mechanism associated assumedly with improved bioavailability of NO.

This study was supported by the VEGA 2/0084/14 and NOREG.

♣ CAN CAROTENOIDS AFFECT AORTIC ENDOTHELIAL OCCLUDIN EXPRESSION DURING INFLAMMATION?

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Bacterial infection and inflammation, accompanied by an oxidative stress, have been considered as an important pro-atherogenic factor during all stages of atherogenesis, affecting mainly vascular endothelium. Novel therapeutic strategies targeting inflammation bear great potential for the prevention and treatment of atherosclerotic vascular diseases. In this context, effects of natural carotenoids, produced by specific yeast strains, in our case *Rhodotorula glutinis*, particularly with regard to vascular endothelial barrier function require attention. Endothelial tight junctions (TJs) play a crucial role in the regulation of paracellular transport of substances from circulating blood to subintimal space. Occludin, one of TJs proteins, is responsible for their proper function. Changes in occludin expression may affect intactness of TJs, increase endothelial permeability and finally lead to development of atherosclerotic plaque. Therefore, in our pilot study we examined antioxidant effects of carotenoids produced by yeast biomass on aortic endothelial occludin expression of endotoxemic Wistar rats. The inflammation was induced by a single dose of bacterial lipopolysaccharide (LPS) (1 mg/kg i.p.). Subsequently, rats were fed with a yeast biomass containing carotenoids (10 mg/kg/day) for 10 days. LPS decreased occludin expression and its location in endothelium comparing with the controls. It was associated with impaired endothelium-dependent relaxation of aorta and increased activity of NOS in aorta. Interestingly, gene expression of iNOS and eNOS isoforms was not changed. LPS increased levels of TNF-α and MDA as well as NAGA activity in plasma. Carotenoids increased occludin expression in LPS rats, decreased levels of inflammatory markers and improved aortic relaxation. Our results indicate antioxidant and anti-inflammatory effects of short-term supplementation of rats with yeast biomass containing natural carotenoids what may positively affect barrier function of vascular endothelium.

Supported by VEGA grant 2/0065/13.

♣ EXPRESSION OF miRNAs AND TNF-α IN IRRADIATED RAT MYOCARDIUM AND POTENTIAL TARGETS FOR MITIGATION OF INJURY

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Radiation therapy is one of the methods widely used to treat oncological diseases. Ionizing radiation affects not only cancer tissues, but also surrounding healthy tissues that may cause undesirable changes leading to cell death. The aim of the study was to examine changes in the myocardium at morphological and molecular levels six weeks after application of ionizing radiation in a single dose 10 and 25 Gy on Wistar male rat mediastinum. The rats were treated with selected drugs (Atorvastatin, acetylsalicylic acid (ASA), Tadalafil, Enbrel and molecular hydrogen) to reduce negative impact of radiation on the heart. Changes in expression of selected miRNAs (miR-1, miR-15b and miR-21) were measured by real-time PCR. Levels of TNF-alpha were tested by enzyme-linked immunosorbent assay (ELISA).

The irradiated group showed significant weight loss (about 20 %) in the control group and groups treated with atorvastatin, acetylsalicylic acid and Tadalafil. In Enbrel group similar body weight compared to controls was found. Irradiated rats exhibited non-significant decrease in systolic blood pressure in comparison with non-irradiated groups (about 15%).

Down-regulation of miR-1 has been observed in cardiac hypertrophy and chronic myocardial infarction. Irradiation downregulated miR-1 in irradiated hearts. Mir-1 expression levels in Tadalafil and Atorvastatin groups were decreased compared with control ones. ASA treatment decreased values of miR-1 in both control and irradiated group.

It is known that increase of miR-15b is pro-apoptotic in association with ischaemia. In our study, irradiation downregulated miR-15 by 42%. In treated hearts, Enbrel and ASA did not show significant change in expression of miR-15b as compared to controls.

MiR-21 is involved in ischaemic cardiac protection by reducing cardiac cell apoptosis. After irradiation, miR-21 was increased nearly 10-fold than in control hearts. Atorvastatin and Enbrel slightly increased miR-21 level, but Tadalafil reduced it (about 40%). On the other hand, in ASA group, expression of miR-21 increased more than twice than in a control group.

As compared to untreated control groups, irradiation caused a significant decrease in TNF-α. Enbrel and ASA decreased the level of TNF-α. In the Sildenafil group, there was increase in the TNF-α.

These results suggest possible protective function of Enbrel and Tadalafil on the heart damaged by irradiation as demonstrated by changes in miRNA 1, 15b and 21 values. Administration of hydrogen- enriched water slightly decreased hs-CRP in irradiated rats and revealed protective effect of hydrogen.

This work was supported by grants APVV-0241-11, VEGA 2/0201/15 and partially by NIH R37 HL051045 to RCK and CIHR to PKS.

PROTECTIVE EFFECT OF MOLECULAR HYDROGEN ON THE HEART IN SITUATIONS OF INCREASED PRODUCTION OF OXYGEN FREE RADICALS: RADIATION AND REPERFUSION INJURY

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Effects of radiation and ischemia/reperfusion (IR) on heart have a common denominator, such as formation of oxygen free radicals, which in addition to signaling functions have, at elevated concentrations, damaging effect on all structures of the heart and vessels.

In 2007 Oshawa et al. demonstrated that molecular hydrogen (H2) is able quickly penetrate all biological membranes and can be used as an effective scavenger of deleterious free oxygen species.

The effect of H2 administration was tested in two sets of experiment: A. in irradiated hearts and B. in I/R experiments.

A. Effect of H2 in irradiated rats: The rats were divided into four groups: non-irradiated, non-irradiated with H2, irradiated and irradiated with H2 (irradiation was administered in a single dose of 10 Gy). Rats were sacrificed in three different times (2 days, 9 days and 45 days). Blood samples were used for hs-CRP analysis as an inflammatory marker. Results showed that levels of hs-CRP were slightly downregulated in irradiated rats after application of hydrogen-enriched water given by gavage.

B. Effect of administration of H2 on I/R injury in rat myocardium: Krebs-Henseleit (KH) solution saturated with H2 was used at the onset of reperfusion period. Results showed that in the group treated with hydrogen-saturated KH solution, the size of infarction was markedly decreased as compared with that in the in control hearts.

These results suggest that hydrogen administered in a form H2-enriched water given by gavage or using H2-saturated KH solution at early phase of reperfusion has an anti-inflammatory and anti-apoptotic protective effect on heart damaged by mediastinal irradiation and by I/R injury.

This work was supported by grants APVV-0241-11, APVV-0102-11, VEGA 2/0021/15 and 2/0201/15.

SOCIAL-ISOLATION REARING AND CHANGES OF NITRIC OXIDE SYNTHASE ACTIVITY IN BRAIN AND CARDIOVASCULAR SYSTEM.

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Background: Nitric oxide (NO) has been shown to play a key role in the cardiovascular system, including modulation of vascular dilator tone and local cell growth. Now it is evident that NO exerts not only its peripheral vasodilatory action but it is involved in central regulations as well. Recently it was shown that NO play an important role in the development of social stress that may eventually lead to schizophrenia. Thus, we aimed to study the effect of social isolation on NO synthase (NOS) activity and behavioral parameters in rats.

Methods: In our study 32 weeks old male Wistar Kyoto rats were used. The animals were obtained after weaning (21 days postnatal) and were randomly divided into two groups. The first group was reared singly (RS), while the second group was reared 3 rats per cage (RG). After 29 weeks, body weight, blood pressure, locomotor activity and exploratory behaviors, acoustic startle reactivity, prepulse inhibition (PPI) and habituation were measured. NOS activity was determined by measuring the formation of L-[^{3}H] citrulline from L-[^{3}H] arginine in the heart, aorta and brain.

Results: The body weight of RS rats was significantly higher than that of RG rats. The blood pressure was not changed significantly within the groups. In the open-field test, the locomotor activity of RS rats was significantly higher, while immobile time was significantly lower in comparison with RG rats. Entries and time spend in central space were increased in RG rats as well. Habituation in RS rats was decreased and PPI was significantly impaired. Activity of NOS in the heart and aorta of RS rats did not change significantly, while it markedly decreased in the brain in comparison with RG rats.

Conclusion: Although impaired behavior and decreased brain NOS activity were seen in rats with social isolation, no changes in blood pressure and NOS activity in the cardiovascular system were determined.

EFFECT OF LPS-INDUCED INFLAMMATION ON RENAL Na,K-ATPase IN MALE RATS

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Inflammation induced by bacterial infection has been shown to disturb the maintenance of intracellular sodium homeostasis by Na,K-ATPase in various organs such as the pulmonary airway system, liver, heart, aorta, central nervous system, and kidney. This enzyme plays a crucial role in cell homeostasis because it maintains Na^+ and K^+ gradients between the intracellular and extracellular milieu, which are necessary for maintenance of the cell volume. The most important role in the regulation of sodium homeostasis in the whole organism is ascribed to the tubular Na,K-ATPase in renal tissue.

Measurements of enzyme kinetics of renal Na,K-ATPase were used for characterization of ATP- and Na^+ -binding sites in rats that were subjected to 10 days of moderate inflammation that was induced by a single dose of Escherichia coli lipopolysaccharides (LPSs) at a dose of 1 mg kg^{-1} body weight. We hypothesized that LPSs might initiate a malfunction of renal Na, K-ATPase. We also investigated the potential effect that fish oil (FO) has in the prevention of Na,K-ATPase alterations by administering FO daily at a dose of 30 mg kg^{-1}. The Na,K-ATPase was slightly altered in the vicinity of the ATP-binding site as suggested by the 9% increase of the concentration of ATP necessary for half-maximal activation of the enzyme, thus indicating a deteriorated binding of ATP as a consequence of inflammation. Daily supplementation of FO partly attenuated LPS-induced injury, hence maintaining the activity of renal Na,K-ATPase to the level of healthy control animals.

In conclusion, our findings showed that FO prevented an excessive malondialdehyde production in LPS-treated animals and stabilized renal Na,K-ATPase.

The study was supported by the Slovak Grant Agency VEGA by grants improving 2/0065/13 and 2/0141/13.

MYOCARDIAL mRNA LEVELS OF ANTIOXIDANT ENZYMES IN RATS ADAPTED TO PROTECTIVE AND NON-PROTECTIVE REGIMENS OF CHRONIC HYPOXIA.

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Chronically hypoxic myocardium exhibits an improved tolerance to acute ischemia-reperfusion injury. It has been shown that reactive oxygen species (ROS) formed during adaptation to hypoxia play an important role in the induction of protective cardiac phenotype. On the other hand, excess ROS contribute to tissue damage caused by ischemia-reperfusion (I/R), and this effect can be attenuated by cellular antioxidant defense systems. The aim of our study was to determine the transcription activity of major antioxidant genes in rat hearts adapted to three different hypoxic regimens in a normobaric chamber (FIO_2 = 0.1) for 3 weeks. Adult male Wistar rats were exposed to continuous hypoxia (without reoxygenation; CNH), intermittent hypoxia for 8 h/day (INH8) or intermittent hypoxia for 23 h/day (INH23). Control group was kept under normoxic conditions. The expression of mRNAs was measured by Real Time PCR in left ventricular myocardium at the end of adaptation. The separate groups of rats were subjected to 20-min coronary artery occlusion and 3-h reperfusion for infarct size (IS) determination. CNH as well as INH8 significantly reduced IS from 63 ± 3% of the area at risk in the normoxic group to 41 ± 4 % and 46 ± 4 %, respectively. Adaptation to INH23 did not induce infarct size-limiting effect (66 ± 2 %). Both cardioprotective regimens increased mRNA level of cytosolic peroxiredoxine 2 and elevated gene expression of glutathione reductase, manganese superoxide dismutase, thioredoxine 2 and its reductase, i.e. the antioxidants mostly located in mitochondria. Non-protective INH23 regimen had practically no effect on antioxidant gene expression. These results suggested that the improved cardiac ischemic tolerance of chronically hypoxic rats is likely related to increased myocardial antioxidant capacity.

Supported by APVV-SK-CZ-2013-0075

♣ INVESTIGATING THE REGENERATIVE POTENTIAL OF HUMAN PERICARDIAL ADIPOSE TISSUE-DERIVED STEM CELLS

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Cardiovascular diseases are the leading cause of morbidity and mortality worldwide and as such represent a significant burden to global healthcare. Regenerative medicine signifies a new strategy for treating these diseases by creating living, functional tissue that can restore cardiac and vascular function. Stem cells have become central in the development of regenerative medicine techniques due to their differentiation potential and ability to support endogenous repair through paracrine mechanisms. Currently, adult mesenchymal stem cells derived from bone marrow are the most common cell type used in regenerative medicine but adult stem cells derived from adipose tissue are emerging as a favourable alternative. Adipose tissue contains a higher proportion of adult stem cells compared with bone marrow, requires a less invasive harvesting procedure, and is easily expendable from the body. Adult stem cells have been derived from many different sources of adipose tissue but pericardial adipose tissue has yet to be investigated. The aim of my PhD is to successfully extract, characterise, and expand a novel population of stem cells derived from pericardial adipose tissue and assess their potential utility for regenerative medicine applications. This will involve optimising the extraction method for the cells, assessing their growth, expansion, and phenotypic characteristics *in vitro*, and testing their differentiation potential. All tissue samples will be obtained as a waste product from cardiac surgeries, and an additional aspect of this PhD is to investigate how patient risk profile affects the reparative potential of these cells. Ultimately, the cells will be seeded onto biodegradable scaffolds for use in bioengineered regenerative cardiovascular applications, specifically myocardial reparative patches for patients with acute or chronic myocardial infarction and bioengineered vascular grafts for patients with chronic vascular conditions requiring replacement therapy.

This research is funded by The National Institute for Health Research (NIHR).

EXPRESSION OF CALCIUM BINDING PROTEINS IN SKELETAL AND HEART MUSCLES OF RATS WITH ALTERED THYROID STATUS

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Thyroid hormones modify MyHC isoform content and this can result in transformation of slow to fast muscle fibers or vice versa. However, for the correct performance, other physiologically important components of ECC machinery should occur. We have therefore investigated whether the alterations of thyroid hormone levels will alter expression of selected calcium binding proteins in the slow soleus (SOL) and the fast extensor digitorum longus (EDL) hind limb muscles and in the heart of adult female inbred Lewis strain rats. HY rats were treated with 0.05 % solution of methimazole (2-mercapto-1- methylimidazole, Sigma) in drinking water, the TH status was induced by intraperitoneal injections of 3, 3',5-triiodo-L-thyronine (Sigma, sodium salt, T_3, 150 μg/kg body weight) 3 times a week. Protein levels were determined by SDS-PAGE followed by western blot analysis and gene expression was assessed using reverse transcription and subsequent real time polymerase chain reaction (RT-PCR). In the poster, we describe the protein and mRNA transcript levels for calsequestrin 1 and 2, parvalbumin and phospholamban in SOL and EDL muscles of euthyroid, hypothyroid and hyperthyroid rats.

This study was supported by MYORES LSH-CT-2004-511978, GACR 304/08/0256 and 7AMB14SK123 grants and by Research Project RVO: 67985823 (AV0Z 50110509).

THE ROLE OF MATRIX-METALLOPROTEINASES IN RELATION TO FUNCTIONAL HEART FAILURE.

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BACKGROUND: Extracellular matrix in the heart is a subject of extensive study due to its dynamic nature and its major role in development of several diseases. Matrix metalloproteinases (MMPs) are responsible for the degradation and remodelling of the extracellular matrix. They are also suggested to play an important role in the pathogenesis of heart failure (HF).

OBJECTIVES: The aim of this study was to determine the activity of circulating MMP-2 and MMP-9 in patients with HF and healthy controls in respect of gender, comorbidities and treatment.

METHODS: MMP-2 and MMP-9 activities were determined using gelatine zymography in plasma collected from 51 participants.

RESULTS: Gelatine zymography did not reveal any differences in circulating MMP-2 and MMP-9 activities in patients with HF and healthy controls in general. However, when patients were divided into subgroups according to gender, treatment and co-morbidities, significant differences in MMP-2 activity were found. There was a decrease in gelatinolytic activity of MMP-2 in treated hypertensive participants versus healthy ones. In contrast, we observed increased MMP-2 activity in participants suffering from hypertension with coexistent HF versus hypertensive participants without HF. In addition, a decrease in MMP-2 activity was shown in female participants suffering from HF versus males suffering from HF.

CONCLUSIONS: Potential inhibitory effect of antihypertensive treatment on MMP-2 activity was found. Coexistent HF with hypertension probably reduces the inhibitory effect of antihypertensive treatment on MMP-2 activity. Potential cardioprotective factors in females may influence the activity of MMP-2.

This work was supported by VEGA 1/0032/14 and 2/0108/15 grants.

SIX-WEEK SUPPLEMENTATION WITH N-3 POLYUNSATURATED FATTY ACIDS DID NOT SIGNIFICANTLY AFFECT ALTERATIONS OF LIPID METABOLISM INDUCED IN MALE INBRED STRAIN LEWIS RATS BY ALTERED THYROID STATUS.

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The results presented represent a part of our study comparing the effect of n-3 polyunsaturated fatty acids (n-3 PUFA) supplementation (200 mg/kg of body weight/day) on lipid metabolism and myocardial characteristics in Lewis, SHR and WKY rats with altered thyroid status (1-6). Euthyroid (EU), hypothyroid (HY) and hyperthyroid (HT) status of male inbred Lewis rats was well defined by plasma levels of triiodothyronine, activity of liver mitochondrial glycerol-3-phosphate dehydrogenase and by anatomical parameters. Fasting blood glucose levels were significantly higher in the HT compared to the EU and HY rats (5.0 ± 0.2 *vs.*3.7 ± 0.4 and 4.4 ± 0.2 mmol/l, respectively). The concentration of plasma postprandial triglycerides in HT animals was increased compared to EU and HY males (0.9 ± 0.1 *vs.* 0.5 ± 0.1 and 0.4 ± 0.1 mmol/l, respectively). On the other hand, hypothyroidism compared to euthyroid and hyperthyroid status was associated with elevated plasma levels of total cholesterol (2.6 ± 0.2 *vs.* 1.5 ± 0.1 and 1.6 ± 0.1 mmol/l, respectively), LDL cholesterol (0.9 ± 0.1 *vs.* 0.4 ± 0.1 and 0.2 ± 0.1 mmol/l, respectively) and HDL cholesterol (1.6 ± 0.1 *vs.* 1.0 ± 0.1 and 1.3 ± 0.1 mmol/l, respectively). Six week supplementation of n-3 PUFA did not significantly modify none of the above parameters, although it showed tendency to ameliorate thyroid hormone induced myocardial changes. Preliminary measurements of n-3PUFA effects on lipid metabolism in WKY and SHR rats suggest similar results.

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Supported by GAČR 304/12/0529, VEGA 0046/12, APVV-SK-CZ-0027-11, MSCT CR MSM0021620858 and 7AMB14123 grants and by the Research Project RVO: 67985823 (AV0Z 50110509).

♣ CELLULAR RECRUITMENT AND MATRIX REMODELLING OF BIOLOGICAL VASCULAR GRAFTS FOLLOWING TRANSPLANTATION IN A PORCINE CAROTID MODEL

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Background
Established surgical treatment for coronary and peripheral vascular disease (PVD) consists of bypass surgery. However, about 75% of the coronary grafts consist of autologous saphenous veins (SVG) with 50% patency rate only at 10 years. PVD is treated either with SVG or <6mm artificial conduits with a patency rate of 50% at 2 years. This poor outcome is mostly due to intimal hyperplasia and early thrombus. Bio-engineering of small vascular grafts is emerging as a possible alternative technology mostly based on experiment *in vitro*. However, very little is known about the impact of *in vivo* conditions such as blood pressure, flow dynamics, level of oxygenation or inflammation in determining cell recruitment and matrix remodelling in these grafts following *in vivo* implantation in a model relevant to human anatomy and physiology.

Purpose and aim
In this study we set to investigate the fate of plain biological vascular grafts fashioned from CorMatrix (CM), Porcine (PP) and Bovine (BP) pericardium at 1 month following implantation in a porcine carotid model.

Methods
Sterile CM, PP, and BP biological patches were fashioned in 5mm diameter vessels (25mm long) using 7-0 prolene surgical sutures. The obtained grafts were transplanted in 70kg pigs using an established carotid replacement models (n=7; 3CM, 2PP, 2BP) under standardised anaesthetic conditions. Each animal underwent bilateral implant of the same scaffold type. In all animal grafts were interposed end-to-end in the carotid artery on the right and in the carotid vein on the left, for a total of 17 implants. Animals received 75mg Aspirin/day. At one month, grafts were explanted under controlled anaesthesia and divided in 6 segments: proximal native vessel, proximal anastomosis, proximal graft, distal graft, distal anastomosis, and distal native vessel. All sections were fixed in 4% PFA, embedded in wax, and cut into 5µm sections. EVG and H&E staining was used to assess the differences in matrix remodelling between groups and native vessels. Comparative evaluations included graft patency, transmural cell density/recruitment, lumen area, neo-intima thickness. Additionally, immunostaining with antibodies against endothelial cells (ECs) and smooth muscle cells (SMCs) was used for vascular phenotyping among groups.

Results
At 1 month the arterial CM, PP, and BP grafts were all patent, whereas all the venous grafts were occluded due to neo-intima formation. The size of the lumen area in arterial grafts was comparable to the native arteries. All the biological grafts were infiltrated by a large amount of cells with a density comparable with native arteries. There was a trend for reduced thickness of matrix for CM and PP, but not for BP grafts. Vascular phenotyping demonstrated a complete ECs layer as well as a thick layer of sub-endothelial SMCs in all the grafts in arterial position; whereas there was no evidence of ECs in most of the grafts implanted in venous position.

Conclusion
This pilot study suggests that plain biological vascular grafts undergo a marked cellular and matrix remodelling in a porcine carotid model. This may impact significantly on the effectiveness of artificial grafts bioengineered *in vitro* when implanted *in vivo*. The study also suggests that difference in blood pressure, flow dynamics, and oxygenation level between arterial and venous implantations might impact on graft remodelling, early patency rate, and vascular phenotyping. Further work is required to ascertain mechanistic insights to be able to control and manage the fate of bioengineered grafts after transplantation in *in vivo* models relevant to human anatomy and physiology.

♣ CALCITRIOL MODULATES VASCULAR EXPRESSION OF RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS AND MONOAMINE OXIDASE IN EXPERIMENTAL DIABETES

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Background and aim: Calcitriol (1,25-cholecalciferol), the active form of vitamin D, has been reported to improve vascular function in patients with diabetes mellitus but the precise mechanism(s) underlying its beneficial effects have not been fully elucidated. The aims of the present study were to investigate the effects of calcitriol on vascular reactivity and on the expression of monoamine oxidase (MAO) and Receptor-for-Advanced-Glycation-Endproducts (RAGE) respectively, in aortic segments harvested from diabetic rats. **Material and methods:** To this aim, aortic rings isolated from streptozotocin-induced diabetic rats (50mg/kg, single dose, intraperitoneal) and the non-treated controls were incubated 24 hours in the absence or presence of 1,25-cholecalciferol (0.1μM). Subsequently, the rings were suspended in organ chambers and used for isometric force measurements. Endothelium-dependent relaxation to increasing concentrations of acetylcholine (ACh) was recorded together with the vascular contractility to endothelial nitric oxide synthase (eNOS) inhibitor L-NAME (Nω-Nitro-L-arginine-methyl-ester-hydrochloride, 10μM). The effect of vitamin D on MAO and RAGE expression was assessed by quantitative RT-PCR and immunehistology. **Results:** Incubation with calcitriol modulated the vascular tone by reducing the contractility and improving the endothelium-dependent relaxation by 30%. Contraction in the presence of the eNOS inhibitor was also significantly increased in diseased vessels and partially normalized by vitamin D treatment. Moreover, 24 h incubation with vitamin D resulted in decreased MAO and RAGE expressions in vascular preparations. **Conclusions:** Vitamin D partially improved the vascular relaxation in vitro and reduced the MAO and RAGE expression in aortic rings harvested from rats with experimental type 1 diabetes. Further investigations aimed at characterizing the mechanisms underlying vitamin D action are warranted.

The work was funded by the POSDRU grant no. 159/1.5/S/136893 titled: "Strategic partnership for the increase of the scientific research quality in medical universities through the award of doctoral and postdoctoral fellowships–DocMed.Net_2.0"

♣ EFFECT OF DECELLULARIZATION PROTOCOL OF HUMAN SAPHENOUS VEINS ON CYTOTOXICITY AND MATRIX COMPONENT

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Rationale for the study:

It is proposed that development of arterial-like vascular conduits using decellularised venous extracellular matrix scaffolds seeded with cells are desirable to overcome the problem of early vein graft thrombosis, late vein graft thickening, and artificial grafts infection of autologous, xeno- and artificial graft conduit used for coronary artery bypass surgery. The aim of this study was to compare the effect of different decellularisation protocols on vein matrix components and cytotoxicity on seeded cells.

Methods:

Segment of human saphenous vein were decellularized by using Sodium Dodecyl Sulfate (SDS) of various percentage (0.01%, 0.025%, 0.05%, 0.075% and 0.1%). Residual cellular and extracellular matrix composition was studied with histological staining; H&E and EVG. To assess cytotoxicity and biocompatibility of the decellularised scaffold, Human Adipose Derived Stem Cells (hADSC), Human Umbilical Vein Endothelial Cells (HUVEC) and Human Smooth Muscle Cells (hSMC) were seeded and cultured on decellularised vein segments.

Results:

All concentration of SDS resulted in complete decellularisation of the vein without detrimental loss of collagen and elastin content and morphology. 50 000 cells was seeded on disc of decellullarised veins (4mm in diameter; 0.01% SDS; n=6) where proliferation occurred after 24 hours and significantly 2-fold higher numbers of hSMC proliferation was detected compared to ADSC.

Conclusions:

The decellularization protocol was efficient and did not affect the extracellular matrix of veins and was observed to be non-cytotoxic to ADSCs, HUVECs and hSMCs.

♣ OMEGA-3 FATTY ACIDS MODERATE SUSCEPTIBILITY OF THE HEART TO LETHAL ARRHYTHMIAS IN AGED MALE AND FEMALE SPONTANEOUSLY HYPERTENSIVE RATS

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Background: Reduced connexin (Cx43) expression, elevated activity of matrix metalloproteinase (MMP) and adrenergic beta1 autoantibody (b1-AAB) production are implicated in the development of heart failure and increased incidence to lethal arrhythmias. Low omega-3 (ω-3) index was suggested to be a risk factor for cardiovascular diseases and sudden cardiac death. Aim of this study was to explore the effect of ω-3FA intake on ω-3 index, Cx43, MMP, b1-AAB and susceptibility to arrhythmias in aged male (♂) and female (♀) spontaneously hypertensive rats (SHR).

Methods: 1 year old SHR and age-matched healthy Wistar rats (WR) fed with ω-3FA (Vesteralens, Norway, EPA+DHA 200mg/day/2month) were compared with untreated rats. Gas chromatography was used for analysis of red blood cells ω-3FA and ω-6FA composition. Blood plasma was used for the detection of adrenergic beta1 AAB (b1-AAB). Left ventricular tissue was taken for Cx43 and PKC-epsilon expression using Western blot method and for MMP-2 activity using zymography. Inducible ventricular fibrillation (VF) was examined using Langendorff-mode perfused heart.

Results: Compared to healthy WR ω-3 index was lower in both ♂ and ♀ SHR, i.e. 0.73% and 0.44% vs. 1.75% and 1.17%. This parameter was significantly increased due to ω-3 FA intake to 2.38% and 3.34% in both sexes of SHR. ♂ and ♀ SHR also exhibited a significant increase of serum levels of b1-AAB, activity of MMP2, decrease of Cx43 protein and its functional phosphorylated forms as well as corresponding PKC-epsilon. Non-treated ♂ and ♀ SHR were much prone to inducible VF (100% males and 65% females) comparing to WR (65% males and 35% females).This propensity, expression of Cx43 and PKC-epsilon as well as production of b1-AAB, MMP2 activity were significantly normalized in ♂ and ♀ SHR due to ω-3FA intake.

Conclusions: These findings suggest multiple cardio-protective effects of omega-3 intake that can contribute to decreased susceptibility of the heart to VF.

This study was supported by VEGA 2/0167/15, VEGA 2/0046/12

♣ EFFECT OF TREATMENT WITH ASPIRIN AND ATORVASTATIN ON MYOCARDIAL CONNEXIN-43 AFTER IRRADIATION OF RAT HEART.

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Background: Direct cell-to-cell communication in the heart is maintained via gap junction channels. They are composed of proteins – connexins (Cx), while the most abundant Cx in the heart is Cx43. Radiotherapy that is often used to treat cancer has been shown to up-regulate Cx43 in various tissues, although, the exact mechanism is not fully understood. Moreover, it is not known whether post-irradiation treatment with cardioprotective compounds may affect myocardial Cx43 expression.

Purpose: The aim of this study was to explore the effect of single chest irradiation on cardiac Cx43 and PKC signaling, and the effect of treatment with aspirin, atorvastatin and sildenafil (Viagra).

Materials and methods: Adult, male Wistar rats were divided into non-irradiated control rats and irradiated rats subjected to single dosage radiation on mediastinum at 25 Gy. Both groups were treated during six weeks with aspirin (3 mg/day), atorvastatin (0.25 mg/day), sildenafil (0.3 mg/day) and compared to untreated rats. In the end of the experiment biometrical parameters were registered. Left and right ventricular tissues were taken for determination of total Cx-43 expression and its functional phosphorylated forms (PCx43) using Western blot. Expression of PKC-epsilon, which phosphorylates Cx43 and pro-apoptotic PKC-delta was also determined.

Key Results: Body weights were significantly decreased in irradiated rats regardless the treatment. Neither heart nor left and right ventricular weights were affected due to irradiation and treatment. An increase of total Cx43 expression in left and to lesser extent in right ventricles as well as significantly enhanced levels of PCx43 in both ventricles were found 6-weeks post-irradiation. Aspirin and atorvastatin attenuated elevation of total Cx43 as well as functional PCx43 in left ventricles only. Sildenafil enhanced total but not PCx43 in left ventricles and suppressed total and PCx43 in right ventricles of irradiated rats. Expression of PKC-epsilon was not significantly affected by irradiation or treatment. PKC-delta expression was increased after irradiation in left but not right ventricle, while aspirin and atorvastatin enhanced its expression in right but not left ventricle.

Conclusion: Our results suggest that up-regulation of cardiac Cx43 induced by irradiation is attenuated by treatment with aspirin and atorvastatin while not by sildenafil. Further studies should explore functional consequences of Cx43 alterations and should identify the implication of protein kinase in phosphorylation of Cx43.

This study was supported by APVV 0241/11 and VEGA 2/0046/12, 2/0021/15 grants.

MOLECULAR HYDROGEN REDUCES ISCHEMIA/REPERFUSION INJURY IN THE ISOLATED RAT HEART SUBJECTED TO POSTCONDITIONING: NOVEL CARDIOPROTECTIVE INTERVENTION

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Generation of free radicals through incomplete reduction of oxygen during ischemia/ reperfusion (I/R) has been well described. On the other hand, H_2 reduces oxidative stress due to its ability to react with strong oxidants, such as hydroxyl radical, and easily penetrate by diffusion tissues and cells, without disturbing metabolic redox reactions. It is also known that H_2 has an impact on the prevention of apoptotic and necrotic changes after 30-min ischemia and has a therapeutic potential for the regulation of inflammation in the heart.

This study was designed to evaluate cardioprotective potential of H_2 in a setting of global I/R in isolated rat hearts perfused with Krebs-Henseleit buffer (KHB) and to compare the efficiency of hypoxic postconditioning (H-PostC) with intervention when KHB was saturated with H_2 during H-PostC (H2+H-PostC) in the hearts exposed to 30-min global I/120-min R. H-PostC was induced by 4 cycles of 1-min perfusion with oxygen-free KHB intercepted by 1-min perfusion with normal KHB, at the onset of reperfusion. H2+H-PostC was applied in a similar manner, by 4 cycles of 1-min perfusion with oxygen-free KHB enriched with H_2 intercepted with 1-min perfusion with normal KHB. Size of infarction (IS, TTC staining) expressed in % of area at risk (AR), recovery of function (LVDP, in % of preischemic values) and occurrence of ventricular tachyarrhythmias (VT) served as the end-points of protection.

H-PostC improved post-I/R recovery of LVDP by 78% as compared to that in non-conditioned controls and significantly reduced the size of myocardial infarction from IS/AR 39±1,4% in controls to 25±0,9%. H_2 present in KHB during H-PostC even further decreased IS to 17±0,8% ($p<0,05$ vs. both, controls and H-PostC) and improved LVDP recovery more than two-fold. However, both, H-PostC and H2+H-PostC did not suppress appreciably the severity of reperfusion VT.

Application of hydrogen appears to be cardioprotective in a setting of postconditioning and to facilitate the efficiency of hypoxic postconditioning. Molecular mechanisms behind this effect remain to be elucidated.

Supported by Grants VEGA 2/0201/15, APVV-0102-11 and APVV-0241-11. VEGA 2/0021/15

♣ STRUCTURAL CHANGES OF MICROCIRCULATION IN THE BRAIN AFTER MELATONIN TREATMENT IN CONTROL AND SPONTANEOUSLY HYPERTENSIVE RATS

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Microcirculation is the smallest part of the cardiovascular system and is the most important for the nutrient distribution and blood pressure control. Structural changes of microcirculation can result in cardiovascular syndromes and diseases related to hypertension. Melatonin can have beneficial effects on endothelium and structure of vessels since it can modulate NO availability and has strong antioxidant capacity. To study changes of microcirculation induced by hypertension or melatonin administration, we used 4 groups of animals: control Wistar rats (cWKY), spontaneously hypertensive rats (cSHR), melatonin treated Wistar (mWKY) and SHR rats (mSHR). Melatonin treated rats received melatonin (10mg/kg) in drinking water during the dark-time of the LD 12:12 cycle. The density of capillaries was visualized by immunofluorescence method with labeled lectin (*Fluorescein-lycopersicon esculentum lectin*) and structural changes in the wall thickness were visualized by an antibody for α-smooth muscle actin. The density of capillaries was evaluated in 3 areas of the brain: preoptic area, cerebellum and hippocampus. We observed an increase ($p<0.05$) in wall thickness and wall:lumen ratio in cSHR than cWKY rats in vessels with total area within 300μm^2. However, we did not observe changes in capillary density between cSHR and cWKY rats. After melatonin treatment we found higher capillary density in cWKY than mWKY rats ($p<0.05$) in the preoptic area and the same trend ($p=0.138$) was seen between cSHR and mSHR rats. In other brain areas capillary density was not affected by melatonin. After melatonin treatment we observed a trend ($p=0.057$) to an increase in wall thickness in mWKY rats in comparison to cWKY rats in vessels with total area in range 150-300μm^2 and a tendency ($p=0.133$) in vessels with total area 300-1000μm^2. The wall:lumen ratio was also increased in mWKY rats in vessels with total area up to 150μm^2 ($p=0.035$); 150-300μm^2 ($p=0.102$) and 300-1000 μm^2 ($p=0.153$). Higher capillary density in the preoptic area after melatonin treatment can reflect an enhanced sensitivity of this brain area to be controlled by melatonin. In normotensive rats melatonin increased the vessel wall thickness and more research is needed, which components contribute to this change and if the final effect is beneficial or not.

Supported by APVV 0291-12, VEGA 1/0557/15, GUK/230/2015.

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