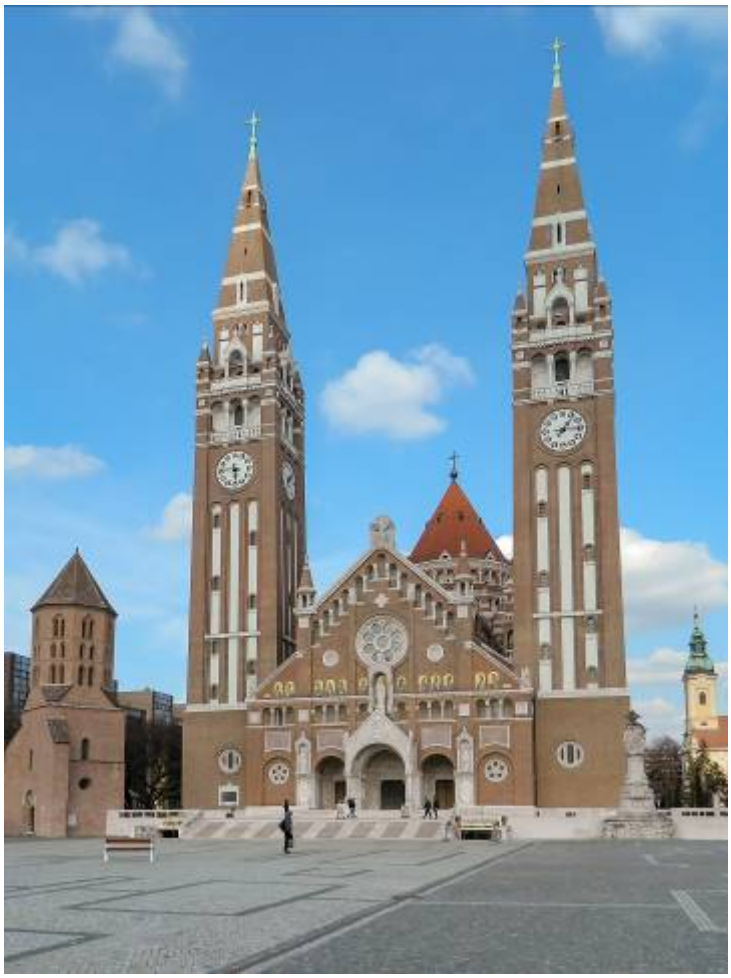
8th European Section Meeting of the International Academy of Cardiovascular Sciences

September 28 – October 1, 2022
Szeged, Hungary



PROGRAMME AND ABSTRACT BOOK

8th European Section Meeting of the International Academy of Cardiovascular Sciences (IACS)

Meeting venues:

Art Hotel Szeged,

Somogyi u. 16, 6720 Szeged, Hungary

https://artszeged.accenthotels.com/en

AND

Szeged Hungarian Academy of Sciences (SZAB) building,

Somogyi u. 7, 6720 Szeged, Hungary

September 28 – October 1, 2022
Szeged, Hungary

8th European Section Meeting of the International Academy of Cardiovascular Sciences (IACS)

Szeged, Hungary, September 28 – October 1, 2022

Programme and Abstract Book

Editors: István Baczkó, Norbert Nagy and Péter Bencsik

Szeged, 2022

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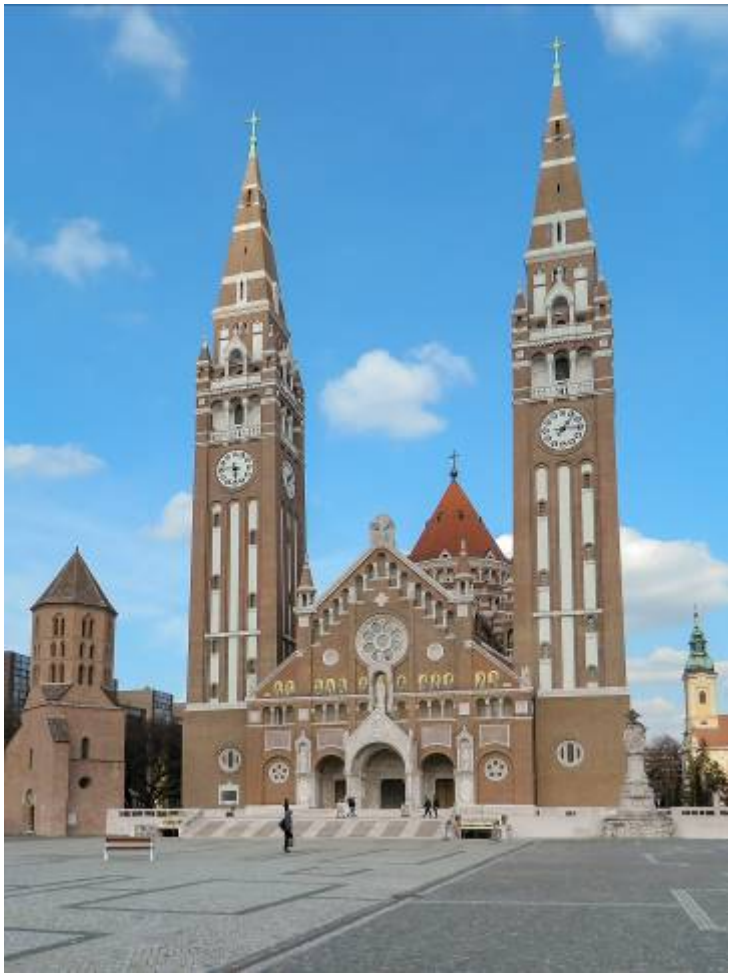
8th European Section Meeting of the International Academy of Cardiovascular Sciences

September 28 – October 1, 2022

Szeged, Hungary



FINAL PROGRAMME

Contact information

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Dear Colleagues,

It is our pleasure to announce that in 2022, the **8th European Section Meeting of the International Academy of Cardiovascular Sciences (IACS-ES)** will be held in Szeged, Hungary, on September 28 – October 1, 2022. We are pleased to invite you to participate in this fascinating scientific meeting focusing on the following scientific topics:

- **Clinical and theoretical aspects of ventricular arrhythmias and sudden cardiac death**
- **Atrial fibrillation: clinical therapy, novel and future strategies of AF management**
- **Clinical and theoretical aspects of heart failure**
- **Cardioprotection, clinical application of cardioprotection**
- **Genetics of cardiovascular diseases**
- **Cardiac metabolism**
- **Novel therapeutic approaches in cardiovascular diseases**
- **Coronary angiogenesis from bench to bedside**

The meeting will feature both basic scientific and clinical sessions, including lectures of invited speakers and free oral communications selected from submitted abstracts. We would like to provide opportunities for a number of young investigators to discuss their latest results and to compete in both oral and poster sessions.

We believe that your participation will greatly contribute to the success of the meeting and provide an opportunity to discuss the latest advances in experimental and clinical cardiovascular research. In addition to high quality science, the organisers wish to provide a friendly atmosphere in the university town of Szeged. We invite you to join us at this meeting, to renew old friendships, and to make new ones!

Looking forward to meeting you in person in Szeged,
With best regards,

Prof. István Baczkó, MD, PhD
President and Chair of the Meeting

Honorary Chair of the Meeting: Prof. Naranjan S. Dhalla, PhD, MD (Hon), DSc (Hon)
Main organizers: Dr. Péter Bencsik, MD, PhD (Vice Chair) and Dr. Norbert Nagy, PhD (Meeting Secretary)

Organizing secretariat:
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Meeting venue

Art Hotel Szeged, Somogyi u. 16, 6720 Szeged, Hungary
https://artszeged.accenthotels.com/en

and

Szeged Hungarian Academy of Sciences (SZAB) building, Somogyi u. 7, 6720 Szeged, Hungary

The two buildings are located 50 m from each other.

Publication opportunity for presenters
The abstracts of all oral and poster presentations will be printed in an abstract book. Presenters will be encouraged to submit a full manuscript based on the material presented at the conference for consideration to be published in a special issue of the *Canadian Journal of Physiology and Pharmacology*. Manuscript submissions will be subjected to the usual peer-review process.

Registration

Please send your completed registration form to the following e-mails: baczko.istvan@med.u-szeged.hu and bencsik.peter@med.u-szeged.hu.

The registration fee will be **100 EUR/person for participants, 50 EUR/person for juniors and 10 EUR/person for undergraduate students**. To qualify for Junior registration fee, the registrant must be a PhD student OR have a PhD degree obtained in 2019 or later (documentation required) AND must not be older than 35. The registration fee covers attendance of the scientific program, welcome reception, gala dinner (except undergraduate registrants) and coffee breaks. The registration fee of the invited speakers is covered by the organizers.

Please indicate your name and city of origin on the payment for easier processing.

Registration deadline

Registration deadline: **July 31, 2022.**
***Payment deadline*:** **July 31, 2022.**

On site registration is possible, at 200 EUR/person for participants and 100 EUR/person for Junior registrants, respectively. All participants will receive an official Certificate of Attendance and an official Conference Registration Fee Invoice. Those who registered online before the deadline can also pay the registration fee in cash on site, at the rate of the early registration.

Young Investigator Award Competition
Young investigators (under the age of 35) were encouraged to submit their CV, oral abstract, and a maximum 2-page summary of the research constituting the basis of their talk to baczko.istvan@med.u-szeged.hu and bencsik.peter@med.u-szeged.hu. A Committee has chosen 6 finalists who will present their work at the Young Investigator Award Session at the meeting. **The submission deadline was: July 31, 2022**.

Poster Awards for Young Investigators
Several poster awards will be available for young investigators (under the age of 35). Please indicate on your Poster that you want to participate in the competition. Stickers for this purpose will be provided on site. The poster board format is portrait, size 160 x 150 cm. Please make sure your poster fits this board format.

Travel grants
Due to the increased costs of conference organization (e.g. extremely high inflation, catering), travel grants are not available at this time.

Accomodation information

All invited speakers will be lodged at the Art Hotel Szeged, their accommodation for 3 nights is covered by the organizers.

For registering conference participants, it is also possible to book rooms at the conference venue Art Hotel Szeged (https://artszeged.accenthotels.com/en).
When booking the room at the Art Hotel, please refer to the password "**IACS-ES Szeged 2022"**.

In addition, we recommend the following hotels in Szeged in the vicinity of the conference venue (we have no agreement with these hotels, therefore please do not refer to the password):

Dóm Hotel (****): https://domhotelszeged.info/en/ ; Science Hotel (****): https://sciencehotel.hu/en/
Mozart Hotel (****): https://www.mozarthotel.hu/en ; Szent János Hotel Szeged (****): https://szentjanoshotel.hu/?eng ; Hotel Novotel Szeged (****): http://novotel.hotelszeged.com/en/

Special acknowledgment to the organizations below for their contributions and sponsorship of the meeting

National Research, Development and Innovation Office

Hungarian Society of Cardiology

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Overview of Conference Programme

DAY 1 (September 28, 2022) ART HOTEL SZEGED, Lecture Hall

13:00- **REGISTRATION**

15:00-15:30 Opening ceremony

Session chairs: **István Baczkó** (Szeged, Hungary; President and Chair of the meeting)
András Varró (Szeged, Hungary; President of IACS-ES)

15:00-15:10 **István Baczkó** (Szeged, Hungary; President and Chair of the meeting)
15:10-15:15 **Gábor Németh** (Szeged, Hungary; Vice Dean of Albert Szent-Györgyi Medical School, University of Szeged)
15:15-15:20 **András Varró** (Szeged, Hungary; President of IACS-ES)
15:20-15:25 **Naranjan Dhalla** (Winnipeg, MB, Canada; Honorary Life President of IACS)
15:25-15:30 **Grant Pierce** (Winnipeg, MB, Canada; President of IACS)

15:30-19:00 PLENARY LECTURES

15:30-16:00 **Roberto Bolli** (Louisville, KY, USA; Past President of IACS)
Cell therapy for heart disease: Quo vadis?
16:00-16:05 Presentation of Scientific Excellence in Cardiovascular Research Award
16:05-16:35 **Grant Pierce** (Winnipeg, MB, Canada; President of IACS)
The value of supplementing a diet with flaxseed

16:35-16:50 *Coffee Break*

16:50-17:35 **Naranjan Dhalla Honorary Lecture**
Igor Efimov (Chicago, IL, USA)
Transient implantable devices to treat arrhythmia: bioresorbable bioelectronics platform
17:35-17:40 Presentation of 2022 Naranjan Dhalla Honorary Lecture Award
17:40-17:45 Presentation of 2021 Naranjan Dhalla Honorary Lecture Award

Previous Naranjan Dhalla Honorary Lecture Awardees:
2016 **Ursula Ravens**, Freiburg, Germany
2017 **David A Eisner**, Manchester, UK
2018 **Gerardus JM Stienen**, Amsterdam, Netherlands
2019 **Grant N Pierce**, Winnipeg, Canada
2021 **Lorrie A Kirshenbaum**, Winnipeg, Canada

19:00- *Welcome Reception (SZAB building)*

DAY 2 (September 29, 2022) SZEGED HUNGARIAN ACADEMY OF SCIENCES BUILDING

Parallel sessions **in Halls A-B-C**

9:00-10:30 SESSION 1: Novel targets for HFrEF therapy (Hall A)

Session chairs: **Michael Czubryt** (Winnipeg, MB, Canada)
Noémi Nyolczas (Budapest, Hungary)

9:00-9:25 **Michael Czubryt** (Winnipeg, MB, Canada)
Fibroblast activation as a target for cardiac fibrosis therapy
9:25-9:50 **Naranjan Dhalla** (Winnipeg, MB, Canada)
Diverse mechanisms of heart failure due to myocardial infarction

9:50-10:15 **Noémi Nyolczas** (Budapest, Hungary)
Novelties in the treatment of heart failure with reduced ejection fraction

10:15-10:30 **Zorislava Bajić** (Banja Luka, The Republic of Srpska, Bosnia and Herzegovina)
The role of treatment with glp-1 receptor agonist liraglutide on isoprenaline-induced myocardial injury in rats

9:00-10:40 SESSION 2: Cardiac remodelling (Hall B)

Session chairs: **Mark Boyett** (Bradford, UK)
Martin Morad (Charleston, SC, USA)

9:00-9:25 **Mark Boyett** (Bradford, UK)
Sinus node and atrioventricular node dysfunction in heart failure – involvement of the immune system and transcriptomic remodelling

9:25-9:50 **Alicia D'Souza** (Manchester, UK)
Why do athletes have cardiac arrhythmias? A view from the lab bench

9:50-10:15 **Martin Morad** (Charleston, SC, USA)
Mutations in CPVT1-associated and CPVT1-unassociated RyR2 Calcium binding residue reveal remodeling of EC-coupling in hiPSC-CMs

10:15-10:40 **Raffaele Coppini** (Florence, Italy)
Human hypertrophic cardiomyopathy: from electrophysiological insights to pharmacological strategies

9:00-10:30 SESSION 3: Natural compounds in cardioprotection (Hall C)

Session chairs: **Ranko Škrbić** (Banja Luka, The Republic of Srpska, Bosnia and Herzegovina)
Dragan Djuric (Belgrade, Serbia)

9:00-9:25 **Dragan Djuric** (Belgrade, Serbia)
Possible cardioprotective effects of vitamins B6 and B9: lessons from cardiometabolic models

9:25-9:50 **Monika Barteková** (Bratislava, Slovakia)
Potential implications of quercetin in cardioprotection

9:50-10:15 **Ranko Škrbić** (Banja Luka, The Republic of Srpska, Bosnia and Herzegovina)
Beneficial cardio-metabolic properties of Pomegranate peel extract: results of clinical and experimental studies

10:15-10:30 **Zsófia Onódi** (Budapest, Hungary)
Distinct patterns of inflammasome signalling in the cardiac and skeletal muscle from murine models of Duchenne muscular dystrophy

10:30-11:00 *Coffee Break*

11:00-12:30 SESSION 4: Metabolic diseases (Hall A)

Session chairs: **Gary Lopaschuk** (Edmonton, AB, Canada)
Danina Muntean (Timisoara, Romania)

11:00-11:25 **Gary Lopaschuk** (Edmonton, AB, Canada)
Preventing cardiac insulin resistance in the failing heart

11:25-11:50 **Danina Muntean** (Timisoara, Romania)
Alleviation of MAO-related oxidative stress by antidiabetic drugs: of mice and men

11:50-12:15 **Tamás Csont** (Szeged, Hungary)
Cardiac effects of diet-induced hypercholesterolemia

12:15-12:30 **Nevena Jeremic** (Kragujevac, Serbia)
The cardioprotective effects of Trametes versicolor polysaccharides on rats with metabolic syndrome

11:00-12:30 SESSION 5: Pathophysiology of atrial fibrillation (Hall B)

Session chairs: **Ursula Ravens** (Freiburg, Germany)
José Jalife (Madrid, Spain)

11:00-11:25 **Ursula Ravens** (Freiburg, Germany)
Mechanosensitive channels, stretch and fibrosis - a pathophysiological role in atrial fibrillation

11:25-11:50 **José Jalife** (Madrid, Spain)
Molecular mechanisms underlying the progression of paroxysmal to persistent atrial fibrillation

11:50-12:15 **Katharine Dibb** (Manchester, UK)
Atrial remodelling in heart failure: consequences and recovery

12:15-12:30 **Tímea Bálint** (Budapest, Hungary)
Atrial fibrillation is not associated with altered left atrial microRNA expression profile in ischemic end-stage human heart failure

11:00-12:15 SESSION 6: Molecular hydrogen and gasotransmitters (Hall C)

Session chairs: **Ján Slezák** (Bratislava, Slovakia)
Jerzy Beltowski (Lublin, Poland)

11:00-11:25 **Ján Slezák** (Bratislava, Slovakia)
Saturation of donor and recipient with molecular hydrogen alleviates graft dysfunction and overall condition after transplantation of the heart

11:25-11:50 **Barbora Kaločayová** (Bratislava, Slovakia)
Molecular hydrogen: new protective tool against acute kidney injury associated with cardiac surgery

11:50-12:15 **Jerzy Beltowski** (Lublin, Poland)
Role of hydrogen sulfide in statin-induced inhibition of insulin secretion

12:35 GROUP PHOTO (Dóm square, in front of the Votive Church, on the front stairs)

12:50-14:00 Lunch break (lunch not provided) – Work lunch for IACS officials in SZAB

14:00-16:00 SESSION 7: Young Investigator Award Competition (Hall A)

Session chairs: **Antonio Zaza** (Milan, Italy)
Paul Volders (Maastricht, The Netherlands)
Gary Lopaschuk (Edmonton, AB, Canada)

14:00-14:20 **Katie Skeffington** (Bristol, UK)
Changes in oxidative stress and calcium signalling pathways in the chronically hypoxic fetal heart

14:20-14:40 **Linda Bartosova** (Bratislava, Slovakia)
Quercetin reduces pro-hypertrophic signaling and mitigates diastolic dysfunction in obese diabetic rats

14:40-15:00 **Dimitra Palioura** (Thessaloniki, Greece)
Pparβ/δ activation protects from mitochondrial degeneration, inflammation and fibrosis in a genetic animal model of heart failure

15:00-15:20 **Branislav Kura** (Bratislava, Slovakia)
Therapeutic effect of molecular hydrogen on radiation-induced overproduction of free radicals in myocardium

15:20-15:40 **Alexandra Polyák**, (Szeged, Hungary)
Cardiac remodeling accompanied by increased arrhythmia susceptibility in a dog model of chronic high-intensity endurance training

15:40-16:00 **Dóra Halmi** (Szeged, Hungary)
Kynurenic acid against simulated ischemia/reoxygenation-induced cardiac cell damage: the possible role of mitoprotection

14:00-16:00 SESSION 8: Ischemia/reperfusion injury: novel directions (Hall B)

Session chairs: **Lorrie Kirshenbaum** (Winnipeg, MB, Canada)
Péter Ferdinandy (Budapest, Hungary)

14:00-14:25 **Péter Ferdinandy** (Budapest, Hungary)
Development of miRNA therapeutics for cardioprotection

14:25-14:50 **Lorrie Kirshenbaum** (Winnipeg, MB, Canada)
Regulation of autophagy and cardiac cell death pathways in the heart

14:50-15:15 **Saadeh Suleiman** (Bristol, UK)
Cardiac pathology and vulnerability to reperfusion injury during open heart surgery

15:15-15:40 **Inna Rabinovich-Nikitin** (Winnipeg, MB, Canada)
Shift work adversely affects myocardial autophagy and cardiac function following myocardial infarction

15:40-16:00 **Norbert Szentandrássy** (Debrecen, Hungary)
TRPM4 in ventricular myocardium, can it be a novel target in cardiovascular disease?

16:15-18:30 Poster Session I (SZAB ACADEMY BUILDING)

Snacks provided

DAY 3 (September 30, 2022) SZEGED HUNGARIAN ACADEMY OF SCIENCES BUILDING

Parallel sessions **in Halls A-B-C**

9:00-10:15 SESSION 9: Myocardial calcium handling (Hall A)

Session chairs: **David Eisner** (Manchester, UK)
Antonio Zaza (Milan, Italy)

9:00-9:25 **David Eisner** (Manchester, UK)
Calcium buffering in the heart: the 99% you don't see

9:25-9:50 **Antonio Zaza** (Milan, Italy)
PLN- R14del, a controversial cardiomyopathy - observations from patient-derived cardiomyoctes and transgenic mice

9:50-10:15 **Zoltán Varga** (Budapest, Hungary)
Inflammasome activation in end-stage heart failure

10:15-10:30 **Balázs Ördög** (Leiden, Netherlands)
Full control of cardiac action potentials by opto-electronic dynamic patch clamping

9:00-10:30 SESSION 10: Pathologies associated with altered hemodynamics (Hall B)

Session chairs: **Bohuslav Ostadal** (Prague, Czech Republic)
Tamás Radovits (Budapest, Hungary)

9:00-9:25 **Bohuslav Ostadal** (Prague, Czech Republic)
Developmental aspects of cardiac adaptation to increased work load

9:25-9:50 **Tamás Radovits** (Budapest, Hungary)

Investigation of pressure overload-induced myocardial remodeling and pressure unloading induced reverse remodeling in rat models

9:50-10:15 **Petr Ostadal** (Prague, Czech Republic)

Hemodynamic effects of extracorporeal membrane oxygenation (ECMO) in cardiogenic shock

10:15-10:30 **Christopher Dostal** (Vienna, Austria)

Dapagliflozin improves haemodynamic recovery after cardioplegic arrest in isolated working mouse heart

9:00-10:30 SESSION 11: Diabetic cardiomyopathy (Hall C)

Session chairs: **Dinender Singla** (Orlando, FL, USA)

Belma Turan (Ankara, Turkey)

9:00-9:25 **Dinender Singla** (Orlando, FL, USA)

BMP-7 attenuates diabetic cardiomyopathy

9:25-9:50 **Belma Turan** (Ankara, Turkey)

Differential effects of the acute or chronic applications of GLP-1 receptor agonists on the remodeling of aging heart

9:50-10:15 **Fatima Mraiche** (Doha, Qatar)

The off-target NHE1 inhibitory effect of SGLT2 inhibitors in cardiac remodeling

10:15-10:30 **Vladimir Zivkovic** (Kragujevac, Serbia)

Hyperbaric oxygen treatment in maintaining of the cardiac function in insulin-treated rats with diabetes type 1

10:30-11:00 *Coffee Break*

11:00-12:15 SESSION 12: Heart failure with preserved ejection fraction (Hall A)

Session chairs: **Róbert Halmosi** (Pécs, Hungary)

Suresh Tyagi (Louisville, KY, USA)

11:00-11:25 **Zoltán Papp** (Debrecen, Hungary)

Pharmacological venodilation to treat pulmonary hypertension complicating heart failure with preserved ejection fraction (PH-HFpEF)

11:25-11:50 **Suresh Tyagi** (Louisville, KY, USA)

Remote ischemia mechanism of heart failure with preserved ejection fraction (HFpEF)

11:50-12:15 **Róbert Halmosi** (Pécs, Hungary)

Role of mitochondrial quality control processes in heart failure

11:00-12:30 SESSION 13: Cardiac arrhythmias: novel mechanisms (Hall B)

Session chairs: **Carol Ann Remme** (Amsterdam, The Netherlands)

Godfrey Smith (Glasgow, UK)

11:00-11:25 **Carol Ann Remme** (Amsterdam, The Netherlands)

Pro-arrhythmic consequences of branched chain amino acid dysregulation

11:25-11:50 **Godfrey Smith** (Glasgow, UK)

Electrophysiological heterogeneity in populations of ventricular cardiomyocytes and the consequences for the action potential response to specific ion channel inhibition

11:50-12:15 **Andrew Trafford** (Manchester, UK)

TRPC6 channels as a driver of cardiac arrhythmias

12:15-12:30 **Paul Volders** (Maastricht, The Netherlands)

Importance of the KCNQ1 S6 region in long-QT syndrome type 1

11:00-12:30 SESSION 14: Novel targets in cardiovascular disease and considerations in cardiotoxicity (Hall C)

Session chairs: **Anikó Görbe** (Szeged and Budapest, Hungary)
Milos Stojiljkovic (Banja Luka, The Republic of Srpska, Bosnia and Herzegovina)

11:00-11:25 **Anikó Görbe** (Szeged and Budapest, Hungary)
Hidden cardiotoxicity - cardiac safety testing in ischemic and comorbid conditions: development of preclinical test platforms

11:25-11:50 **Paramjit Tappia** (Winnipeg, MB, Canada)
Dual role of phospholipase C isozymes during ischemia-reperfusion injury in the heart

11:50-12:15 **Attila Kiss** (Vienna, Austria)
Investigating the cardiac side effects of cancer and cancer associated cachexia in mice

12:15-12:30 **Miloš Stojiljković** (Banja Luka, The Republic of Srpska, Bosnia and Herzegovina)
Organophosphate-induced cardiovascular changes in mammals

12:30-14:00 *Lunch break (lunch not provided) - Work lunch for IACS officials in SZAB*

14:00-16:00 SESSION 15: Cardiac and vascular remodeling (Hall A)

Session chairs: **Róbert Sepp** (Szeged, Hungary)
Georgios Kararigas (Reykjavík, Iceland)

14:00-14:25 **Georgios Kararigas** (Reykjavík, Iceland)
Sex-biased responses in cardiac remodelling

14:25-14:50 **Róbert Sepp** (Szeged, Hungary)
The genetic landscape of hypertrophic cardiomyopathy in Hungary

14:50-15:15 **Péter Bencsik** (Szeged, Hungary)
Novel targets for volume overload induced LV hypertrophy

15:15-15:40 **Dávid Nagy** (Budapest, Hungary)
The decompensation of systolic function in pressure overload-induced left ventricular myocardial hypertrophy is associated with unique microRNA expression profile

15:40-16:00 **Ghassan Bkaily** (Sherbrooke, QC, Canada)
Taurine and high salt-induced vascular smooth muscle remodeling

14:00-16:00 SESSION 16: Myocardial conditioning and cardioprotection (Hall B)

Session chairs: **Ricardo Gelpi** (Buenos Aires, Argentina)
Ágnes Végh (Szeged, Hungary)

14:00-14:25 **Ricardo Gelpi** (Buenos Aires, Argentina)
Molecular basis of myocardial remote ischemic preconditioning

14:25-14:50 **Tanya Ravingerova** (Bratislava, Slovakia)
Physical exercise as a form of non-ischemic „conditioning“: potential molecular mechanisms of cardioprotection

14:50-15:15 **Frantisek Kolar** (Prague, Czech Republic)
Excess ischemic arrhythmias may protect against myocardial infarction

15:15-15:40 **Zoltán Giricz** (Budapest, Hungary)
Extracellular vesicles in cardioprotection

15:40-16:00 **Milan Ivanov** (Belgrade, Serbia)
Effects of NADPH oxidase blockade and hyperbaric oxygen preconditioning on 4-HNE, NGAL, and HO-1 tissue expression in postischemic acute kidney injury induced in spontaneously hypertensive rats

14:00-15:40 **SESSION 17: Mitochondrial function and oxidative stress (Hall C)**

Session chairs: **Ferenc Gallyas** (Pécs, Hungary)
Vladimir Jakovljevic (Kragujevac, Serbia)

14:00-14:25 **Vladimir Jakovljevic** (Kragujevac, Serbia)
Association of oxidative stress and periapical lesions in hypertensive rats

14:25-14:50 **Ferenc Gallyas** (Pécs, Hungary)
Repurposing of desethylamiodarone for cancer therapy

14:50-15:15 **Márta Sárközy** (Szeged, Hungary)
The effects of the preimplantation factor on the development of radiation-induced heart disease in a rat model

15:15-15:40 **Oana Maria Aburel** (Timisoara, Romania)
Targeting mitochondria with methylene blue in human epicardial adipose tissue

16:15-18:30 **Poster Session II (SZAB ACADEMY BUILDING)**
Snacks provided

20:00-24:00 ***Gala Dinner and Award Ceremonies (bus transfer departing from Art Hotel)***

Day 4 (October 1, 2022) **SZEGED HUNGARIAN ACADEMY OF SCIENCES BUILDING**

9:00-10:15 **SESSION 18: Novel targets in cardiovascular disease (Hall A)**

Session chairs: **Devendra Agrawal** (Pomona, CA, USA)
Attila Tóth (Debrecen, Hungary)

9:00-9:25 **Ildikó Bock-Marquette** (Pécs, Hungary)
Enhancing cardiac regenerative therapies by reminding the adult heart on its embryonic state

9:25-9:50 **Devendra Agrawal** (Pomona, CA, USA)
Novel therapeutic approach to prevent maturation failure of arteriovenous fistula

9:50-10:15 **Attila Tóth** (Debrecen, Hungary)
Direct cardiac myosin activators: positive inotropy with potential side effects

9:00-10:15 **SESSION 19: Basic cardiac electrophysiology (Hall B)**

Session chairs: **Thomas Jespersen** (Copenhagen, Denmark)
Norbert Nagy (Szeged, Hungary)

9:00-9:25 **Thomas Jespersen** (Copenhagen, Denmark)
Compromised cardiac electrophysiology caused by simulated obstructive sleep apnea in a porcine model

9:25-9:50 **Balázs Horváth** (Debrecen, Hungary)
Dynamics of the late sodium current under the action potential in canine, guinea pig and human left ventricular cardiomyocytes

9:50-10:15 **Norbert Nagy** (Szeged, Hungary)
The role of the reverse Na^+/Ca^{2+} exchanger and the Ca^{2+}-dependent K^+-current in sinus-node pacemaking

10:30- 10:45 **CLOSING REMARKS (Hall A)**

Officers and Executive Council of the International Academy of Cardiovascular Sciences – European Section

President: András Varró, Szeged, Hungary
Past President: Karl Werdan, Halle, Germany
Vice President: Tatiana Ravingerova, Bratislava, Slovakia
Vice President: Danina Muntean, Timisoara, Romania
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Belma Turan, Turkey
Dragan M. Djuric, Serbia
Ferenc Gallyas, Hungary
Petr Ostadal, Czech Republic
Ranko Škrbić, The Republic of Srpska, Bosnia and Herzegovina
Miloš Stojiljković, The Republic of Srpska, Bosnia and Herzegovina

8th European Section Meeting of the International Academy of Cardiovascular Sciences

September 28 – October 1, 2022
Szeged, Hungary

ABSTRACT BOOK

ORAL PRESENTATIONS

FIBROBLAST ACTIVATION AS A TARGET FOR CARDIAC FIBROSIS THERAPY

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Cardiac fibrosis is a major independent risk factor for adverse patient outcomes, including arrhythmias, cardiac dysfunction and failure, and death. Excessive deposition of extracellular matrix occurs as a result of activation of resident cardiac fibroblasts to myofibroblasts, which greatly increase matrix synthesis, alter their core metabolism, and become contractile in nature. Because this activation process is critical for fibrosis to occur, it is an intriguing target for anti-fibrosis therapies, which are currently completely lacking. We identified scleraxis as a transcription factor that specifically regulates the expression of genes necessary for fibroblast activation. Our previous *in vitro* work showed that scleraxis activates fibroblast to myofibroblast conversion, in part by directly transactivating key pro-fibrotic genes. Conversely, scleraxis loss prevents activation of fibroblasts by pro-fibrotic TGF or cell stretch. We have also shown that scleraxis regulates expression of glutaminase-1, ostensibly providing fuel through glutaminolysis to support myofibroblast function. We now report that scleraxis is required for fibroblast activation during pressure overload *in vivo*: fibroblast-specific scleraxis deletion completely attenuated fibrosis and significantly improved cardiac systolic function. Deletion of scleraxis four weeks after pressure overload induction by thoracic aortic constriction prevented further loss of cardiac systolic function, virtually eliminated fibrosis via myofibroblast loss, and reduced mortality at twelve weeks post-surgery to zero. Our work thus shows that scleraxis regulates fibroblast activation and myofibroblast maintenance, and blockade of scleraxis function shows the potential to arrest or prevent cardiac fibrosis, resulting in improved function and survival, and implicating scleraxis as an important target for anti-fibrosis therapy development.

Keywords: Fibroblast, myofibroblast, gene regulation, metabolism, fibrosis

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DIVERSE MECHANISMS OF HEART FAILURE DUE TO MYOCARDIAL INFARCTION

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It is now well known that heart failure due to myocardial infarction is associated with elevated levels of several vasoactive hormones such as catecholamines angiotensins, endothelins and serotonin. These hormones not only induce cardiac hypertrophy but also produce functional hypoxia, metabolic derangements, oxidative stress, inflammation, subcellular defects for Ca^{2+}-handling abnormalities and arrhythmias in the failing heart. Particularly, prolonged exposure of the hypertrophied heart promotes the oxidation of catecholamines and serotonin by monoamine oxidase as well as activation of NADPH oxidase by angiotensin II and endothelin for the generation of oxyradicals and oxidants which result in oxidative stress. These pathophysiological events affect different signal transduction pathways and play a critical role in the development of apoptosis, necrosis and fibrosis as well as activation of different proteases and dramatic alterations in the extracellular matrix for the occurrence of adverse cardiac remodeling and cardiac dysfunction. There also occurs a loss of adrenergic support for maintaining cardiac function in the failing heart. Thus, heart failure due to myocardial infarction is not only a consequence of the loss of myocardium but also involves a set of complex mechanisms in both viable cardiomyocytes and extracellular matrix. It is suggested that multi-target therapy should be developed for delaying the progression of heart failure.

Keywords: vasoactive hormones, metabolic defects, Ca^{2+}-handling abnormalities, oxidative stress, extracellular matrix.

NOVELTIES IN THE TREATMENT OF HEART FAILURE WITH REDUCED EJECTION FRACTION

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In recent years, clinical practice in the management of heart failure with reduced ejection fraction (HFrEF) has changed significantly. Many new options have emerged for the pharmacological treatment of HFrEF.
A few years ago, ACE inhibitors, beta receptor blockers, and mineralocorticoid receptor antagonists were the first-line treatment for HFrEF. Today, the ACE inhibitors have been at least partially replaced by the angiotensin receptor neprilysin inhibitor, sacubitril-valsartan, and a new class of drugs, SGLT2 inhibitors, has emerged as a first-line treatment. The use of these drugs is now included in the current heart failure guidelines. Both the heart failure guidelines published by the ESC in 2021 and the AHA/ACC/HFSA in 2022 recommend the use of these drugs as a class I recommendation.
Second-line treatment has been supplemented with the soluble guanylyl cyclase stimulator, vericiguat. The above-mentioned guidelines recommend the use of vericiguat as a class IIb recommendation.
The GALACTIC HF study, published in 2021, demonstrated a beneficial effect of omecamtiv mecarbil on the composite primary endpoint of heart failure events and cardiovascular mortality in heart failure patients with a left ventricular ejection fraction of 35% or less. This new agent is not yet included in the current guidelines.
The presentation will review the above-mentioned novelties in the pharmacological management of heart failure with reduced ejection fraction, which are already in clinical practice or close to clinical application.

Keywords: novelties, pharmacological treatment, heart failure with reduced ejection fraction

THE ROLE OF TREATMENT WITH GLP-1 RECEPTOR AGONIST LIRAGLUTIDE ON ISOPRENALINE-INDUCED MYOCARDIAL INJURY IN RATS

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Liraglutide (LIR) improves glycaemia, reduces body weight and has anti-atherosclerotic and anti-inflammatory effects. It improves endothelial function through NO-induced vasodilation and reduced oxidative stress. The aim of the study was to investigate the role of LIR on the isoprenaline (ISO)-induced myocardial injury (MI).
MI in Wistar rats was induced by subcutaneous injection of ISO at a dose of 85 mg/kg of body weight on two consecutive days. The experimental animals were divided into 4 groups: C group, control (received saline on days 1 and 2 + saline for 7 consecutive days), I group (ISO on days 1 and 2 + saline for 7 days), L group (saline on days 1 and 2 + LIR for 7 days) and L+I group (ISO on days 1 and 2 + LIR for 7 days). In this study, myocardial damage, oxidative stress and haemodynamic changes were evaluated.
ISO-induced MI was demonstrated by ultrasound findings of reduced myocardial contractility, increased concentrations of high-sensitive troponin I (hsTnI) and pathohistological changes. The results showed that LIR attenuated biochemical markers and oxidative stress parameters in ISO-induced MI, such as: aspartate aminotransferase (AST), alanine aminotransferase (ALT), thiobarbituric acid substances (TBARS), catalase (CAT) superoxide dismutase (SOD) and reduced glutathione (GSH). LIR also attenuated the ultrasound hemodynamic changes induced by ISO.
Treatment with LIR attenuated oxidative stress, myocardial damage, and haemodynamic changes in ISO-induced MI.

Keywords: liraglutide, isoprenaline, heart failure, oxidative stress, haemodynamic changes

HUMAN HYPERTROPHIC CARDIOMYOPATHY: FROM ELECTROPHYSIOLOGICAL INSIGHTS TO PHARMACOLOGICAL STRATEGIES

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Hypertrophic cardiomyopathy (HCM) is the commonest genetic cardiac disease, with a prevalence of 1/500. It is caused by over 1400 different mutations, mainly involving the genes coding for sarcomere proteins. The main pathological features of HCM are left ventricular hypertrophy, diastolic dysfunction and the increased ventricular arrhythmogenesis. Predicting the risk of heart failure and lethal arrhythmias is the most challenging clinical task for HCM patient management. Moreover, there are no disease-modifying therapies that can prevent disease progression or sudden arrhythmic death in HCM patients. In the last years, cell and animal models and translational studies that have been employed to get insight into the mechanism underlying HCM structural, mechanical and electrophysiological abnormalities, eventually leading to lethal arrhythmias. Preclinical tests of novel or existing drugs in these models are essential for a deeper understanding of HCM pathophysiology and for obtaining meaningful information on novel treatments, in order to improve patient risk stratification and therapeutic management. Guideline-directed therapy of HCM includes non-selective drugs such as disopyramide, non-dihydropyridine calcium channel blockers, or β-adrenergic receptor blockers, mainly used in patients with symptomatic obstruction of the outflow tract. Based on preclinical studies, drugs acting on potential HCM-specific targets were tested in patients. Despite the huge efforts, none of these studies was able to change clinical practice for HCM patients: in recent years, novel compounds have been developed addressing myocardial hypercontractility and altered energetics in a direct manner, through allosteric inhibition of myosin. Hopefully, the impact of these targeted interventions will alter the natural history of the disease in the near future.
During the last 15 years, we have studied the electrical and mechanical properties of cardiomyocytes and intact trabeculae isolated from cardiac samples of over 70 HCM patents with symptomatic outflow obstruction who underwent surgical myectomy. With this approach, we have identified the fundamental ion channel and Ca-handling alterations of HCM myocardium, and tested the effects of a number of traditional and innovative compounds. More recently, we have validated cardiomyocytes differentiated from patient specific induced pluripotent stem cells as a representative model of human HCM, to be used for future drug screening attempts. Finally, computational approaches were developed to help tailoring therapy on the needs of each patient.

POSSIBLE CARDIOPROTECTIVE EFFECTS OF VITAMINS B6 AND B9: LESSONS FROM CARDIOMETABOLIC MODELS

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In order to see if there is a relationship between homocysteine and vitamins B6 and B9 (folic acid, folate), as possible targets in cardioprotection, we established a few models of cardiometabolic diseases in rats (intraperitoneally induced hypermethioninemia and hyperhomocysteinemia, streptozotocin-induced diabetes mellitus, monocrotaline-induced right heart failure, and isoprenaline-induced myocardial infarction) during last time. These cardiometabolic models include complex processes like oxidative stress, inflammation, endothelial dysfunction, gasotransmitters, myocardial injury and cardiovascular remodeling. It was found that plasma levels of homocysteine were significantly increased in experimentally induced hyperhomocysteinemia and isoprenaline-induced myocardial infarction. Although vitamins B6 and/or B9 deficiency can cause hyperhomocysteinemia, and hyperhomocysteinemia is associated with cardiovascular risk or injury, the application of these vitamins, individually or together, confirmed to affect oxidative stress, inflammation, and gasotransmitters. In addition, observational clinical study was also done with an aim to determine the relationship between biomarkers of homocysteine metabolism, inflammation, endothelial dysfunction and severity of coronary artery disease (SYNTAX score) in patients with stable angina pectoris. The increased plasma level of homocysteine, interleukin 6, hs CRP, fibrinogen, and erythrocyte sedimentation rate were detected in patients with high clinical SYNTAX score (>33). Although there is a confirmed role of homocysteine in CVD, the obtained results do not confirm appropriate therapeutic effects of vitamins B6 and B9 in monitored pathophysiological processes.

POTENTIAL IMPLICATIONS OF QUERCETIN IN CARDIOPROTECTION

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Since there are still no cardioprotective drugs on the market for prevention/treatment of myocardial ischemia-reperfusion (I/R) injury, searching for novel cardioprotective compounds is very much needed. Quercetin (QCT), a natural polyphenol enriched in human food, is a promising substance that exerts several beneficial effects in cardiovascular system including preventing cardiac I/R injury. Cardioprotective potential of QCT was largely documented in healthy young animals but only limited data are available regarding cardiac effects of QCT in presence of comorbidities, co-medications and in ageing subjects.
The aim of the current study is to summarize data obtained in our experimental group documenting potential of QCT for preventing myocardial I/R injury in different experimental settings including presence of selected comorbidities, co-medications, and in aged subjects.
QCT in the dose 20mg/kg/day was administered orally for 4/6 weeks to rats of different age and rats with selected comorbidities/co-medications. After the end of treatment hearts were isolated and *ex vivo* exposed to I/R (30-min global ischemia/2-hour reperfusion). Recovery of cardiac function and infarct size were assessed as the physiological outputs of the experiments. Molecular mechanisms of QCT action were evaluated as well.
The results showed that QCT exerts cardioprotective effects in I/R injury in healthy young and doxorubicin-treated rats but it was inefficient in preventing I/R injury in aged rats and in rats with comorbidities (hypertensive/type 2 diabetic). In conclusion, QCT might be potentially cardioprotective in preventing myocardial I/R injury; however, ageing and/or presence of comorbidities may decrease or even abolished anti-ischemic effects of QCT.

Keywords: Ischemia-Reperfusion, Quercetin, Cardioprotection, Ageing, Comorbidities

The study was supported by grants: VEGA 2/0104/20, APVV-18-0548, APVV-21-0194

DISTINCT PATTERNS OF INFLAMMASOME SIGNALLING IN THE CARDIAC AND SKELETAL MUSCLE FROM MURINE MODELS OF DUCHENNE MUSCULAR DYSTROPHY

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The life-limiting complications of Duchenne muscular dystrophy (DMD) include cardiomyopathy, leading to chronic heart failure. The routine treatment for delaying disease progression both in the skeletal and cardiac muscle are glucocorticoids, supporting the hypothesis that inflammation may contribute to the manifestation of cardiac and skeletal muscle dysfunction. However, little is known about the inflammatory mechanisms during the course of the disease, particularly whether inflammatory processes involve both cardiac and skeletal muscle. Thus, the objective of our research was to characterize inflammasome activity in myocardium and skeletal muscle tissue at different time points in two murine models of DMD.

Skeletal muscle and left ventricular myocardial samples were collected from mdx mice and mdx rats (both carrying mutation or deletion in dystrophin gene which produces nonfunctional dystrophin protein) as well as from wildtype littermates. Inflammasome signaling [inflammasome sensors NLRP3, NLRC4 and AIM2, adaptor protein and effectors e.g. interleukin-1 beta (IL-1β), interleukin-18 (IL-18) and gasdermin D (GSDMD)] was assessed by immunoblotting in left ventricular myocardial and skeletal muscle samples collected from the animals at two different time points (month 3 and month 9-10).

Skeletal muscle samples from both species showed a tendency towards elevated expression of GSDMD irrespective of the animal age. Surprisingly, adaptor protein ASC was only elevated in mdx mouse skeletal muscle and heart, but not in mdx rats. Increased expression and cleavage of cytokines was observed in the skeletal muscle of mdx rats. Cytokine expression was not changed in the heart or skeletal muscle of mdx mice. No significant alterations were detected in the expression of inflammasome sensors and caspase-1.

Muscular dystrophy-related inflammatory responses are distinct between skeletal muscle and heart tissue of murine DMD models. Gasdermin D is identified as a robust inflammatory mediator during the disease progression, suggesting its potential as a late stage drug target. Generally, inflammation tends to decrease over time, supporting the clinical observations that the efficacy of anti-inflammatory therapies might be more prominent in the early stage of DMD.

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ALLEVIATION OF MAO-RELATED OXIDATIVE STRESS BY ANTIDIABETIC DRUGS: OF MICE AND MEN

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The global burden of cardiometabolic diseases is expected to increase in the near future with most of related deaths occurring in low and middle-income countries. The major pathomechanisms that underlie these pathologies are chronic oxidative stress and low-grade inflammation that promote each other in a vicious circle leading to both disease progression and the occurrence of complications. Monoamine oxidase (MAO) with two isoforms, A and B, are flavoenzymes located at the outer mitochondrial membrane that have emerged as important sources of cardiovascular oxidative stress. Inflammation is responsible for age-independent increase in MAO expression in the cardiovascular system. A huge body of research demonstrated the role of original antidiabetics in improving the outcome of non-diabetic patients with cardiovascular diseases yet the underlying pathomechanisms remain elusive. Metformin, the central pillar of therapy in type 2 diabetes and Empagliflozin, a largely prescribed SGLT-2 inhibitor, alleviated MAO expression and ROS production in vascular and cardiac preparations from both murine models and humans. Here we provide the evidence for a novel and direct protective effect of antidiabetics in the cardiovascular system, independent of glucose management.

THE CARDIOPROTECTIVE EFFECTS OF TRAMETES VERSICOLOR POLYSACCHARIDES ON RATS WITH METABOLIC SYNDROME

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Mushrooms are untapped resource of species with both nutritive and medicinal potential, undervalued and underexplored until recent history, but now they have been increasingly utilized in conventional medicine, pharmaceutical and food industry. Among many, *Trametes versicolor* (L.:Fr.) Lloyd has been used in Traditional Chinese Medicine to improve immunity, spleen and liver function etc. Nowadays, it has been used in treatment of various types of cancer and respiratory, urinary and digestive tract infections. However its cardioprotective properties, even though are known, have been poorly investigated. Herein, we present the first data regarding cardioprotective properties of *T. versicolor* polysaccharides using *in vivo* model system. This experimental study was conducted on 36 male Wistar albino rats (12 per group, 8 weeks old, bw: 200-250 g) divided into control group, rats with metabolic syndrome (MetS) and MetS rats treated with 100 mg/kg of *T. versicolor* polysaccharides every day for 4 weeks by oral gavage. Parameters of heart function were estimated according to *Langendorff* technique. Moreover, echocardiograph analyses as well as blood pressure and heart rate were examined. Our results showed that four-week treatment with *T. versicolor* polysaccharides alleviated left ventricular hypertrophy and substantially improved *in vivo* cardiac function. Additionally, evaluating the *ex vivo* obtained results we observed remarkably improved cardiac contractility, systolic and diastolic function of MetS rats treated with *T. versicolor*. Taken together, these findings suggest that *T. versicolor* polysaccharides plays significant role in providing cardioprotection of diabetic rats but further investigation is necessary to elucidate the mechanisms which are in the basis of *T. versicolor`s* cardioprotective properties.

Keywords: Trametes versicolor; isolated heart; metabolic syndrome; rat.

ATRIAL FIBRILLATION IS NOT ASSOCIATED WITH ALTERED LEFT ATRIAL MICRORNA EXPRESSION PROFILE IN ISCHEMIC END-STAGE HUMAN HEART FAILURE

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In chronic heart failure (CHF), atrial remodeling is frequently accompanied by atrial fibrillation (AF). Nevertheless, some patients with CHF remain in sinus rythm (SR) despite of the pathological alterations of the left atria (LA). Recent studies have shown that changes in microRNA (miRNA) expression may contribute to the remodelling of the LA. However, the direct role of miRNA expression dysregulation in the development of AF has not yet been investigated independently of pathologically altered LA.
Hence, we aimed to characterize miRNA expression in LA samples from end-stage heart failure patients with AF or SR.
LA samples were collected from male, non-diabetic patients with end-stage ischemic HF who underwent heart transplantation (n=24). There were no differences in the age, ejection fraction, LA diameter and NYHA class of our groups. As a marker of atrial strain, mRNA expression of atrial natriuretic peptide (ANP) was measured by qRT-PCR. A histological examination was performed to characterize the degree of atrial fibrosis. Global miRNA expression profiling was performed using the Nanostring technology.
The mRNA expression of ANP was similar between the CHF-AF and CHF-SR groups, which suggests that the atrial load was the same in the two groups. Furthermore, no difference in atrial collagen content was observed between the groups, which confirms that the fibrotic alteration was similar. The miRNA measurement showed no difference in atrial miRNA expression between the two study groups.
AF is not associated with different left atrial miRNA expression in end-stage CHF with a similar degree of LA dilatation, ANP expression and interstitial fibrosis.

Keywords: atrial fibrillation, microRNA, heart failure, left atrium

APPLICATION OF DONOR HEART AND RECIPIENT WITH MOLECULAR HYDROGEN ALLEVIATES GRAFT DYSFUNCTION AND OVERALL CONDITION AFTER SIMULATED TRANSPLANTATION OF THE PIG HEART

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Heart transplantation has become a routine method of end-stage heart failure treatment but restoring the graft full function often requires repeated electrical defibrillation shocks until the heart resumes the full physiological function. This critical transient period of ventricular fibrillation is largely due to the increased formation of oxygen free radicals during cumulative actions of anesthesia, cardiac ischemia reperfusion, extracorporeal circulation (ECC), and defibrillation after cold asystole storage. It has recently been shown that molecular hydrogen (H_2 gas) selectively reduces $^{\bullet}OH$ radicals and modifies several inflammatory pathways. Benefits of H_2 in treating oxidative stress related dysfunctions are here assessed.
Two main pig groups were established: a group with simulated heart transplantation, and a similar group but supplemented with hydrogen-rich air (4% of hydrogen, >40% of oxygen) in anesthesia administered during the whole experiment. Markers of inflammation, tissue damage, and oxidative stress were determined. Protein expression of total and phosphorylated connexin 43, protein kinase C epsilon type, and activity of matrix metalloproteinases 2 and 9 were measured. Furthermore, the histochemistry of hypoxic/ischemic injury sensitive enzymes were analyzed and electron microscopy of cardiac tissue was evaluated.
In addition to the donor's heart, the recipient's entire body is affected by long-term anesthesia, hyperoxia, circulatory dyshomeostasis caused by ECC. A substantial body of our experimental evidence suggests that hydrogen can significantly alleviate transplantation-related ischemia-reperfusion injury and may have a protective effect against hyperoxia and ROS formation during anesthesia as well as against heart damage induced by repeated electric shocks during cardiac defibrillation. The present study should encourage well-designed clinical trials aimed to test the efficacy of this strategy.

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

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MOLECULAR HYDROGEN: NEW PROTECTIVE TOOL AGAINST ACUTE KIDNEY INJURY ASSOCIATED WITH CARDIAC SURGERY

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Cardiac surgery-associated acute kidney injury (CS-AKI) is common postoperative complication following cardiac surgery. Since oxidative stress is hypothesized to be one of the causes of CS-AKI, molecular hydrogen (H_2) supplementation has been proposed as a novel and promising antioxidant for the prevention of CS-AKI. Our study was performed on an *in vivo* model of simulated porcine heart transplantation. Animals in the H_2 group received H_2 in gaseous form (3%) during inhalation of anesthesia and throughout oxygenation of blood in extracorporeal circulation. The levels of creatinine, urea, and phosphorus were measured in plasma. Renal tissue samples were analyzed by the Western blot method (Nrf2; Keap-1; SOD) as well as by measuring enzyme kinetics of Na,K-ATPase. After cardiac surgery selected plasma biomarkers were elevated. The use of H_2 was followed by the normalization of all these parameters. Our results suggest activation of Nrf2/Keap1 pathway as well as increased SOD protein expression in the H_2-treated group. Regarding Na,K-ATPase we detected a significant decrease of the V_{max} in the H_2 group. Our results support the effectiveness of H_2 supplementation in CS-AKI, especially in terms of normalization of plasma biomarkers to control levels. The protective effect of H_2 might be linked to its activity against oxidative stress via Nrf2/Keap1 pathway modulation. H_2 treatment resulted in decrease in renal Na,K-ATPase activity indicating decreased sodium reabsorption, which appears to be a novel regulatory mechanism of an important membrane transporter during CS-AKI in the H_2-treated pig model.

Keywords: Molecular hydrogen, Cardiac surgery, Kidney injury, Oxidative stress

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ROLE OF HYDROGEN SULFIDE IN STATIN-INDUCED INHIBITION OF INSULIN SECRETION

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Statins inhibit cholesterol synthesis and are used in the prevention and treatment of cardiovascular diseases. However, recent studies indicate that statins may increase the incidence of type 2 diabetes. Hydrogen sulfide (H_2S) is the important endogenous. It has been demonstrated that H_2S inhibits insulin secretion by pancreatic beta cells by activating ATP-sensitive K^+ channels. In addition, by inhibiting coenzyme Q (CoQ) synthesis, statins increase H_2S level in some tissues by impairing its mitochondrial oxidation by sulfide:quinone oxidoreductase (SQR) We examined the effect of statins on insulin secretion and the possible involvement of H_2S. Wistar rats were treated with atorvastatin (20 mg/kg/day) or rosuvastatin (5 mg/kg/day) for 1 week. Neither atorva- or rosuvastatin had any effect on insulin sensitivity measured by hyperinsulinemic euglycemic clamp. However, both statins reduced glucose-induced insulin secretion both in vivo and ex vivo by isolated islets. Statins increased net H_2S production by isolated islets, however, had no effect on the expression or activity of H_2S synthesizing enzymes. In contrast, statins reduced mitochondrial H_2S oxidation. Statins had no effect on mitochondria density, inner membrane potential or SQR activity but decreased CoQ concentration in both plasma and pancreatic islets. The effect of statins on insulin secretion was mimicked by H_2S donor, Na_2S, and was attenuated by the inhibitor of H_2S synthesis, propargylglycine, ATP-sensitive K^+ channel blocker, glibenclamide, or CoQ supplementation. In conclusion, although statin-induced up-regulation of H_2S signaling may be beneficial for the cardiovascular system, H_2S may contribute to diabetogenic effect of these medications.

Keywords: statins, hydrogen sulfide, diabetes mellitus, insulin

CHANGES IN OXIDATIVE STRESS AND CALCIUM SIGNALLING PATHWAYS IN THE CHRONICALLY HYPOXIC FETAL HEART

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Cyanotic congenital heart disease (CHD) is associated with a greater risk of adverse outcomes following cardiac surgery (Najm et al. *J Thorac Cardiovasc Surg* **119**:515,2000). Research suggests that many cyanotic CHD patients will also have had reduced oxygen levels before birth (Sun et al. *Circulation* **131**:1313,2015). Whether exposure to cyanosis during development causes molecular changes in the heart which may affect surgical outcomes is unknown.
Pregnant sheep were exposed to normoxia (N) or hypoxia (H: 10% O_2) between days(d) 105-138 of pregnancy (term ~145 days, n=16 per group). At d138, fetal hearts were frozen for molecular analysis or mounted on a Langendorff preparation to determine function, followed by fixation for stereology.
131 proteins were differentially expressed in the left ventricle (LV) of H fetuses. Ingenuity Pathway Analysis (IPA) highlighted superoxide radical degradation as a significantly enriched canonical pathway ($p=3.84 \times 10^{-2}$) due to the upregulation of the antioxidant SOD2, a finding validated by Western blotting. Functional and stereological analyses showed that H fetuses have impaired diastolic function but maintain contractility despite thinning of the LV wall. IPA analysis in H fetuses also highlighted calcium signalling as a significantly enriched function ($p=1.84 \times 10^{-2}$), showing β-tropomyosin upregulation, which has been linked to diastolic dysfunction (Muthuchamy et al. *J Biol Chem* **270**:30593,1995) and HDAC8 downregulation, linked to compensatory maintenance of systolic function (Meraviglia et al. *Int J Mol Sci* **19:** 419, 2018).
Proteomic analysis deepens insight into molecular mechanisms underlying changes in cardiac development in the hypoxic fetus.

Supported by The British Heart Foundation

QUERCETIN REDUCES PRO-HYPERTROPHIC SIGNALING AND MITIGATES DIASTOLIC DYSFUNCTION IN OBESE DIABETIC RATS

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With its potent anti-inflammatory and ROS scavenging properties, quercetin (Que) is being implicated in spectrum of different pathological phenotypes including cardiac remodeling. However, the precise mechanism of its action remains far from being clarified as well as the exact molecular pathways Que interferes with in certain cardiac pathologies. For that reason, we tested out the effects of Que in the model of 1-year-old Zucker Diabetic Fatty rats which combines type 2 diabetes mellitus and obesity. Animals were divided into the following experimental groups: control (fa/+) vehicle-treated, diabetic (fa/fa) vehicle-treated, control (fa/+) Que-treated and diabetic (fa/fa) Que-treated group. Que was administered to respective groups at a dose of 20 mg/kg/day during 6 weeks. Echocardiography examination was performed prior to the onset of the treatment and at the end of sixth week. Subsequently, we carried out a series of assays in excised left ventricular tissues – hydroxyproline assay, SDS-PAGE and immunoblotting. On the level of echocardiography, Que was able to normalize increased E/A ratio, suggesting diastolic dysfunction, in diabetic rats to the level of controls. In addition, Que promoted a decrease in overall wall's mass. Moreover, a significant decrease in total collagen content was associated with such reduction. On the protein level, Que significantly reduced pro-hypertrophic transcriptional pathway – MEF2/HDAC4 in diabetic animals as well as other transcriptional factors – GATA4, NFAT3 and its regulator Calcineurin A. Taken altogether, Que showed capability to ameliorate diastolic dysfunction and to suppress pro-hypertrophic signalization and therefore produced beneficial effects in obese diabetic rat hearts.

Keywords: quercetin, hypertrophy, diabetes, diastolic dysfunction

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PPARβ/δ ACTIVATION PROTECTS FROM MITOCHONDRIAL DEGENERATION, INFLAMMATION AND FIBROSIS IN A GENETIC ANIMAL MODEL OF HEART FAILURE

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PPARβ/δ is a primary transcriptional regulator of cardiac energy metabolism with pleiotropic properties, including anti-inflammatory, anti-oxidative and cardioprotective action. The aim of the present study was to investigate whether pharmacological activation of PPARβ/δ could ameliorate cardiac tissue damage in desmin null mice (*Des*$^{-/-}$), a genetic model of heart failure and explore the potential effects on the impaired mitochondrial network. In *Des*$^{-/-}$ mice, ultrastructural abnormalities with severely damaged mitochondria, massive cardiomyocyte death, an early acute inflammatory response and severe cardiac remodeling lead to dilated cardiomyopathy and eventually heart failure. Our findings demonstrate that PPARβ/δ activation protects from extensive cardiac inflammation, fibrosis and cardiac remodeling, all hallmarks of the *Des*$^{-/-}$ heart. Importantly, PPARβ/δ activation stimulates mitochondrial biogenesis, protects mitochondria from exacerbated degeneration and improves the deranged mitochondrial network as observed in transmission electron microscopy images of *Des*$^{-/-}$ hearts. Concomitantly, PPARβ/δ restores the balance in fission/fusion protein markers, attenuates ATP depletion and enhances mitochondrial functionality in *Des*$^{-/-}$ hearts. Furthermore, PPARβ/δ activation alleviates oxidative stress as evidenced by almost 50% decrease in superoxide levels through transcriptional activation of the antioxidant regulator, Nrf2 and major ROS scavengers, in the failing myocardium. In conclusion, pharmacological activation of PPARβ/δ during myocardial degeneration and heart failure in *Des*$^{-/-}$ hearts demonstrates cardioprotective action by preserving the structural and functional quality of the mitochondrial network and alleviating inflammation and fibrosis.

Keywords: heart failure; inflammation; mitochondria; peroxisome proliferator-activated receptor β/δ

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THERAPEUTIC EFFECT OF MOLECULAR HYDROGEN ON RADIATION-INDUCED OVERPRODUCTION OF FREE RADICALS IN MYOCARDIUM

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Overproduction of free radicals and subsequent formation of oxidative stress is a common denominator of many cardiovascular diseases. Ionizing radiation (IR) is widely used in medicine, mostly in radiotherapy or various imaging technics. The radiation induces cells damage mostly through the formation of aggressive hydroxyl radicals. In the last 15 years, many researchers paid attention to molecular hydrogen (H_2) as an effective and selective scavenger of free radicals, and as potentially therapeutic substance in many diseases.
The main goal of this study was to examine a potential therapeutic effect of H_2 against free radicals produced by IR directly applied to the mediastinum area of rats. The study also aimed to compare the effectiveness of two different methods of H_2 administration – hydrogen-rich water (HRW) and inhalation. Rats (male, 3 months old) were irradiated with a single dose of 10 Gy. These were treated by HRW (2 mg/L) or inhalation (30 min. of 4% H_2 gas) daily three times for two and nine days.
The inhalation of H_2 and HRW administration significantly improved all measured blood biochemical parameters (lactate dehydrogenase, alanine aminotransferase, uric acid, etc) where inhalation proved more effective. Also, irradiation-induced increased levels of selected markers of oxidative stress (superoxide dismutase, catalase, malondialdehyde) and inflammation (TNF-α and NF-κB) were significantly downregulated after H_2 treatment, while inhalation was more effective here as well.
Based on these results, it could be concluded that hydrogen is a protective therapeutic substance against irradiation-induced heart damage and that inhalation is more effective than drinking hydrogen-rich water.

Keywords: heart, inhalation, irradiation, molecular hydrogen, oxidative stress

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CARDIAC REMODELING ACCOMPANIED BY INCREASED ARRHYTHMIA SUSCEPTIBILITY IN A DOG MODEL OF CHRONIC HIGH-INTENSITY ENDURANCE TRAINING

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Despite the cardiovascular benefits of regular physical exercise, chronic high-level exercise can evoke malignant arrhythmias, including ventricular fibrillation and even sudden cardiac death, especially in young top athletes. In some cases the underlying mechanisms are unclear.
The goal of this study was to assess mechanisms underlying cardiac structural-electrical changes and arrhythmia vulnerability by high-level vigorous exercise training in animal species that are electrophysiologically relevant to the human heart.
Beagle dogs were randomly assigned to matched sedentary (‘Sed’, n=12) or intensive exercise-training (‘Ex’, n=12) groups. ‘Ex’ dogs underwent a 4-month-long intensive treadmill-running protocol (5 days a week, 6 hours a day at a speed of 14-21 km/h with an inclination 5-12%). *In vivo* echocardiography and electrophysiological measurements were performed. Proarrhythmic sensitivity was tested and the autonomic alterations were examined. At study end, arrhythmia susceptibility was tested with high-frequency burst stimulation in open-chest anaesthetized dogs. This was followed by cardiac excision and cardiomyocyte isolation, formalin preservation for histology and snap-freezing in liquid nitrogen for molecular biology.
The vigorous endurance training was resulted in increased left ventricular end-diastolic diameter, increased septal wall thickness and greater left ventricular mass index (LVMi ‘Sed’ vs. ‘Ex’: 98±12 vs. 136±7 g/m2, $p<0.05$). Some degree of enhanced fibrosis was observed. Endurance training decreased heart rate both in whole animal and *in vitro* dog experiments. ECG recordings presented enhanced heart rate variability parameters, prolonged PQ (‘Sed’ vs. ‘Ex’: 98.3±2.9 vs. 116.7±3.6 ms, $p<0.05$), QRS (‘Sed’ vs. ‘Ex’: 60.5±2.4 vs 70.8±1.6 ms, $p<0.05$), QTc (‘Sed’ vs. ‘Ex’: 213.6±2.8 vs. 237.1±3.4 ms, $p<0.05$), Tp-Te (‘Sed’ vs. ‘Ex’: 27.9±2.5 vs.36.5±1.7 ms, $p<0.05$) intervals associated with significantly enhanced QT interval variability (eg. QT-STV ‘Sed’ vs. ‘Ex’: 2.5±0.2 vs. 3.6±0.4 ms, $p<0.05$), reflecting elevated level of repolarization dispersion. Ectopic activity was also enhanced in the exercised dog ventricle. Atropine treatment resulted in moderate heart rate increase in the ‘Ex’ animals. Chronic endurance exercise elevated the proarrhythmic risk and consequent ventricular fibrillation in dogs subjected to burst electrical stimulation.
We developed a new animal model that shares similarities with the human endurance-trained athlete’s heart. The model represents increased arrhythmia susceptibility, an important clinical paradigm, and explores potential underlying mechanisms, including vagal enhancement, increased repolarization dispersion and enhanced fibrotic changes. Increased arrhythmia susceptibility is supported by the enhanced arrhythmia incidence in the exercised group. Similar changes may be present in young human top athletes, however further investigations are required.

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KYNURENIC ACID AGAINST SIMULATED ISCHEMIA/REOXYGENATION-INDUCED CARDIAC CELL DAMAGE: THE POSSIBLE ROLE OF MITOPROTECTION

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Acute myocardial infarction (AMI) is a life-threatening condition that belongs to the leading causes of death worldwide. Analysis of agents which potentially increase the tolerance of cardiac cells against the harmful effects of ischemia/reperfusion supports the development of new treatment strategies. In this study, we aimed to investigate the potential mitoprotective effect of kynurenic acid (KYNA) and its possible involvement in the agent's previously uncovered cardiocytoprotective effect against simulated ischemia/reoxygenation (SI/R)-induced damage of H9c2 cardiomyoblasts. H9c2 cells were exposed to 6 hours of ischemia, followed by 2 hours of reoxygenation. 64 μM KYNA treatment was performed throughout the SI/R protocol for the investigation of the potential mitoprotective effects of KYNA. The rate of oxidative stress was measured on both cellular (DHE staining) and mitochondrial (MitoSox) levels at the end of the experiment. Mitochondrial function was analyzed via the detection of potential alterations in the mitochondrial membrane potential (JC-1 staining) and investigations on the efficiency of mitochondrial respiration (high resolution respirometry; Oroboros O2k). Alterations in the structure and distribution of mitochondria were investigated via immunocytochemistry and electronmicroscopic morphometry. Our results demonstrated that SI/R caused an increase in the level of both cellular and mitochondrial oxidative stress. SI/R-induced depolarization of mitochondrial membranes was observed compared to the normoxic control groups. SI/R-induced functional damage of mitochondria affected the baseline respiration and decreased the activity of respiratory complexes. Alterations both in the distribution and structure of mitochondria were detected as well. All the above-mentioned SI/R-induced harmful effects were attenuated by the applied KYNA treatment. Our data suggest that attenuation of mitochondrial damage might be involved in the cytoprotective effect of KYNA against SI/R induced cardiac cell damage.

Keywords: mitochondria, mitoprotection, kynurenic acid, ischemia/reperfusion

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NOVEL THERAPEUTIC APPROACH TO PREVENT MATURATION FAILURE OF ARTERIOVENOUS FISTULA

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An autologous arteriovenous fistula (AVF) is the preferred vascular access in hemodialysis; however, a high rate of maturation failure that is characterized by inadequate dilation and inadequate blood flow in the outflow vein renders the fistula not useful for hemodialysis after initial adequate blood flow after AVF creation. Inflammation, neointimal hyperplasia and failure of outward remodeling are the major causes of AVF maturation failure accounting for 60% of all the newly created AVF. Proliferation, migration, and phenotypic changes of vascular smooth muscle cells and extracellular remodeling due to increased matrix metalloproteinases play a crucial role in the pathogenesis. In this study, AVF was created in Yucatan miniswine by anastomosis of femoral artery and femoral vein. TLR-4-mediated inflammation was examined using its inhibitor, TAK-242, to investigate the effect on vessel remodeling and AVF maturation. The expression level of several proteins, vein outward remodeling, artery and vein diameter, blood flow through the fistula and femoral artery and vein, and vessel thickness involved in AVF were assessed by ultrasound, angiography, optical coherence tomography, immunohistochemistry, and histomorphometry. The TLR-4 inhibition with TAK-242 attenuated inflammation, decreased neointimal hyperplasia, and favored femoral artery and vein remodeling; the features favoring AVF maturation. The bulk RNA sequencing and the Ingenuity Pathway Analysis revealed changes in many transcription factors and microRNAs that are involved in angiogenesis, vascular smooth muscle cell proliferation, migration, and phenotypic changes, endothelial cell proliferation and function, oxidative stress, vessel remodeling, immune responses, and inflammation. These findings suggest that not only the luminal factors but also the mediators from surrounding structures like muscles mediate vascular cuffing contributing to vessel thrombosis and AVF maturation failure via early thrombosis. Therefore, targeting the key regulatory sites, including TLR4, may have therapeutic potential.

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MUTATIONS IN CPVT1-ASSOCIATED AND CPVT1-UNASSOCIATED RYR2 CALCIUM BINDING RESIDUE REVEAL REMODELING OF EC-COUPLING IN HIPSC-CMS

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Reports on 3-dimentional near-atomic Cryo-EM images of skeletal ryanodine receptor have revealed identity and location of Ca^{2+} and Caffeine binding sites residues. Mutating RyR2 Ca^{2+}-binding residues in homologous HEK cell, though revealing the roles of binding residues on RyR2 function, fail to account for possible remodeling of other cellular Ca^{2+}-signaling pathways, likely to occur in diseased states. The remarkable similarity of Ca^{2+}-signaling in human stem-cell derived cardiomyocytes (hiPSC-CMs) to adult cardiomyocytes led us to examine the effects of CPVT1-associated or CPVT1-unassociated mutations of the putative RyR2 calcium binding site residues on EC-coupling consequences of hiPSC-CMs Ca^{2+}-signaling. We introduced Q3925E mutation associated and E3848A mutation unassociated with CPVT1 in hiPSC-CMs using CRISPR/Cas9 gene editing and determined their EC-coupling phenotypes.
In TIRF-imaged and voltage-clamped WT and mutant hiPSC-CMs infected with SR-targeted ER-GCaMP6 probe: 1) I_{Ca} densities were comparable (7-10pA/pF) in mutant- and WT-cells; 2) I_{Ca-} and caffeine-triggered Fura-2 (cytosolic calcium) or ER-GCaMP6 (SR Ca^{2+} release) signals were significantly suppressed in both mutants; 3) Arrhythmic Fura-2 signals in either mutant were not accompanied by ER-GCaMP6 Ca-release signals; 4) Even though caffeine failed to trigger Ca^{2+} release in mutant voltage-clamped cells, only ~20% to ~70% of cells responded respectively to 5 & 20mM caffeine in intact cells, but these responses were delayed, slow, and 2-APB- or ruthenium red-sensitive. Mutations of RyR2 Ca^{2+}-binding residues, irrespective of CPVT1 association, reveal interaction between Ca^{2+} and caffeine binding-sites and unmask remodeling of EC-coupling in heart cells that accounts for arrhythmogenic Ca-transients of mutant cells.

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PLN- R14DEL, A CONTROVERSIAL CARDIOMYOPATHY - OBSERVATIONS FROM PATIENT-DERIVED CARDIOMYOCTES AND TRANSGENIC MICE

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Phospholamban (PLN) phyis the natural inhibitor of SERCA2a function, decreases its Ca^{2+} affinity and mediates the effect of its phosphorylation-dependent modulation. The heterozygous R14del PLN mutation (PLN-R14del) is associated with an arrhythmogenic dilated cardiomyopathy (DCM) with clinical onset at middle age. Initial heterologous expression studies (microsomal preparations) detected a sharp decrease in SERCA2a affinity for Ca^{2+} in the presence of heterozygous PLN-R14del; this led to interpret DCM as the consequence of SERCA2a "super-inhibition" by mutant PLN. This interpretation has been assumed in a number of later studies attempting therapeutic approaches. We have recently evaluated intracellular Ca^{2+} dynamics in cardiomyocytes derived (hIPSCMs) from a symptomatic heterozygous PLN-R14del carrier. The results obtained surprisingly pointed to enhancement of sarcoplasmic reticulum (SR) Ca^{2+} transport, i.e. opposite to what expected from SERCA2a superinhibition. To test whether these results might have a more general value, we have further investigated Ca^{2+} dynamics in a transgenic PLN-R14del murine model. Preliminary results from this model are fully consistent with our observations in hiPSCMs. My presentation will discuss these new findings and their impact on the interpretation of the mechanism by which PLN-R14del results in DCM.

REALTIME AND COMPLETE (RE)SHAPING OF CARDIAC ACTION POTENTIALS ON A MULTICELLULAR LEVEL BY OPTO-ELECTRONIC DYNAMIC PATCH CLAMPING

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All living cells possess a membrane potential (Vm), which has been implicated in the regulation of diverse biological processes, e.g. cell cycle, proliferation and volume control. In cardiomyocytes, dynamic Vm changes give rise to action potentials (APs) spreading throughout the heart to regulate contractions. Little is known, however, about the role of cardiac Vm in maintaining cardiomyocyte homeostasis or driving pathological conditions. To gain insight into such matters, one needs to meticulously control cardiac Vm on a multicellular level. To acquire such control, we developed an adaptive, feedback loop-controlled experimental system (APqr) using dynamic patch clamping and optogenetics. To exploit its novel research possibilities, we applied APqr to realize instant restoration of disturbed AP morphology. We show that under optimized conditions, drug-prolonged (4AP) AP durations (APD80, CTL: 228±41ms → 4AP: 325±19ms, $p<0.05$, $n=4$) were restored immediately using APqr (232±49ms) in monolayers of immortalized human atrial myocytes. APD20 and APD50 values were similarly corrected. Furthermore, the average Vm difference over one AP was as high as 33±6mV when comparing drug-prolonged APs to CTL, which was reduced to 5±3mV by our APqr method ($n=4$, $p<0.05$), indicating accurate AP reshaping. In conclusion, our data show that APqr is able to restore and maintain AP morphology in the presence of disturbance. APqr may be at the root of taking full control of the cardiac Vm in various conditions, including multicellular preparations from acute to chronic settings, to not only unravel the role of Vm in homeostatic regulation, but also in disease mechanisms and treatment.

Keywords: action potential, dynamic patch clamping, optogenetics, human immortalized atrial myocytes, cellular electrophysiology

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DEVELOPMENTAL ASPECTS OF CARDIAC ADAPTATION TO INCREASED WORKLOAD

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The heart is capable of extensive adaptive growth in response to its function, because its performance as a pump is directly determined by the mass of its contractile elements. In agreement with the developmental approach of cardiac adaptation, normal cardiac growth represents the adaptive response to the increased energy demand. Growth response of the cardiac muscle changes significantly during phylogenetic and ontogenetic development. Cold-blooded animals maintain the ability of cardiac myocyte proliferation even in adults; the remarkable potential of cardiac proliferation is often associated with lifelong ability of such species to grow even after reaching sexual maturity. On the other hand, in warm-blooded animals the normal cardiac growth is biphasic: whereas fetal or neonatal cardiac myocytes express proliferative potential (hyperplasia), after birth proliferation substantially declines and the heart growth is accomplished almost exclusively by hypertrophy of cardiac cells. Proliferation of cardiac myocytes peaks during embryonic life and then decreases until birth. After birth, proliferation of cardiomyocytes declines dramatically; the rapid switch from myocyte hyperplasia to hypertrophic growth occurs between day 3 and 4 in rat and mouse heart. The data on the final number of cardiomyocytes in humans vary from 1 month after birth to young adults. Interest in the developmental aspects of cardiac cellular response to the increased workload steadily increases. The main reason seems to be the effort to determine the mechanisms responsible for the plasticity of the immature heart in order to provide new strategies to promote proliferation of adult cardiomyocytes in the injured myocardium.

DAPAGLIFLOZIN IMPROVES HAEMODYNAMIC RECOVERY AFTER CARDIOPLEGIC ARREST IN ISOLATED WORKING MOUSE HEART

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Cardioplegic arrest in cardiac surgery is associated with temporary or long-term myocardial contractile dysfunction. Beyond the glucose-lowering effects, sodium glucose cotransport 2 inhibitors (SGLT2-i) have shown dramatically beneficial cardiovascular effects in the last years. However, whether SGLT2 inhibitors protect the heart after cardioplegic arrest and global cold ischaemia is unknown.
Adult male C57BL/6J mice were anaesthetised, the hearts were excised and then mounted in a perfused isolated working heart system. Cold ischaemia (4°C) for 100 min was induced by St. Thomas cardioplegia. The cardioplegic solution was applied every 20 min, followed by 30 min reperfusion. Cardiac hemodynamic variables were continuously recorded. Isolated hearts were randomised to cardiac arrest with/without applying Dapagliflozin (control n=5, SGLT2-i n=4, respectively; 0.1 µg/ml per gram bodyweight) directly to the perfusion buffer prior to cardiac arrest.
Cold ischaemia and reperfusion resulted in a significant reduction in aortic flow (63%±6%, $p<0.05$) in addition to systolic and diastolic parameters such as dP/dt_{max} (75%±8%, $p<0.05$) and dP/dt_{min} (76%±9%, $p<0.05$) compared to baseline. In contrast, a significantly higher rate of systolic (dP/dt_{max} 88%±2%, $p<0.05$) and diastolic (dP/dt_{min} 104%±10%, $p<0.01$) recovery was measured in the SGLT2-i group compared to the control group. Similar improvement in aortic flow recovery was shown (73%±3%, $p<0.05$) compared to control.
Our study emphasises the novel, proof-of-concept character of SGLT2 inhibitor administration before cardiac arrest, which subsequently improves hemodynamic recovery. These results pave the way for a new area of using SGLT2 inhibitors in elective cardiac surgery.

BMP-7 ATTENUATES DIABETIC CARDIOMYOPATHY

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Diabetic cardiomyopathy is a complex disease that involves progression of hyperglycemia, oxidative stress, and inflammation. However, the role of inflammation-induced pathological mechanisms are still evolving. Therefore, we will discuss inflammation-induced cell death pyroptosis, inflammasome formation, and downstream pathways leading to tissue inflammation. Additional mechanisms of cell death pathways will be presented with specific emphasis on the involvement of Nek7-GBP5 pathway in cell death. Next, we will discuss inflammatory cellular infiltration, and the role of tissue inflammation mediated structural cardiac remodeling and cardiac dysfunction in streptozotocin-induced diabetes. Data in this study was confirmed using Immunostaining, Western blotting, H&E, and Masson's trichrome staining on diabetic hearts. Furthermore, BMP-7 treatment attenuated a series of inflammatory, pathophysiological, and structural adverse effects together with improving cardiac function.

DIFFERENTIAL EFFECTS OF GLP-1 RECEPTOR AGONIST APPLICATIONS ON THE REMODELING OF AGING-HEART

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Glucagon-like peptide-1 receptor (GLP-1R) agonists improve cardiovascular dysfunction via the pleiotropic effects behind their receptor action. However, it is unknown whether they have cardioprotective action in aging-heart. Therefore, we examined the effects of GLP-1R agonist liraglutide treatment (4 weeks) on systemic parameters of aged rats (24-mo-old) compared to those of adult rats (6-mo-old) such as electrocardiograms (ECGs) and blood pressures. At cellular levels, action potential (AP) parameters, ionic currents, and Ca^{2+}-regulation were examined in freshly isolated ventricular cardiomyocytes. The liraglutide treatment of aged rats significantly reserved the prolongations in ECG parameters and increases in both systolic and diastolic pressures together with recoveries in plasma oxidant and antioxidant status. The prolonged AP durations and depolarized membrane potentials of the isolated cardiomyocytes from the aged rats were found to be normalized via recoveries in K^+-channel currents with liraglutide treatment. The alterations in Ca^{2+}-regulation including leaky-ryanodine receptors could be also reserved via recoveries in Na^+/Ca^{2+}-exchanger currents with this treatment. Direct treatment of isolated aged-rat cardiomyocytes with liraglutide could recover the depolarized mitochondrial membrane potential, the increase in both reactive oxygen and nitrogen species (ROS and RNS), and cytosolic Na^+-level, although Na^+-channel currents were not affected by aging. Interestingly, the liraglutide treatment of aged-rat cardiomyocytes provided significant inhibition of activated SGLT2 and recoveries in the depressed insulin receptor substrate 1 (IRS1) and increased protein kinase G (PKG). The recovery in the ratio of phospho-endothelial nitric oxide (eNOS) level to eNOS protein level in the treated ventricular cardiomyocytes implies the involvement of liraglutide-associated inhibition of oxidative stress-induced injury via IRS1-eNOS-PKG pathway in aging-heart. Interestingly, marked irregular atypical fibrillations and significant prolongation of the QT intervals in in situ ECGs were observed following an acute GLP agonist application. Unlikely to adult group, GLP agonism prolonged APs, reduce K-currents and increased the Ca^{+2}-spark frequency in the aged cardiomyocytes, while Na-channels become resurgent after GLP activation implying higher propensity for arrhythmias. Overall, our data, for the first time, provide important information on the direct cardioprotective effects of GLP-1R agonism in the hearts of aged rats in a differential manner as chronic or acute effects.

HYPERBARIC OXYGEN TREATMENT IN MAINTAINING OF THE CARDIAC FUNCTION IN INSULIN-TREATED RATS WITH DIABETES TYPE 1

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The side effects of hyperbaric oxygen (HBO) treatment, such as oxidative stress and oxygen toxicity, have long been of interest. However, there are no comprehensive studies evaluating such toxic effects in diabetes mellitus (DM). The purpose of this study was to determine the effects of HBO on glucose homeostasis and cardiac function in experimentally induced diabetes and insulin treated and non-treated rats.
Diabetes was induced by intraperitoneal administration of 60 mg/kg streptozotocin to Wistar albino rats. A total of 48 male Wistar rats were randomly divided into 4 groups: 1) Control group, no diabetic induction without HBO treatment; 2) HBO group, exposed to 100% oxygen at 2.8 ATA (atmosphere absolute) for 1 h once daily, for 5 days (two weeks); 3) DM group, diabetes induced by streptozotocin (STZ) injection; and 4) DM + HBO group, received both STZ injection and HBO exposure; 5) DM+INS group, NPH insulin 5U/day, 6) DM+HBO+INS, received both NPH insulin and HBO exposure for 2 weeks.
The efficacy of the insulin therapy or HBO treatment was evaluated by comparing cardiac parameters among the experimental groups, as well as glycemia measurement. Glycemic levels, dp/dt max and min, SLVP, DLVP, HR and CF were reestablished in diabetic rats treated with insulin (ITD). We observed a 30% improving of cardiodynamics in the treated diabetic rat with insulin and HBO. We established an insulin therapy that consists of a daily insulin dose for all animals to maintain most of them at or near normoglycemia. Our results provide, what is to our knowledge, the most detailed schedule of insulin therapy for treating STZ-diabetic rats and HBO treatment.

Keywords: diabetes mellitus type 1, streptozotocin, hyperbaric oxygen therapy, neutral protamine hagedorn (NPH) insulin

ELECTROPHYSIOLOGICAL HETEROGENEITY IN POPULATIONS OF VENTRICULAR CARDIOMYOCYTES AND THE CONSEQUENCES FOR THE ACTION POTENTIAL RESPONSE TO SPECIFIC ION CHANNEL INHIBITION

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Studies on ventricular myocytes isolated from rabbit hearts suggests that single myocytes from the same region of a heart show highly variable electrophysiology and variable response to ion-channel blocking drugs. In-silico modelling of the electrophysiology of populations of cells suggests that the underlying cause is an even higher cell-to-cell variation in expression of ion-channels. This work suggests that action potentials occur because the relative expression of ion channels (rather than absolute) is regulated. Therefore, predisposition to arrythmias from genetic or environmental causes may be due to disrupted pattern of co-expression. This work indicates alternative strategies to treat dysfunction, namely manipulations of the activities of ion channel activities in a network to restore the normal pattern of co-expression.

TRPC6 CHANNELS AS A DRIVER OF CARDIAC ARRHYTHMIAS

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Cardiac arrhythmias are a significant cause of cardiovascular morbidity and mortality and the link between perturbed intracellular calcium homeostasis and cardiac arrhythmias is long-established. For example, overloading of the intracellular calcium store, the sarcoplasmic reticulum (SR), or alterations in the ability of the SR to retain calcium leads to the spontaneous release of calcium from the SR into the cytoplasm in the form of calcium sparks and calcium waves. Some of this cytosolic calcium is then extruded from the cell by the electrogenic sodium-calcium exchanger generating an inward, depolarising, membrane current and triggered arrhythmias. Whilst this mechanism is firmly established, a key question remains regarding how calcium enters the cell to allow the SR to reach the critical threshold calcium content at which spontaneous arrhythmogenic calcium release occurs. Previous work from our group [1] had suggested that a mechanism distinct from the L-type calcium channel and reverse mode sodium calcium exchange supported a background calcium entry into cardiac myocytes. However, the lack of available pharmacological and molecular tools at the time of this original study did not allow us to identify the cause of this background calcium influx. More recently, it was suggested that canonical transient receptor potential (TRPC) channels [2] may mediate a background calcium influx in mouse ventricular myocytes. Notably, TRPC6 channels are inhibited by protein kinase G (PKG) dependent phosphorylation [3]. Given these considerations, the first aim present study was designed to identify the nature of the background calcium influx that *a priori* is required to maintain spontaneous calcium release from the SR and give rise to calcium dependent triggered arrhythmias. The second aim of the study was then to determine if the antiarrhythmic effect of acute phosphodiesterase 5 inhibition, and thus augmented PKG activity, we have recently reported [4] is, at least in part, due to an effect on the source of background calcium influx potentially occurring via TRPC6 channels. Experiments were performed in isolated sheep ventricular myocytes and Langendorff perfused mouse hearts and we used, respectively, high external calcium concentrations and programmed electrial stimulation protocols to induce arrhythmic behaviour. In isolated cell experiments we found that background calcium influx was exclusively carried by TRPC6 channels as inhibition of L-type calcium channels, sodium calcium exchange and TRPC1,4,5 channels had no effect on calcium overload induced calcium wave formation or background calcium influx. At the whole heart level, catecholamine exposure and programmed electrical stimulation reliably induced arrhythmias and arrhythmia scores were statistically attenuated by manoeuvres leading to an increase in PKG activity. Additionally, on exposure to the PDE5 inhibitor sildenafil, the frequency of calcium sparks was reduced in isolated myocytes. Together, these results indicate a specific role for TRPC6 mediated background calcium entry in the genesis of calcium dependent arrhythmias in ventricular myocytes. Additionally, our data demonstrates that targeting PKG activity also attenuates calcium dependent arrhythmias which is at least conceptually consistent with the role of PKG dependent inhibition of TRPC6 channel activity.

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

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Unexpected cardiac adverse events are one of the leading causes of interruption of clinical trials and drug withdrawals. It has been shown that cardiovascular risk factors and comorbidities (such as aging, metabolic diseases, etc) and their medications (e.g. nitrates, antidiabetic drugs, statins, etc) may interfere with cardiac ischemic tolerance and molecular signaling of endogenous cardioprotection. Indeed certain drugs may exert adverse events on the diseased heart that is hidden in the healthy myocardium. Hidden cardiotoxic effects of drugs may occur due to (i) enhancement of unwanted signaling due to ischemia/reperfusion injury and/or the presence of risk factors and/or (ii) inhibition of cardioprotective signaling pathways, both of which may lead to ischemia-related cell death and pro-arrhythmic events. This led to novel concept of “hidden cardiotoxicity”, i.e. cardiotoxity seen only in the diseased heart, i.e. ischemia/reperfusion injury and/or its major comorbidities (Ferdinandy et al, Eur Heart J, 2018). Hidden cardiotoxicity cannot be revealed by the routinely used cardiac safety testing methods in “healthy” test systems, moreover, the mechanism of hidden cardiotoxocity is largely unknown. Therefore, we aimed to develop a preclinical in vivo and vitro platform and test already withdrawn drugs with hidden cardiotoxic properties (Brenner et al, Cells, 2020; Weber et al, Pharmaceuticals, 2022) and new drugs with potential cardiotoxic properties. Here we summarize the current knowledge on hidden cardiotoxicity and urge the need for development of novel cardiac safety testing platforms for early detection of yet “hidden” cardiotoxicity.

NOVEL TARGETS FOR VOLUME OVERLOAD INDUCED LEFT VENTRICULAR HYPERTROPHY

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Volume overload (VO)-induced cardiac eccentric hypertrophy occurs in mitral or aortic valve regurgitation as well as during remodeling after myocardial ischemia. Although, anti-remodeling treatment is available, there is no specific treatment to stop or reverse cardiac hypertrophy. Therefore, our aim was to identify novel players in the development of VO-induced eccentric hypertrophy by using transcriptomics and bioinformatics. In this study, VO was induced by an aorto-caval fistula in 2-month-old male Wistar rats. Sham operated animals served as control. Functional parameters were measured by transthoracic echocardiography at termination, 4- or 8-months after induction of VO. We found hypertrophic remodeling, which was accompanied by mechanical dysfunction and increased cardiomyocyte stiffness. Total RNA was isolated from LV samples and microRNA deep sequencing was performed to identify altered microRNA profile. Via bioinformatic target prediction, mRNA targets possessing at least 4 connections to altered microRNAs were selected for further analyses. Out of 752 microRNAs being present in LV samples during deep sequencing, 22 microRNAs showed significant down- and 12 microRNAs significant up-regulation according both log2 fold-change and adjusted p value between the 8m-VO as compared to 8m-sham group. Bioinformatic target prediction by using microRNA-mRNA network analysis identified 3 mRNA targets, Nova1, Btg2 and Rock2 connected to 5 differentially expressed microRNAs as well as further 12 mRNAs possessing 4 connections to altered microRNAs. Biological validation of the results at mRNA and/or protein level is required, however, it seems that Nova1, Btg2 and Rock2 may play a pivotal role in the development of eccentric ventricular hypertrophy induced by VO.

Keywords: left ventricular eccentric hypertrophy, volume overload, aorto-caval fistula, microRNA profile, bioinformatic target prediction

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THE DECOMPENSATION OF SYSTOLIC FUNCTION IN PRESSURE OVERLOAD-INDUCED LEFT VENTRICULAR MYOCARDIAL HYPERTROPHY IS ASSOCIATED WITH UNIQUE MICRORNA EXPRESSION PROFILE

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MicroRNA (miRNA) expression is dysregulated in pressure overload (PO)-induced left ventricular (LV) myocardial hypertrophy (LVH). However, whether the altered miRNA expression might contribute to the decompensation of systolic function in PO-induced LVH is still debated. Thus, we aimed to characterize miRNA expression in PO-induced LVH with and without systolic heart failure (HF).
Aortic banding (AB) was performed in male rats to induce PO. Sham-operated animals served as controls. Functional and morphological alterations were assessed by echocardiography and histology. At the end of the experimental period, rats in the AB group were subcategorized based on ejection fraction [EF] into AB_{LVH} (EF>40%) and AB_{HF} groups (EF<40%). Global miRNA expression profiling was performed using next generation sequencing. Bioinformatic network analysis was carried out to predict miRNA-target interactions. Expression of selected target genes was measured by qRT-PCR.
Increased heart weight-to-tibial length, LV mass and fibrosis confirmed the development of pathological LVH in both AB_{LVH} and AB_{HF} groups. Nevertheless, increased lung weight-to-tibial length, chamber dilatation and impaired EF were noted only in the AB_{HF} group, when compared to controls. 50 miRNA showed different expression in the AB_{HF} compared to the AB_{LVH} group. Based on this altered expression profile, bioinformatic analysis predicted over 3000 target genes. 15 genes with high regulation strength were selected for target validation. Fmr1, Zfpm2, Wasl, Ets1 and Atg16l1 showed decreased mRNA expression levels in the AB_{HF} group versus AB_{LVH}.
Decompensation of systolic function in PO-induced LVH is associated with unique miRNA profile leading to differential gene regulation.

Keywords: heart failure; decompensation; pressure overload-induced left ventricular hypertrophy; microRNA; bioinformatic network analysis

HIGH SALT-INDUCED HUMAN VASCULAR ENDOTHELIAL AND SMOOTH MUSCLE REMODELING

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Salt sensitivity is a factor that contributes to an increase in blood pressure of at least 10%. It is a complex phenomenon whose cellular and molecular mechanisms remain controversial despite the various epidemiological studies and animal models developed. The dogma is that this salt sensitivity is primarily due to a renal defect in salt excretion. Recently, it has been suggested that an increase in plasma sodium of 2-4 mM induced by a high salt diet may lead to vascular dysfunction by directly exposing smooth muscle cells to extracellular sodium overload. The increased [Na+]e induces remodeling of human vascular endothelial and smooth muscle cells (hVSCs) and intracellular calcium homeostasis. Chronic increase in [Na+]e leads to a significant concentration-dependent increase in hVEChVSMC volume. This increase in cell volume is associated with an increase in the number of NTTs (nuclear T-tubules). By exposing the cells to 4 mM NaCl, there is an increase in the basal intracellular Ca^{2+} level of both hVECs and hVSMCs.Furthermore, salt-pre-sensitized cells are more sensitive to sodium than non-sensitized cells. The morphological and functional remodeling induced by the chronic increase of [Na+]e is irreversible in the short term. In conclusion, chronic exposure to extracellular sodium is associated with the remodeling of ionic and morphological homeostasis.

Keywords: salt sensitivity, hVSMC, sodium, calcium, NTT, hypertrophy, cell remodeling.

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PHYSICAL EXERCISE AS A FORM OF NON-ISCHEMIC „CONDITIONING“: POTENTIAL MOLECULAR MECHANISMS OF CARDIOPROTECTION

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Although ischemic preconditioning (IPC) is the most robust adaptive intervention protecting the heart of all animal species against ischemia/reperfusion (I/R) injury, its application in humans is limited to elected operations due to technical requirements, such as chest opening, short-term duration and unpredictable occurrence of AMI. However, other forms of “conditioning” do not require invasive intervention.
We explored preventive interventions applied *in vivo* aimed to increase cardiac resistance to I/R prior to AMI *ex vivo* using non-invasive approach in the adult male Wistar rats: voluntary exercise-induced PC (EPC). For EPC, adult male animals were placed in the cages equipped with wheels for free running, while control sedantary animals stayed in the standard cages. At the beginning and the end of the protocol, heart structure and function was evaluated by ECHOcardiography and except reduced body weight did not reveal functional changes. After 2 weeks, the efficacy of EPC was tested in the Langendorff-perfused hearts exposed to 30 min global ischemia/2 hrs reperfusion, focused on the postischemic recovery of function, arrhythmogenesis and extent of lethal injury (infarct size, IS/AR, TTC staining). In parallel groups, heart tissue samples were obtained for the investigation of the levels and activity of several proteins involved in “pro-survival” RISK cascade. EPC significantly reduced contractile dysfunction, IS size and the incidence and severity of reperfusion arrhythmias. Protective effects were associated with a significant up-regulation of selected pro-survival RISK proteins, such as PKB, PKCε, eNOS, anti-apoptotic and anti-oxidative effects. Beneficial effects of sub-chronic free running suggest its potential in the management of ischemic heart disease.

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EXCESS ISCHEMIC ARRHYTHMIAS MAY PROTECT AGAINST MYOCARDIAL INFARCTION

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C-reactive protein (CRP) is not only an inflammation marker, but it also exerts direct cardiovascular actions. Here we analyzed the effects of CRP overexpression on cardiac susceptibility to ischemia/reperfusion (I/R) injury in adult spontaneously hypertensive rats (SHR) expressing human CRP transgene (SHR-CRP). Using an in vivo model of coronary artery occlusion, we found that transgenic expression of CRP predisposed SHR-CRP to prolonged ventricular tachyarrhythmias. The proarrhythmic phenotype in SHR-CRP was associated with altered heart and plasma eicosanoids, myocardial composition of fatty acids in phospholipids, and autonomic nervous system imbalance. Interestingly, excess ischemic arrhythmias in SHR-CRP led to a significant reduction in infarct size (IS) compared with progenitor SHR. To explain this unexpected finding, we performed metabolomic analysis of plasma before and after ischemia. Acute ischemia in SHR-CRP markedly increased plasma levels of multiple potent cardioprotective molecules that could reduce IS at reperfusion. We also determined cardiac ischemic tolerance in hearts subjected to remote ischemic conditioning (repeated occlusions of both hind legs during ischemia) which provided IS-limiting effect in SHR that was comparable with myocardial infarction observed in naïve SHR-CRP. In hearts ex vivo, IS did not differ between the strains, suggesting that extra-cardiac factors play a crucial role in cardioprotection. Our study shows that transgenic expression of human CRP predisposes SHR-CRP to excess ischemic ventricular tachyarrhythmias associated with a drop of pump function that triggers myocardial salvage against lethal I/R injury likely mediated by protective substances released to blood from hypoxic body tissues.

Keywords: C-reactive protein; metabolomics; myocardial infarction; remote ischemic conditioning; ventricular arrhythmias.

EFFECTS OF NADPH OXIDASE BLOCKADE AND HYPERBARIC OXYGEN PRECONDITIONING ON 4-HNE, NGAL, AND HO-1 TISSUE EXPRESSION IN POSTISCHEMIC ACUTE KIDNEY INJURY INDUCED IN SPONTANEOUSLY HYPERTENSIVE RATS

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Kidney disease represents a serious global health problem. Free radicals and prooxidants produced during ischemic acute kidney injury (AKI) may further aggravate the course of the disease and play a role in the pathogenesis of subsequent complications. The aims of our study were to examine the importance of NADPH oxidase blockade and to determine the effect of hyperbaric oxygen preconditioning on the immunohistochemical analysis of 4-hydroxynonenal (4-HNE), neutrophil gelatinase-associated lipocalin (NGAL), and heme oxygenase-1 (HO-1) tissue expression in postischemic acute kidney injury induced in spontaneously hypertensive rats (SHR). Twenty-four hours before AKI induction, HBO preconditioning was carried out by exposing to pure oxygen (2.026 bar) twice a day, for 60 min in two consecutive days. Acute kidney injury was induced by removal of the right kidney while the left renal artery was occluded for 45 min by atraumatic clamp. NADPH oxidase blockade was performed by Apocynin (40 mg/kg body weigh)t, intravenously, 5 min before reperfusion. We showed increased 4-HNE renal expression in postischemic AKI compared to Sham-operated (SHAM) group. Apocynin treatment, with or without HBO preconditioning, improved creatinine and phosphate clearances, in postischemic AKI. This improvement in renal function was accompanied with decreased 4-HNE, while HO-1 kidney expression restored close to the control group level. NGAL renal expression was also decreased after apocynin treatment, and HBO preconditioning, with or without APO treatment. Considering our results, we can say that 4-HNE tissue expression can be used as a marker of oxidative stress in postischemic AKI. On the other hand, NADPH oxidase blockade and HBO preconditioning reduced oxidative damage, and this protective effect might be expected even in experimental hypertensive condition.

Key words: acute kidney injury, oxidative damage, spontaneously hypertensive rats

CORRELATION BETWEEN APICAL PERIODONTITIS AND HEART FUNCTION IN HYPERTENSIVE RATS

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The aim of this study was to investigate the link between apical periodontitis (AP), cardiac function and oxidative stress in rats under conditions of hypertension and normotension.
The study included 48 *Wistar rats*, divided into four experimental groups: I-control, II-normotensive rats with AP, III-spontaneously hypertensive rats without AP, and IV-spontaneously hypertensive rats with AP. The pulp chambers of the mandibular first molars were exposed to the oral environment for inducing AP. After four weeks, the rats were sacrificed and hearts according to the *Langendorff technique* were perfused at a gradually increased coronary perfusion pressure of 40-120 cmH_2O. Hemimandibular tissue samples were used for radiographic, histopathological, histomorphometric analysis, and examination of the concentration of proinflammatory cytokines. Cardiac tissue was analyzed histopathologically. Markers of systemic and cardiac oxidative status were determined.
From the radiographic, pathohistological and histomorphometric aspects, the periodontal ligament thickness, alveolar bone and apical cementum resorption, intensity of inflammatory infiltrate and radiographic AP area, were significantly higher in the hypertensive rats. The concentration of proinflammatory cytokines in AP was significantly higher in hypertensive conditions. AP was associated with impaired cardiodynamics and pathohistological changes in isolated rat hearts in hypertensive conditions. Also, they were correlated with increased values of prooxidants and decreased mobility of antioxidants in a hypertensive state.
AP and hypertension were correlated in a rat model. Results may help to better understand the association between AP and cardiac function in hypertensive conditions, supporting the hypothesis that lesions may adversely affect cardiac function and potentially disrupt oxidative status homeostasis in an experimental rat model.

Keywords: Apical periodontitis; Hypertension; Cardiodynamics; Oxidative stress.

TARGETING MITOCHONDRIA WITH METHYLENE BLUE IN HUMAN EPICARDIAL ADIPOSE TISSUE

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Coronary heart disease (CHD) is the leading cause of mortality due to myocardial infarction and of morbidity due to heart failure. Epicardial adipose tissue (EAT) is considered a biologically active organ that has been extensively studied in the past decades in relation with CHD. Monoamine oxidases (MAOs) are mitochondrial enzymes that have been unequivocally recognized as sources of cardiovascular oxidative stress. Mitochondrial dysfunction and the related oxidative stress are central pathomechanisms in CHD, thus targeting mitochondria has emerged as promising therapeutic approach. Methylene blue (MB) is a redox agent reported to protect cardiac mitochondria, yet no data are available about its effects on adipose tissue. The present study, performed in EAT harvested from patients subjected to cardiac surgery (n=25), was aimed to assess the effects of MB in preventing the bioenergetic failure and oxidative stress by modulating ETC and MAOs expression. EAT samples incubated with MB (0.1 µM for 24h) were used for the assessment of MAO gene and protein expression (RT-PCR and immune-fluorescence) and ROS production (spectrophotometry and confocal microscopy). High-resolution respirometry was also performed on fresh EAT samples in the presence *vs.* absence of MB (0.1 µM). We report that MAO-A is the predominant isoform in the EAT. MB was able to reduce MAO expression and ROS generation and to increase all mitochondrial respiratory parameters. In conclusion, methylene blue is a potential candidate for drug repurposing by alleviating oxidative stress and mitochondrial stress in human epicardial adipose tissue in patients with coronary heart disease.

Keywords: methylene blue; epicardial adipose tissue; mitochondria; monoamine oxidase; oxidative stress.

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ENHANCING CARDIAC REGENERATIVE THERAPIES BY REMINDING THE ADULT HEART ON ITS EMBRYONIC STATE

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Our dream in defeating the processes of organ damage and aging has challenged scientists for many years. Although the goal is to successfully treat the body as a whole, steps towards regenerating individual organs such as the heart are even considered significant. We propose interconnecting our collective knowledge regarding aging and embryonic development which may lead to the discovery of molecules providing alternatives to effectively reverse cellular damage.
In our studies, we utilized Thymosin beta-4 (TB4) to support our hypothesis. We found TB4 is widely expressed in the developing heart. In vitro and in vivo animal studies demonstrated TB4 promotes myocardial cell migration and survival in embryonic tissue. In adults, the peptide enhanced myocyte survival and improved cardiac function following coronary artery ligation. Moreover, intravenous injections of TB4 altered the morphology of the adult epicardium, and the changes resembled the characteristics of the embryo as it resulted in the thickening of the epicardial monolayer. Re-activation of the embryonic program was equally reflected by the increased number of capillaries and mature cardiac vessels and by the alteration of the gene expression profile typical of the embryonic state. Strikingly, our analyses via heterozygous capsulin/LacZ animals revealed the effect is independent of hypoxic injury.
In conclusion, by observing the broad spectral capacity of TB4, we believe it is not the only molecule which nature conceals to our benefit. Thus, the discovery and postnatal administration of developmentally relevant candidate molecules such as TB4 may likely result in reversing aging processes of the human body.

Keywords: Regeneration, Aging, Heart, Thymosin beta-4

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TRPM4 IN VENTRICULAR MYOCARDIUM, CAN IT BE A NOVEL TARGET IN CARDIOVASCULAR DISEASE?

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TRPM4 is a unique member of melastatin subfamily of the transient receptor potential channels as it is permeable to only monovalent cations. TRPM4 is activated by the increase of intracellular Ca2+, PIP2, and membrane potential. The presence and role of TRPM4 in human cardiac conducting tissue is well established, but much less is known about TRPM4 in working myocardium. The controversial role of TRPM4 in ventricular hypertrophy, heart failure and ischemia-reperfusion injury will be discussed. A major difficulty of TRPM4 research is the need of a specific and selective inhibitor. Although TRPM4 knock-out animal models provide valuable information, they cannot be used in large animals like the dog, which is a good electrophysiological model of human heart. TRPM4 expression was low but detectable in ventricular myocardium in rats and mice, and we show TRPM4 protein expression in canine isolated left ventricular cells. We recorded APs in isolated left ventricular canine cells using sharp microelectrode and applied whole-cell patch clamp technique in both conventional and AP voltage clamp modes. We described poor selectivity of both the recently discovered CBA and the previously widely used 9-phenanthrol. CBA proved to be slightly better as it blocked Ito and late sodium currents, while 9-phenanthrol reduced many major potassium currents including Ito, IK1, and IKr. The contribution of TRPM4 to canine cardiac AP still needs to be further studied. Clearly more experiments are needed to establish TRPM4 as a novel ventricular target of cardiovascular diseases.

Keywords: TRPM4, CBA, 9-phanenthrol, action-potential, canine left ventricular cell

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ENDOGENOUS INHIBITION OF NEPRILYSIN

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Introduction: Neprilysin is a membrane-bound zinc-dependent metalloprotease with a secreted form in the blood. Its activity has been found in human and rat tissues (predominantly in the kidneys) and in the Vero lineage cells (CCL81) which are obtained from the kidney epithelia of an African green monkey. Neprilysin plays a role in the transformation of signaling molecules, such as natriuretic peptides and angiotensin I and II. Heart failure patients have increased natriuretic peptides which is beneficial as they play a positive role in cardiovascular homeostasis, but as they are inactivated by neprilysin their supply is limited. Neprilysin inhibitors have been introduced in clinical practice to treat heart failure.
Methods: Neprilysin activity was measured using a chromogenic kinetic assay. We used human blood obtained from venous puncture. Cells of the Vero CCL81 lineage were also cultured, measurements were then carried out on the intact cells as well as the supernatant of the homogenized cells. Surprisingly, neprilysin activity (endogenous secreted enzyme) was not measurable in human blood. In line with that, the activity of exogenous recombinant neprilysin was completely inhibited by serum, suggesting the presence of a high-affinity endogenous inhibitor.
Results: There was little to no neprilysin activity in the human blood. In contrast, Vero CCL81 cells maintained the same activity in their intact state and homogenized form. The addition of human sera to Vero CCL81 cells completely inhibited neprilysin activity, suggesting that the blood contains an endogenous inhibitor suppressing circulating neprilysin. Our search for the potential inhibitors revealed the inhibitory effect of bovine and human albumin on the activity of neprilysin in blood and on recombinant neprilysin. Albumin inhibited recombinant neprilysin activity by (% of maximal inhibition here), with an affinity of (IC50 values here).
Conclusions: Our results suggest that (in contrast with the general view) neprilysin inhibiting medical drugs are not acting on the secreted (circulating) neprilysin enzyme. In contrast, circulating neprilysin activity is completely inhibited by endogenous inhibitors, such as serum albumin in humans. Our results suggest that neprilysin can only act in the tissues, where serum proteins have limited access. Endogenous neprilysin inhibition, therefore, restricts natriuretic peptide metabolism to specific tissues. These findings suggest a complex endogenous regulation of neprilysin activity and natriuretic peptide-mediated regulation of blood volume. Dysregulation of neprilysin can lead to hypertension (for which the direct cause of the disease is unknown in most cases).

DYNAMICS OF THE LATE SODIUM CURRENT UNDER THE ACTION POTENTIAL IN GUINEA PIG, CANINE AND HUMAN VENTRICULAR MYOCARDIUM

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Many aspects of the late sodium current ($I_{Na,late}$) are still poorly understood. We studied the true profile of $I_{Na,late}$ in different species. We also aimed to find out if there is a sharp difference in frequency-dependent $I_{Na,late}$ blockade between the so-called "selective late sodium current inhibitors" and "general" class I/B sodium channel blockers.
$I_{Na,late}$ was defined as tetrodotoxin-, mexiletine-, or GS458967-sensitive current, recorded under action potential voltage clamp (APVC) or conventional voltage clamp. Action potential measurements were carried out with sharp microelectrodes.
Under APVC conditions the density of canine and human $I_{Na,late}$ monotonically decreased, whereas in guinea pig, it continuously increased during the AP plateau. Conventional voltage clamp experiments revealed that the "increasing" $I_{Na,late}$ profile in guinea pig is determined by the slow decay of $I_{Na,late}$ in this species.
In guinea pig cells, facilitation of $I_{Na,late}$ prolonged APD and induced arrhythmogenic activities; triggered by spontaneous Ca^{2+} release from the sarcoplasmic reticulum.
CaMKII inhibition using KN-93 reduced $I_{Na,late}$ magnitude throughout the time course of AP. Meanwhile, increasing Ca^{2+}-load did not further increase $I_{Na,late}$, indicating that the Ca^{2+}-CaMKII modulation of $I_{Na,late}$ can already be saturated under baseline condition.
Like mexiletine, GS458967 inhibited both the early and late components of the sodium current. On the basis of its kinetic properties, GS458967 can be classified as a I/B antiarrhythmic drug in Vaughan-Williams classification. Based on our experiments, the sharp distinction between "selective" $I_{Na,late}$ inhibitors and the frequency-dependent $I_{Na,late}$ inhibition described for "general" class I/B sodium channel inhibitors is highly questionable.

Keywords: late sodium current, cardiac myocyte, action potential, antiarrhythmic drug

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THE ROLE OF THE REVERSE NA+/CA2+ EXCHANGER AND THE CA2+-DEPENDENT K+-CURRENT IN SINUS-NODE PACEMAKING

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Sinus node (SN) pacemaking is driven by a close interaction of surface membrane ion-channels and intracellular Ca^{2+}-handling. The reverse mode of the Na^{+}/Ca^{2+} exchanger (NCX) and the small-conductance Ca^{2+}-activated K^{+}-channel (I_{SK}) could be important players of this system, however, the exact roles of these components are not fully clarified.
Whole-cell and perforated patch-clamp experiments were performed on rabbit SN cells supplemented with fluorescent Ca^{2+}-tracking. NCX was assessed by specific block with 1 μM ORM-10962, I_{SK} was inhibited by apamin. The ECG R-R intervals were obtained by Langendorff-perfusion method.
Active reverse NCX caused larger Ca^{2+}-transient amplitude due to larger SR Ca^{2+}-content. Spontaneous action potential (AP) frequency was enhanced in the presence of active reverse NCX. When reverse NCX was facilitated by 1 μM strophantin the $Ca^{2+}{}_{i}$ and spontaneous rate increased. ORM-10962 applied prior to strophantin prevented $Ca^{2+}{}_{i}$ and AP cycle change. SK2 channel expression was verified by immunoblot technique in rabbit SN cells and patch-clamp experiments revealed apamin-sensitive current. However, we found no change in the action potential parameters nor in the ECG R-R interval after application of 100 nM apamin.
Our results indicate that the reverse NCX activity may provide additional Ca^{2+}-influx that could increase SR Ca^{2+}-content, leading to enhanced pacemaking activity. Therefore, the reverse mode of the NCX may contribute in normal SN pacemaking increasing the robustness of the automaticity. In contrast, our data indicate that I_{SK} has no role in SN pacemaking under normal condition.

Keywords: sinus-node, pacemaking, Na-Ca exchanger, apamin,

POSTER PRESENTATIONS

Poster Session I

September 29, 2022

16.15-18.30

ANXIETY, INTERNET ADDICTION AND PULSE WAVE ANALYSIS IN MEDICAL STUDENTS

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Psychosocial factors are related to cardiovascular risk. The present study aimed to assess the relationship between anxiety, internet addiction, and variables resulting from pulse wave analysis in medical students.
A total of 30 medical students, aged 24±0.6 years, 80% female, underwent pulse wave analysis using a Mobil-O-Graph. Anxiety level and internet addiction were assessed using the Hamilton Anxiety Rating Score (HAM-A) and Internet Addiction Assessment questionnaire (IAA), respectively.
Central systolic blood pressure (SBPc), augmentation index and pressure (AI, AP), pulse wave velocity (PWV), IAA, and HAM-A scores were, as follows: 103 ± 9.62 mmHg, 25 ± 10.62%, 7 ± 3.16 mmHg, 5 ± 0.38 m/s, 21 ± 1.96 and 29 ± 8.2, respectively. The AI and PWV were increased for age in 50% and 23% of the study participants, respectively, and early vascular aging (EVA) was detected in 60% of the students. Significant correlations were obtained between IAA and several pulse wave variables, especially SBPc ($r=0.432$, $p<0.0001$), PWV ($r=0.21$, $p<0.0001$), and AP ($r=0.242$, $p<0.0001$). EVA was detected especially in participants with severe, moderate, and mild anxiety (39%, 39%, and 11%, respectively). More than half of the students with EVA (55.55%) had probable internet addiction.
Internet addiction and anxiety are associated with impaired pulse wave variables, early vascular aging, and an increased cardiovascular risk in medical students. Lifestyle must be carefully analyzed in young subjects in order to enable cardiovascular prevention.

Keywords: pulse wave analysis, pulse wave velocity, augmentation index, internet addiction, anxiety

THE ROLE OF *HELICHRYSUM ARENARIUM* EXTRACT IN CARDIOPROTECTION DURING ISCHEMIA/REPERFUSION INJURY

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Beside the widespread traditional use of *Helichrysum arenarium* in the treatment of numerous diseases and conditions, its potential in prophylaxis of cardiovascular diseases has still not been fully clarified. Therefore, the aim of the present investigation was to assess the effects of the *H. arenarium* extract on the redox status and cardiac function of the isolated rat heart. Sixteen male *Wistar albino* rats were divided into two groups: control and *H. arenarium* group, which included animals treated with *H. arenarium* extract *per os* for 14 days. After completing the 14-day protocol hearts from all animals were isolated and subjected to *ex vivo* ischemia/reperfusion injury. The parameters of cardiac function including the maximum and minimum rate of pressure development, systolic and diastolic left ventricular pressure and heart rate were registered. Coronary flow was measured flowmetrically. Levels of superoxide anion radical, hydrogen peroxide, nitrites and index of lipid peroxidation were determined spectrophotometrically in plasma samples. In the erythrocytes lysate, parameters of the antioxidative defense system such as the level of reduced glutathione, and activities of catalase and superoxide dismutase were determined. Our findings indicate that intake of *H. arenarium* extract for 14 days preserved cardiac contractility and relaxation and prevented ischemia-induced deterioration of systolic function. Moreover, the consumption of *H. arenarium* extract was associated with improved activity of the antioxidative defense system. The findings of the present study suggest a possible role of *H. arenarium* in cardioprotection, however further studies are necessary to completely reveal the therapeutic possibilities of this plant species.

Keywords: *Helichrysum arenarium*, ischemia/reperfusion, oxidative stress, heart, rat

RELATIONSHIP BETWEEN ION CURRENTS AND MEMBRANE CAPACITANCE IN CANINE VENTRICULAR MYOCYTES

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Current density, the membrane current value divided by membrane capacitance (C_m) is a widely used parameter in describing transmembrane ionic currents. Calculating current density is often referred to as "normalizing" the current values. This convention assumes that C_m and ion current magnitudes are linearly related for any given ion current, however, this assumption has not been thoroughly investigated so far on cardiac muscle.
Therefore, we have investigated the dependence of amplitudes and integrals of the major cardiac ion currents on C_m, using linear regression, under conventional voltage clamp (CVC) and action potential voltage clamp (APVC) conditions.
The relationship between C_m and ion current parameters was characterized by correlation analyses. Under CVC conditions the correlation was good for I_{K1}, moderate for I_{Kr} and $I_{Ca,L}$, while negligible for I_{Ks}. In the case of $I_{to1,}$ the correlation between the peak amplitude and C_m was negligible when analyzing all cells together, however, correlations were high when the cells were analyzed separately for subepicardial, subendocardial and mid-myocardial origin. Under APVC conditions high correlations were observed in the case of $I_{K1,}$ I_{Kr} and $I_{Ca,L}$. For I_{NCX}, $I_{Na,late}$ and I_{Ks} there were low-to-moderate correlations between C_m and the current parameters. The linear regression indicated a true linear relationship between C_m and current amplitudes or integrals.
In conclusion, we found good correlation between ion current amplitudes or integrals and C_m. Limited correlations are likely consequences of spatial inhomogeneity of ion current density and/or non-ideal experimental conditions. This must be considered when interpreting ion current measurements in cardiac cells.

Keywords: cardiac ion currents, membrane capacitance, current densities, current integrals, dog myocytes

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INVESTIGATING THE ROLE OF THE ECM IN CARDIO PROTECTION: PRODUCTION AND CHARACTERIZATION OF A BIOACTIVE RECOMBINANT AGRIN

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Mature cardiomyocytes (CM) are unable to proliferate, therefore injury to the myocardium results in permanent damage. However, in 2017 the extracellular matrix protein agrin was found to successfully induce myocardial repair in rodent models of cardiac infarction, futher confirmed in porcine models in 2019. It was proposed that agrin increases CM proliferation by interacting with the cell surface receptor αDystroglycan (αDG). To better understand this interaction, the following downstream processes and agrin's regenerative capacity we have produced and purified a recombinant, bioactive form of agrin which we have named, DBAF (dystroglycan binding agrin fragment), which contains only the domains that interact with αDG. DBAF has been characterised using a variety of biochemical and biophysical methods including solid phase binding, and small angle x ray scattering (SAXS). We have found that DBAF is stable upon both chemical and physical denaturation. SAXS analysis has shown that DBAF is compact, and futher compacted in the presence of calcium (Ca^{2+}). Solid phase binding indicated DBAF binds tightly to αDG in a Ca^{2+} dependant manner. Based our molecular model predicting the Ca^{2+} binding site we produced and purified two single mutants and a double mutant of specific residues within the predicted Ca^{2+} binding site. Solid phase experiments revealed that αDG binding is strongly reduced in the mutants, confirming the involvement of such residues in coordinating Ca^{2+} and SAXS experiments futher showed that Ca^{2+} has an effect in compacting the protein.

Keywords: Agrin, cardiomyocyte, regeneration, recombinant agrin, Ca2+

PLACENTAL MITOCHONDRIAL RESPIRATION AND MONOAMINE OXIDASE EXPRESSION ARE INCREASED IN ATYPICAL SEVERE PREECLAMPSIA: A CASE PRESENTATION

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Preeclampsia (PE) is a major complication of pregnancy defined by a blood pressure increase in previously normotensive women and new-onset organ dysfunctions. One of the presentations of severe PE is the HELLP syndrome (hemolysis, elevated liver enzymes and low platelet count). Mitochondrial dysfunction and increased oxidative stress are central pathomechanisms underlying the abnormal placentation in PE but the source of reactive oxygen species (ROS) is far from being elucidated. We report here a case of a of 28 years old woman, 34 weeks of pregnancy diagnosed with atypical PE/HELLP syndrome due normal blood pressure associated with severe vomiting, hepatocytolysis, hemolysis, low platelet count, proteinuria; the fetus was delivered prematurely at 35 weeks of pregnancy, the fetal weight was normal according to the gestational age. The clinical-biological features improved after the delivery. The placental tissue was assessed after delivery for: i) mitochondrial respiratory function, ii) ROS production and iii) monoamine oxidase (MAO) enzyme expression. Mitochondrial respiration of placental mitochondria was assessed by means of high-resolution respirometry according to a protocol adapted to measure complex I and complex II-dependent respiration. Placental samples were incubated with dihydroethidium for ROS measurement in confocal microscopy. Placental MAO gene (RT-PCR) and protein (immune histochemistry) expressions were assessed. Placental mitochondria showed a significant increase in active and maximal uncoupled respiration for both complexes. Placental ROS and MAO expression were also increased. In conclusion, the atypical PE case was associated with increased placental mitochondrial respiration (compensatory?) and augmented local oxidative stress, at least partially dependent on MAO expression.

Keywords: atypical preeclampsia, placenta, mitochondria, high-resolution respirometry, monoamine oxidase

CELL- PERMEABLE SUCCINATE RESCUES PLATELET MITOCHONDRIAL RESPIRATION IN COVID-19 PATIENTS

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The COVID-19 pandemic remains a global health issue since its outbreak. Hyperinflammation with "cytokine storm", endothelial dysfunction, and more recently, mitochondrial dysfunction were identified as main pathomechanisms. Platelet respiratory dysfunction has recently emerged as a peripheral biomarker that could mirror organ mitochondrial dysfunction. NV118, a cell-permeable succinate, is a new prodrug able to support mitochondrial function by enhancing complex II-supported respiration.

The present pilot study performed in platelets harvested from patients hospitalized for various forms of SARS-CoV2 infection was double-aimed: i) to assess the mitochondrial respiratory dysfunction; ii) to investigate whether NV118 can rescue it.

Nineteen patients with moderate (n=5) and severe (n=14) disease were included. Peripheral blood was collected and used for platelet isolation. Platelet respiration was measured at 37°C using high-resolution respirometry according to a SUIT protocol for permeabilized platelets allowing the measurement of the following respiratory parameters: routine, active, non-phosphorylating and maximal uncoupled respiration. Intact platelets (n=15) were acutely exposed to NV118 (*vs* DMSO) and routine, leak and maximal uncoupled respiration were measured.

The severe forms of disease expressed a significant decrease in platelet active respiration for both respiratory complexes and an increase of routine, leak and uncoupled respiration. Moderate forms of disease present only a significant decrease in active respiration, particularly for CI. Cell-permeable succinate elicited a significant increase in routine, leak and maximal uncoupled respiration for both mitochondrial complexes.

In conclusion, COVID-19 differentially impairs platelet bioenergetics, according to the severity of the disease, eliciting mitochondrial respiratory dysfunction that can be rescued by cell-permeable succinate.

Keywords: COVID-19, platelets, mitochondria, high-resolution respirometry, cell-permeable succinate

RELAXING EFFECTS OF IMIDAZOBENZODIAZEPINE MP-III-058 ON RAT AORTA AND TRACHEA

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The role of $GABA_A$ receptors in the periphery has become increasingly important. Considering the molecular evidence of expression of the α5 subunit of the $GABA_A$ receptor in vascular and airway smooth muscle, the relaxant potencial of MP-III-058 (methyl (R)-8-bromo-6-(2-fluorophenyl)-4-methyl-4H-benzo[f]imidazo[1,5-a][1,4]diazepine-3-carboxylate), a selective ligand with hight efficiency on α5-containing $GABA_A$ receptors has been investigated.
The isometric tissue bath system was used to test the ability of MP-III-058 to relax the isolated rat aortic and tracheal rings. The rings were precontracted with phenylephrine ($3x10^{-6}$ M) or acetylcholine (3×10^{-5} M). Additionally, the effects of ligand MP-III-058 on phenylephrine-induced contraction were studied in two concentrations (10^{-5} M and 10^{-4} M).
The maximal relaxant effects of MP-III-058 (92.88±6.82% for aortic rings (n=7) and 53.21±7.02% for tracheal rings (n=12)) were achieved at the highest concentration of 10^{-4} M, and were significantly different ($p < 0.001$) from the respective vehicle controls (15.83±4.23% (n=6) and 6.31±3.39 % (n=4)). Also, there were statistically significant differences ($p < 0.001$) in phenylephrine-induced contractions in the presence of MP-III-058, compared to the control response. At both applied concentration, ligand MP-III-058 produced a significant rightward shift and decreased the maximal contraction in the phenylephrine concentration-response curves.
The present work emphasizes the role of peripheral $GABA_A$ receptors in vascular and airway smooth muscles relaxation. However, further *in vitro* studies are required to determine preclinical relevance for MP-III-058.

Keywords: Concentration-relaxation curve; Selective α5-containing $GABA_A$ ligand; tissue bath experiments

INVESTIGATION OF THE EFFECTS OF ACUTE NICOTINE EXPOSURE AND LONG TERM SMOKING ON HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ENDOTHELIAL CELLS FROM IDENTICAL TWINS WITH DM.

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The 20% of global mortality is caused by smoke-related diseases. Human induced pluripotent stem cells (hiPSC) provide a personalised link between clinical and in vitro cardiovascular models. hiPSC lines were generated from a pair of identical twins with diabetes mellitus to test long and short-term effects of nicotine.
Mononuclear cell fractions were reprogramed into hiPSC with Yamanaka factors (Cytotune 2.0) via Sendai viral transduction. Genotype and pluripotency of the iPSC were checked. hiPSC line from non-diabetic healthy control was used as control. The hiPSC lines were differentiated into endothelial cells (ECs). hiPSC-derived ECs were treated with e-cigarette liquid (vehicle: propylene glycol PG & vegetable glycerin VG 1:1, nicotine concentration: 18 mg/ml) in EC growth medium, 1:80 (ECL18) and the same vehicle without nicotine (ECL0) or nicotine in EC growth medium (0.225 mg/ml). The cells were analysed with high content microscopy (HCS, Opera), using Hoechst, apoptotic marker caspase3/7, mitochondrial TMRM and endothelial CD31 dyes.
On the clinical side, the smoking member of the twins (“C”) was diagnosed and treated with a severe coronary disease, whilst the other (“D”) was a non-smoker and does not suffer from any coronary artery disease. C cell line showed a higher endothelial-to-mesenchymal transition rate and non-endothelial drift as compared to D line at 3 passages after FACS sorting for CD31. The toxicity of ECL18 treatment resulted in higher cell death rate in the control and in D cell line compared to C. Caspase intensity histogram became bimodal as frequencies at higher caspase intensity values arise in response to nicotine treatments, especially in ECL18 treated wells.
Our developed patient-specific in vitro hiPSC model showed alteration in endothelial phenotype associated with nicotine exposure. A scalable platform can provide further cohort level information on toxic agent like nicotine to the cardiovascular system.

Keywords: hiPSC, identical twins, smoking, nicotine, endothelial cells

WILD GARLIC ESSENTIAL OIL AS A NUTRITIONAL STRATEGY FOR COUNTERACTING DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS

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The aim of the study was to estimate the impact of two-week wild garlic (Allium ursinum) essential oil (WGO) consumption on doxorubicin (DOX)-induced cardiotoxicity in rat model.
24 male *Wistar albino* rats were randomly divided into the following groups: healthy control (CTRL), doxorubicin (DOX), and DOX+WGO. Rats were pretreated with WGO (dose of 100 mg/kg/day for 2 weeks orally, for 2 weeks) before injection of a single dose of 15 mg/kg DOX. Three days following DOX application, echocardiographic examination was performed for assessment of in vivo cardiac function. Afterwards, all rats were sacrificed, blood samples were collected and hearts were isolated and perfused on *Langendorff* apparatus for monitoring *ex vivo* myocardial function. Systemic level of pro-oxidants were determined in plasma samples, while cardiac redox status was assessed from coronary venous effluent samples. Antioxidant enzyme values were measured in erythrocyte lisate, whereas heart tissue samples were subjected to histological analysis.
Our results highlighted that DOX induced prominent depression of cardiac function, whereas WGO intake markedly recovered heart contractility and relaxation force. WGO considerably decreased level of the most of measured pro-oxidants both in blood and coronary venous effluent and was also capable of enhancing activity of antioxidant enzymes. Histological injury after DOX injection were significantly alleviated by WGO therapy.
WGO exerted promising effects in diminishing DOX-induced cardiotoxicity predominantly via strong antioxidant property. Our findings suggest WGO as a novel nutritional strategy for cardioprotection.

Keywords: wild garlic oil, doxorubicin-induced cardiotoxicity, rat

MOLECULAR INSIGHT INTO THE LATE ANTIARRHYTHMIC EFFECTS OF NITRITES

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We have evidence that the inorganic sodium nitrite provides a significant antiarrhythmic effect: nitrite given 24 hours before coronary artery (LAD) occlusion and reperfusion (I/R) ('late effect') significantly decreased the incidence of severe ventricular arrhythmias and increased survival in dogs. The sudden increase of free radicals and the impaired calcium homeostasis could be the sources of arrhythmias upon reperfusion; therefore, we have focused on the mitochondria since mitochondria plays a substantial role in the regulation of intracellular reactive oxygen species (ROS) formation and calcium level. Our previous results have revealed that sodium nitrite attenuated ROS production by altering the mitochondrial respiration, decreased the diastolic calcium level during reperfusion and prolonged the effective refracter period which might contribute to this antiarrhythmic effect.
The present study focused on the possible molecular mechanisms: our objective was to examine the role of protein S-nitrosylation in this late protection.
Dog and rat left ventricular tissue samples were collected in the sham-operated (n=3) and nitrite treated groups (at 3h, 6h, 12h and 24h (n=3/time point)). In these samples we have assessed the total protein S-nitrosylation (SNOs) by biotin switch method.
Our results have shown that the administration of sodium nitrite significantly increased the total protein SNOs at 24h in dogs and rats compared to the sham-operated control group.
Our previous and present results suggest that protein S-nitrosylation might play a role in the late antiarrhythmic effect of sodium nitrite. In the future, we aim to identify the proteins (e.g. MCU and mNCX) altered by nitrite administration.

Keywords: arrhythmia, S-nitrosylation, mitochondrial bioenergetics, biotin switch

STELLARIA MEDIA TEA MAY IMPROVE CARDIAC DYSFUNCTION IN DIABETIC RATS WITHOUT AFFECTING THE GLUCOSE HOMEOSTASIS

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Although in folk medicine common chickweed (*Stellaria media*) tea has traditionally been applied for treating diabetes, evidence is still missing. We aimed to assess the effect of *Stellaria media* tea on glucose homeostasis and cardiac function in a diabetic rat model.
Diabetes was induced by fructose-enriched diet and a single injection of low dose streptozotocin. Half of the animals received hot water extract of *Stellaria media* tea (100 mg/kg) by oral gavage. At the end of the 20-week experimental period, blood samples were collected and isolated working heart perfusions were conducted.
Increased fasting serum glucose level, diminished glucose tolerance and decreased cardiac output were in diabetic rats. *Stellaria media* tea did not affect fasting hyperglycemia or glucose intolerance; nevertheless, it attenuated diabetes-induced deterioration of cardiac performance. In association, cardiac STAT3 phosphorylation induced by diabetes was prevented by *Stellaria media* extract.
We demonstrated for the first time that *Stellaria media* tea may improve diabetic cardiac dysfunction without affecting glucose homeostasis. STAT3 signaling may be implicated in the protection of *Stellaria media* tea against diabetic cardiomyopathy.

Keywords: cardiovascular prevention, medicinal herb, diabetic co-morbidity, cardioprotection

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MiRP2 RESCUES LONG QT SYNDROME TYPE 5

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LQT5 is caused by loss of function mutations in the KCNE1 gene. KCNE1 encoding minK, a regulatory subunit of I_{Ks} channel. The minK-related peptides (MiRPs), encoded by members of the KCNE gene family, is also can modulate I_{Ks} channel function.
In this study, we aimed to investigate the effect of MiRP2 on the development of the LQT5 phenotype. KvLQT1, WT-minK, LQT5-minK variant (G52R-minK) and MiRP2 were co-expressed in different combinations. The currents were characterized by whole-cell patch clamp technique. The NanoBiT assay was applied to explore, whether MiRP2 and minK are represented in a distinct ion channel population or they co-assemble in the same ion channel complex.
Average current density was significantly lower in group 3 (KvLQT1+WT-minK+G52R-minK) compared to the group 1 (KvLQT1+WT-minK) and group 2 (KvLQT1+WT-minK+MiRP2). However, the mean current density in the presence of MiRP2 was significantly increased in group 4 (KvLQT1+WT-minK+G52R-minK+MiRP2) compared to the group 3. The KvLQT1 and minK were co-expressed with varying amount of MiRP2 for the NanoBiT experiments. The KvLQT1:minK:MiRP2 ratio was 1:2:0 (group 1), 1:2:1 (group 2) and 1:2:2 (group 3). Average relative luminescence (RLU) was 194 in group 1 which was not significantly different from group 2 (129.3 RLU). However, group 3 showed significantly lower RLU (96.7) compared to group 1.
We conclude that MiRP2 rescues the inhibitory effect of the LQT5-minK variant. Furthermore, MiRP2 is probably able to replace minK within the macromolecular complex of the I_{Ks} ion channel, therefore, MiRP2 possibly modulate the development of the LQT5 phenotype in patients.

Keywords: LQT5, slow component of the cardiac delayed rectifier potassium channel, minK- related peptide 2, NanoLuc® Binary Technology

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LIRAGLUTIDE PROVIDES PROTECTION IN AGING HEARTS

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GLP-1 agonism has several beneficial effects in the human body, including lowering body weight, improving glucose metabolism and insulin resistance, lowering blood pressure, and preventing atherosclerotic plaques, all of which appear to lead to greater protection of function and cardiovascular system. For this reason, it is frequently used in diabetes and in the treatment of insulin resistance. However, it is unknown whether GLP1agonism has cardioprotective effects in the elderly heart in insulin resistance that develops with aging. Therefore, systemic parameters of aged rats (24 months) and ionic parameters at the cellular level were investigated by treatment with the GLP-1R agonist liraglutide (LG: 4 weeks old). Action potential (AP) parameters, ionic currents and Ca^{2+}-regulation at cellular levels were studied in freshly isolated ventricular cardiomyocytes. It significantly improved increases in both SBP and DBP along with improvements in oxidant and antioxidant status. Extended AP times and depolarized membrane potentials of cardiomyocytes isolated from aged rats were normalized by LG treatment through recoveries in K^{+}-channel currents. Changes in Ca^{2+}-regulation, including leaky ryanodine receptors (RyR2), can also be ameliorated by this treatment through recoveries in $Na^{+}/Ca2^{+}$-exchanger currents. Overall, our data provide, for the first time, important insights into the direct cardioprotective effects of LG and GLP-1R agonism in the hearts of aged rats.

CAN SHORT-TERM PRECONIDITIONING WITH LEMON BALM EXTRACT PROTECT THE HEART FROM I/R INJURY?

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The role of phytochemicals as a safe and efficient cardioprotective strategy is gaining scientific attention. Thus, this study aimed to explore whether 8-day application of ethanolic *M. officinalis* extract can salvage the heart from deleterious effects of myocardial ischemia-reperfusion injury (I/R).
The research involved 32 male *Wistar* rats randomly divided into following groups (n=8): I/R- nontreated rats with I/R injury, ME50, ME100 and ME200- rats with I/R injury treated with either 50, 100 or 200 mg/kg of ME for 8 days *per os.* After accomplishing the protocol, the animals were sacrificed, the hearts were isolated and mounted on *Langendorff* apparatus to continuously monitor cardiodynamic parameters. After 20-min stabilization, we induced 20-min ischemia, followed by 30-min reperfusion. Oxidative stress parameters were determined from both coronary venous effluent (O_2^-, H_2O_2, NO_2^-, TBARS) and the samples of heart tissue homogenate (TBARS, SOD, CAT and GSH).
Lemon balm extract induced improvement of cardiac contractility via increase in dp/dt max and dp/dt min, improved SLVP and CF, with no effect on heart rate compared to I/R group. Additionally, significant reduction of prooxidants, O_2^-, H_2O_2 and index of lipid peroxidation (TBARS) was noticed, while NO_2^- was significantly increased in treated groups compared to I/R group. No significant changes on antioxidant enzymes were observed. In general, most prominent effects were noticed in ME200 group.
This study`s results suggest that even 8-day administration of lemon balm may improve cardiac function and mitigate oxidative stress in I/R conditions. Thus, this plant extract may be considered a useful cardio-preventive strategy.

Keywords: Melissa officinalis; lemon balm; Oxidative stress; isolated rat heart; I/R injury

DEVELOPMENT OF A URINE ANGIOTENSIN-CONVERTING ENZYME (ACE) ACTIVITY MEASURING TECHNIQUE

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Introduction: The expression of renal angiotensin-converting enzyme (ACE), thus the function of the local renin-angiotensin aldosterone system can be altered in hypertension and various kidney diseases. ACE is also excreted in the urine, but its activity cannot be measured due to the presence of endogenous inhibitors, so its role as a biomarker is less studied and known in cardiovascular and renal diseases.
Aim: The aim was to identify endogenous inhibitors that affect the measurement of urinary ACE activity and to develop a new measurement method that could help to clarify the role of urinary ACE as a biomarker.
Methods: ACE activity of first morning urine samples was measured by fluorescent kinetic assay after filtration (5, 10, 30 kDa pore size) or appropriate sample dilution (3-1000-fold). Ethanol precipitation and RP-HPLC separation were used for separation and identification of endogenous inhibitors.
Results: ACE activity in native urine samples from healthy individuals cannot be measured; behind this 3 endogenous, reversible ACE inhibitory compounds were isolated. By diluting urine at least 70-fold, endogenous inhibitory activity can be significantly reduced, which technique has been proved to be remarkably faster and more reproducible than conventional mechanical purification using a 10kDa pore size filter. Uric acid reduces urinary ACE activity by 25-40% (IC_{50}= 7mM) in the physiological range (1.48-4.43mM), while urea is responsible for 8-23% of endogenous inhibition (IC_{50}= 848mM) under physiological conditions (100-300mM). The third inhibitor, a non-ionic compound of less than 5kDa, is under further investigation. Under pathological conditions (>30µM), the ACE inhibitory effect of the urobilinogen also becomes pronounced (>10%).
Conclusion: Significant steps have been taken to develop a new method to measure urinary ACE activity, which can be used to routinely determine this parameter. Using our method, the role of urinary ACE activity as a potential biomarker can be better elucidated.

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VENO-VENOUS ECMO-INDUCED KIDNEY INJURY – DEVELOPMENT OF A LARGE ANIMAL MODEL

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Veno-venous extracorporeal membrane oxygenation (vvECMO) is a life-saving intervention in severe respiratory distress. Our aim was to establish a large animal model to study the pathomechanism of complications and the development of vvECMO-induced acute kidney injury (AKI) in a clinically relevant time frame.
The experiments were performed on anaesthetised minipigs. In group 1 (n=6) 24-hr vvECMO was started after l.a. cannulation, followed by a 6-hr post-ECMO period. In the control group (n=6) the animals were observed for 30 hr, without ECMO initiation. Haemodynamics were recorded, renal artery flow (RAF) was measured post-ECMO with ultrasound flowmeter. At the end of the experiments, renal biopsies were taken for histopathological examination, mitochondrial functional measurements (by high-resolution respirometry), and detection of myeloperoxidase and xanthine-oxidoreductase activities. Plasma and urine samples were collected for neutrophil gelatinase-associated lipocalin (NGAL) determination.
RAF was decreased in post-ECMO period (96.3±21 vs 223.6±32 ml/min) and hourly diuresis was lower compared to the controls (3.25±0.4 vs 4.83±0.6 ml/h/kg). Kidney histology demonstrated ischemic structural lesions. In the vvECMO group, urine (4.24±0.25 vs 2.57±0.26 ng/ml) and plasma (4.67±0.1 vs 3.22±0.2 ng/ml) NGAL levels, xanthine-oxidoreductase (5.88±0.8 vs 2.57±0.2 pmol/min/mg protein) and myeloperoxidase (11.93±2.5 vs 4.34±0.6 mU/mg protein) activity were increased. A decrease in complex I-dependent oxidative capacity (174.93±12.7 vs. 249±30.07 pmol/s/ml) indicated mitochondrial dysfunction.
This 30-hrs experimental protocol provided clear evidence for significantly impaired renal function with explicit signs of structural damage and mitochondrial dysfunction. The established large animal model offers basis for studying the pathomechanism, biomarker combinations or potential therapeutic options for vvECMO-linked AKI.
Keywords: veno-venous ECMO, acute kidney injury, mitochondrial function, experimental model, inflammation

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INVESTIGATION OF THE EFFECT OF PREIMPLANTATION FACTOR IN A DOXORUBICIN-INDUCED *IN VITRO* CARDIOCITOTOXICITY MODEL

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Introduction: Nowadays, increasing incidence of cancer is a major problem worldwide. Doxorubicin (DOXO) is an effective chemotherapeutic agent used in order to kill the malignant tumor cells. However, application of DOXO has limits because of the severe consequences that mainly affect the cardiovascular system. Therefore, the investigation of the detailed underlying mechanisms and discovery or development of potential drugs that can help to avoid or decrease the DOXO-induced cardiovascular side effects has a huge clinical potential. Our group focus on the examination of endogenous molecules which can act as potential cytoprotective agents on DOXO-induced cytotoxicity. Preimplantation factor (PIF) is a 15-aminoacid peptide which is in correlation with the viability of pregnancies. PIF has been reported to exert antioxidant and antiapoptotic qualities, so it could be used as a potentially protective molecule againts DOXO-induced cardiocitotoxicity.
Aim of the study: To investigate the possible cytoprotective effects of PIF in a doxorubicin-induced *in vitro* model of heart damage.
Materials and methods: We used H9c2 rat cardiomyoblast cell line to test 0.5 and 24 hours pretreatment with a broad range of PIF (0.3-5000 ng/mL) followed by 24 hours of parallel DOXO and PIF treatment. To detect the viability at the end of the protocol, MTT viability assay and measurement of superoxide level with dihydroetidium assay were performed. DAPI staining was applied in order to visualize the apoptotic morphological changes and γ-H2AX immuncytochemistry was used for examining the DNA double strand breaks.
Results: Thirty min pretreatment with 160 ng/ml PIF significantly reduced the DOXO-induced cell death and oxidative stress (cell death in DOXO vehicle%: 80±3%). In our longer pretreatment model the 10 ng/ml PIF decreased the elevated cell death and oxidative stress caused by DOXO (cell death in DOXO-vehicle %: 73±7%). We detected higher number of apoptotic nuclei and DNA double breaks in the DOXO treated group, which was improved by both of the PIF pretreatments.
Conclusion: PIF may decrease dose-dependently the doxorubicin-induced cardiac cell damage including cell viability, superoxide level and DNA damages. In the future, we plan to investigate the possible intracellular mechanisms behind this protective effect.

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UTILIZING SMALL RNA AND PROTEIN EXPRESSION PROFILING IN THE DETECTION OF PERSONALIZED REGENERATIVE CAPACITIES

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Physical training is a challenge to the whole-body homeostasis, preferably leading to improvements in performance and health. Responses in our systems to exercise load are undeniably complex, and influenced by environmental and genetic factors. A critical element for repeated physical performance is our capability for rapid regeneration, most likely individual specific. In this study, our goal was to reveal novel small molecules, such as micro RNAs, capable of enhancing positive physical regeneration in males.
In our screen, we collected plasma samples from three willfully volunteered subjects, who participated in a 40 km running competition. Specimens were collected prior to, directly after, one-hour after balneotherapy and one day following physical stress. Whole mRNA was isolated and miRNA expression profile was determined using NG sequencing. Protein alterations were detected via 2D gel electrophoresis. Fold changes between certain conditions were calculated by statistical testing.
Our results revealed significant alterations regarding numerous miRNA and miR-related protein targets of various biological processes and molecular functions prior to and following exercise, including resting. miRNA heat map analyses clearly demonstrated, significant initial age-related miRNA differences among the volunteers became equalized one day following the physical load. Moreover, our data revealed, one hour of balneotherapy following physical exercise may prove beneficial in enhancing regeneration.
Our investigations suggest utilizing miRNA profiling prior to and following physical load may reveal novel marker molecules, which sensitively detect and state the physiological conditions and regenerative capacities of our body in a uniquely personalized fashion.

Keywords: Physical exercise, miRNA profiling, Regeneration, Personalized training

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EFFECTS OF QUERCETIN ON SELECTED CARDIOVASCULAR PARAMETERS AND ISCHEMIA-REPERFUSION INJURY OF THE MYOCARDIUM IN AGED RATS

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Quercetin (QCT) is a polyphenolic compound that has been studied for its cardioprotective potential. In previous studies QCT exerted cardioprotective effects on ischemia-reperfusion (I/R) injury in healthy young animals without associated comorbidities. The aim of the current study was to reveal potential beneficial cardioprotective effect of QCT on aged rats.
QCT (20 mg/kg/day, 6 weeks) was administered 24-months old Wistar rats. Blood pressure was measured by tail-cuff pletysmography before the start and at the end of QCT administration. Isolated perfused hearts were exposed to global I/R (30/120min) followed by evaluation of infarct size using TTC staining method. Molecular mechanisms of QCT effects in the heart were analyzed in left ventricles by Western Blot monitoring protein expression of RISK (Reperfusion Injury Salvage Kinases) signaling pathway and markers of apoptosis (Bax/Bcl-2) and antioxidative enzymes (SOD1, SOD2).
Our results showed, that QCT had no effect neither on biometric and biochemical parameters, nor on blood pressure. QCT exerted no cardioprotective effect against I/R and even worsened the trend of post-ischemic recovery of heart function. QCT impaired antioxidant capacity by causing a significant increase in FRAP and AOPP parameters, but had no effect on expression of antioxidative enzymes. QCT did not induce global activation of the RISK pathway, moreover decreased PKC-ε expression.
The effect of QCT as an antioxidant in aged animal model appears to be a rather negative, but its anti-apoptotic properties were promoted. These results are significant in terms of studying the response to QCT as a cardioprotective agent in older individuals.

Keywords: quercetin, myocardium, aging, antioxidant, apoptosis

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CYTOPROTECTIVE EFFECT OF KYNURENIC ACID INVOLVES THE MODULATION OF APOPTOTIC PATHWAYS AGAINST SIMULATED ISCHEMIA/REOXYGENATION INJURY OF CARDIAC CELLS

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Acute myocardial infarction (AMI) is a leading cause of disability and death worldwide, which is caused by multifactorial damage of cardiac cells. Until now, the only therapeutic approach to minimalize the cardiac injury is reperfusion therapy, which is necessary for survival. However, it might initiate further cardiac damage, therefore the analysis of molecules/processes, which potentially increase the tolerance of cardiac cells against ischemic injury have a great therapeutic potential in the field of experimental cardiology. In this study, we aimed to investigate the underlying mechanisms of the barely known and previously uncovered cytoprotective effect of kynurenic acid (KYNA) against simulated ischemia/reoxygenation (SI/R)-induced injury of H9c2 cardiac cells, focusing on apoptosis-related intracellular events. To investigate the anti-apoptotic effects of KYNA, H9c2 cardiac cells were subjected to 6 hours of simulated ischemia and 2 hours of reoxygenation (SI/R) in the presence or absence of 64 µM KYNA, and several markers of apoptosis (membrane blebbing, cell viability, cellular morphology, expression of apoptotic proteins) were assessed using light microscopy, confocal microscopy, western blots, immunocytochemistry and biochemical assays. According to our results, SI/R caused worsening of irreversible membrane blebbing and increased apoptosis related DNA damages (e.g. micronuclei formation, chromatin condensation, frequency of DNA double strand breaks), which outcomes were attenuated by administration of KYNA. The SI/R-induced increase of proapoptotic and decrease of antiapoptotic protein expressions were reverted by KYNA. In concordance with these findings, the activation of effector caspases (caspase-3, -7) increased upon SI/R, which effect was diminished by the applied KYNA treatment, suggesting potential anti-apoptotic processes mediated by KYNA. These data suggest that KYNA exerts its cytoprotective effect against SI/R-induced cardiac cell injury via modulation of apoptotic processes.

Keywords: kynurenines, tryptophan metabolite, programmed cell death, cellular damage, cardiomyocytes

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Kv1.3 POTASSIUM CHANNELS INVOLVED IN THE ANTIHYPERTENSIVE EFFECTS OF RESVERATROL

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Vascular diseases such as hypertension, atherosclerosis, pulmonary hypertension and diabetes mellitus (DM) are commonly associated with functional changes of vascular smooth muscle cells (VSMC) mostly caused by decreased function of certain potassium (K) channels. VSMCs Kv1.3 channels, on contrary, tend to be upregulated, which can contribute to phenotypic modulation (PM) and unwanted vascular remodelling. Besides, Kv1.3 channels represent potential target for macrophage-dependent endothelial dysfunction in angiotensin II-induced hypertension. Resveratrol (RSV), for decades main research object in the fields of cardioprotection, oncology and endocrinology, represents specific drug which useful features are exerted predominantly through modulation of different ion channels, including Kv1.3. This might explain important pharmacological actions attributed to RSV, such as reducing blood pressure. In a clinical setting, addition of RSV to standard antihypertensive therapy was sufficient to reduce blood pressure without the need for additional antihypertensive drugs. Further, meta-analysis have confirmed its antihypertensive efficacy, mostly when used in high doses (>300 mg/day) and in diabetic patients. This is due to upregulation of endothelial NO and, in case of DM, improving insulin sensitivity and acting on VSMCs ion channels, probably Kv1.3. Additionally, in a smooth muscle cells of human umbilical vein, altered expression of Kv1.3 in a model of pregnancy-induced hypertension (PIH) might contribute to adverse pregnancy outcomes. Also, inhibition of Kv1.3 in human lymphocyte might suppress inflammation, contributive factor in all of the above mentioned diseases. All together, it is important to investigate the precise mechanism of RSV modulation of Kv1.3 and the impact of that synergy in other models of VSMSc, such as bypass grafts of diabetic patients.

Keywords: resveratrol, hypertension, vascular smooth muscle cell, Kv1.3, cardioprotection.

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CARDIAC CHANGES AND DECREASED CARDIAC EXPRESSION OF FOXP2 MEDIATED BY DIET-INDUCED MODERATE OBESITY CANNOT BE AFFECTED BY MAO-B INHIBITION.

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Obesity is a major risk factor for the development of cardiovascular diseases, which is associated with oxidative stress and chronic inflammation. Monoamine oxidases (MAOs) are major sources of mitochondrial reactive oxygen species. We have shown previously that MAO-B selective inhibitor selegiline reduces visceral adiposity in obesity, however, it has not been assessed if selegiline can alleviate cardiac oxidative stress. Therefore, we investigated the effects of selegiline on cardiac redox homeostasis and cellular damage in a high-fat high-sucrose diet (HFD)-induced obesity model of rat. We demonstrate that specific MAO-B inhibition by selegiline reduces cardiac mitochondrial ROS production in healthy, but not in HFD obese rats. Although HFD did not affect pro-survival, pro-death and oxidative stress-related mechanisms, it decreased sequestosome-1 level and B-cell lymphoma 2-associated X protein/B-cell lymphoma 2 (Bax/Bcl-2) ratio, and increased TNF and NF-kB expressions. Selegiline did not affect any of these HFD-induced alterations. Simulated hypercholesterolemic treatment disrupted mitophagy in H9c2 cardiomyocytes which was not restored by selegiline. Both SERCA2a and its upstream modulators were affected by HFD and selegiline, however it did not manifest in altered cytosolic calcium dynamics. In addition, we identified a previously unknown cardiac signaling molecule, forkhead box P2 gene (Foxp2), which was decreased in obesity, but not restored by selegiline. In conclusion, MAO-B inhibition is of no significant therapeutic value to alleviate cardiac consequences of obesity.

Keywords: cardioprotection, obesity, MAO-B, selegiline

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

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Current therapies of congenital long QT syndrome (LQTS) fail to prevent arrhythmic events in up to 40% of the patients. Docosahexaenoic acid (DHA), a polyunsaturated fatty acid activates the repolarizing I_{Ks} current if both α- (KvLQT1) and β (KCNE1) –subunits to I_{Ks} are functionally intact.
The potential beneficial (repolarisation-normalizing) effects of DHA in transgenic LQT1 (KCNQ1-Y315S, loss of I_{Ks}), LQT2 (HERG-G628S, loss of I_{Kr}), LQT5 (KCNE1-G52R, decreased I_{Ks}) and LQT2-5 (loss of I_{Kr}/decreased I_{Ks}) rabbits were investigated.
In vivo telemetric ECG analyses in wild-type (WT), LQT1, LQT2, LQT5 and LQT2-5 rabbits were performed at baseline and after 10μM/kg DHA i.m. to assess changes in heart rate corrected QT (QTc) and short term variability of QT (STV_{QT}). *Ex vivo* monophasic action potential measurements in Langendorff-perfused hearts were carried out to investigate DHA-induced (20μM) changes in action potential duration (APD_{75}) and action potential (AP) triangulation (APD_{90}-APD_{30}).
Baseline QTc (ms±SEM) was significantly longer in LQT1, LQT2 and LQT2-5 than in WT (166±3.8, 165±3.7, and 167±12.1 vs. 144±14.3; $p<0.05$). Baseline STV_{QT} (ms±SEM) was increased only in LQT2. *In vivo*, DHA shortened QTc through activation of I_{Ks} only in WT (-12.0±1.9, $p<0.01$) and in LQT2 (-20.7±1.7, $p<0.01$). Furthermore, in LQT2, DHA normalized STV_{QT}. Similarly, *ex vivo*, DHA shortened APD_{75} (ms±SEM) only in WT and in LQT2 (-12.3±2.2 and -18.1±3.5, $p<0.01$). Moreover, AP triangulation was decreased by DHA in LQT2 (-5.8±1.8, $p<0.01$). Importantly, DHA didn't increase the spatial dispersion of repolarisation (QT and APD_{75} dispersion).
DHA exerts a beneficial repolarisation-normalizing effect through activation of I_{Ks} in LQT2 with intact α- and β-subunits to I_{Ks}. DHA could thus represent a novel therapeutic tool in LQT2 syndrome.

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

ANALYSIS OF NECROPTOSIS AND AUTOPHAGY SIGNALLING IN ACUTE MYOCARDIAL ISCHEMIA/REPERFUSION INJURY: A ROLE OF RIP3

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RIP3 is a core mediator of necroptotic cell loss which has been shown to underlie some phenotypes of myocardial ischemia/reperfusion (IR) injury. However, the extent to which necroptosis contributes to such damage under short reperfusion remains elusive. Thus, under conditions of acute myocardial IR we provided a comprehensive analysis of necroptotic and autophagic signalling as there are indications for their interplay. Langendorff-perfused rat hearts subjected to 30-min ischemia and 10-min reperfusion exhibited impaired cardiac function which was not ameliorated by RIP3 inhibition. Immunoblotting analysis revealed that the detrimental effects of IR were unlikely mediated by necroptosis, since neither the canonical RIP3–MLKL nor the non-canonical CaMKIIδ–mPTP and PGAM5–Drp1 pathways were activated. Although the signalling involved in autophagy inhibition was unaffected, autophagy activation was suppressed by IR as evidenced by decreased expression of Beclin-1, pSer555-ULK1, pSer555-ULK1/ULK1 ratio, and LC3-II/LC3-I ratio. RIP3 inhibition prevented the IR-induced plasma membrane rupture and delayed mPTP opening which was associated with modulation of XO and MnSOD. Additionally, LC3-II expression in IR hearts was suppressed by RIP3 inhibition, indicating some effect on autophagosome processing. Conclusively, this is the first study suggesting that early reperfusion of previously ischemic heart is not associated with execution of necroptosis. Furthermore, we showed that RIP3 very likely underlies this cardiac damage via the modulation of oxidative stress- and mitochondrial function, rather than promoting cell loss due to necroptosis. Lastly, the relationship between necroptosis and autophagy under such acute IR settings is unlikely, apart from the potential autophagosome regulation.

Keywords: necroptosis, autophagy, ischemia/reperfusion injury, oxidative stress

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THE HIDDEN ELECTROPHYSIOLOGICAL CARDIOTOXIC EFFECTS OF ROFECOXIB ON RABBIT VENTRICULAR PREPARATIONS

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Unexpected ischaemia-induced cardiac adverse events are major contributors to clinical trial discontinuation and drug attrition. However, the proarrhythmic effect of drug candidates is exclusively studied in healthy cells, tissues, or in healthy experimental animals. Thus, the aim is to develop a sensitized animal model that can reliably screen for the arrhythmogenic effects of a compound. Here we show that the selective COX- 2 inhibitor rofecoxib possesses cardiac electrophysiological adverse effects only revealed during ischaemia/reperfusion, a phenomenon we termed “hidden cardiotoxicity”. Our group has previously reported the hidden cardiotoxic effect of rofecoxib on rat ventricular preparations. Given the significant differences in cardiac electrophysiological properties between rats and humans, the human extrapolation of arrhythmological results obtained from rats is limited, so our aim was to investigate the proarrhythmic effect of rofecoxib in a sensitized rabbit model.
Action potentials were registered from rabbit right ventricular papillary muscles using the conventional microelectrode technique and the effects of 10 μM rofecoxib upon test ischaemia and reperfusion were investigated.
Rofecoxib (10 μM) did not alter electrophysiological parameters in normoxic conditions. However, following 30 minute ischaemia the APD_{90} was significantly decreased during reperfusion compared to APD_{90} in the vehicle-treated group. Following sI/R, a decrease in impulse conduction velocity was also measured in the rofecoxib- treated group, but the differences were not statistically significant.
Under pathological conditions, rofecoxib may increase the incidence of reperfusion arrhythmias. Consequently, the sensitized rabbit model may be suitable for investigating the "hidden cardiotoxic" effect of a drug candidate compound. Significant differences were observed in the effect of rofecoxib on repolarization between the rat and rabbit models. However, due to the well- known electrophysiological differences between the two species, the human relevance of the results obtained in rabbits is more reliable.

Keywords: hidden cardiotoxicity, proarrhythmia, ischaemia/reperfusion

INSIGHT INTO PATHOGENESIS OF SEVERE MITRAL VALVE REGURGITATION IN A PATIENT WITH OBSTRUCTIVE HYPERTROPHIC CARDIOMYOPATHY

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Hypertrophic obstructive cardiomyopathy (HOCM) is one of the most frequent hereditary heart diseases. The severely hypertrophied interventricular septum, together with the systolic anterior movement (SAM) of the mitral valve (MV), frequently cause a significant pressure gradient in the left ventricular outflow tract, as well as varying degrees of mitral regurgitation (MR). We report the case of a 64 years-old female patient, diagnosed with HOCM two years ago, admitted to our clinic with dyspnea with low intensity activity and fatigue. Transthoracic echocardiography showed a concentric, asymmetrical left ventricular hypertrophy, an elongated anterior mitral leaflet (AML), with a significant SAM inducing a severe regurgitation. Fibrosis was observed on both leaflets with calcification of the posterior mitral ring. Shear stress on the mitral valve apparatus in this process may activate molecular mechanisms that cause early valvular tissue degeneration, altering mitral valve function. Monoamine oxidase (MAO) with 2 isoforms has emerged as an important cardiovascular source of reactive oxygen species (ROS), inducing fibrosis, but data about its expression in valvular tissue is scarce. In this respect, we assessed ROS production and MAO A and B expression in a sample of diseased MV harvested during surgery. We found an increased production of ROS and MAO expression which was further augmented after *ex vivo* incubation with AII and was alleviated in the presence of MAO-A and B inhibitors. In conclusion, MAO-related oxidative stress may play a role in the pathogenesis of mitral regurgitation in patients with HOCM.

Keywords: hypertrophic obstructive cardiomyopathy, mitral regurgitation, oxidative stress, monoamine oxidase

VITAMIN B6 SLIGHTLY AFFECT CARDIAC OXIDATIVE STRESS AND HISTOLOGICAL MARKERS IN MONOCROTALINE-INDUCED RAT HEART FAILURE

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Heart failure (HF) induced by monocrotaline (MCT) is well-known by the pulmonary arterial vessels remodeling mechanisms with increased pulmonary resistance and oxidative stress markers. The purpose of this study was to validate the hypothesis that four week treatment with vitamin B6 could affect HF by modulating oxidative stress biomarkers, and structure of the rat heart. The male Wistar albino rats were divided in 3 investigated groups: blank solution-exposed control (C, physiological saline 1 ml/kg 28 days ip., n=8), B6 (vitamin B6 7 mg/kg/day 28 days ip., n=8), and MCT+B6 (MCT 50 mg/kg once ip. plus vitamin B6 7 mg/kg/day 28 days ip., n=8). Activities of enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX), glutathione content, parameters of oxidative damage of proteins, thiol- and carbonyl groups, nitrotyrosine content, and total S-glutathionylation, as well as histomorphometric and certain immunohistochemical parameters of rat cardiac tissue were determined. The activity of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPX) did not change, whereas the total glutathione (GSH) was significantly decreased in the MCT+B6 group. This was followed by slightly decreased level of the total glutathionylation observed in the MCT+B6 group. The parameters of protein oxidative damage (reactive carbonyl derivates, thiol groups and nitrotyrosine) did not significantly change in the MCT+B6 group. There was observed an increasing trend in right ventricle and left ventricle wall thickness in the MCT+B6 compared with C and B6 groups as well as in immunohistochemical markers of cell proliferation (Ki67 and PCNA) positivity.

Keywords: Heart failure, Monocrotaline, Vitamin B6, Oxidative stress, Rat

DIALLYL TRISULFIDE ATTENUATES DOXORUBICIN-CARDIOTOXICITY IN RATS

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Diallyl trisulfide (DATS) is a natural donor of hydrogen sulfide isolated from garlic. The aim of the study was to examine the effects of DATS against doxorubicin (DOX)-induced cardiotoxicity in rats.
Thirty rats were divided into three groups: CTRL (healthy untreated rats, n=10), DOX (rats injected with a single dose of doxorubicin 15 mg/kg ip on the 14th day of experiment, n=10), DOX+DATS (rats treated with 16 mg/kg DATS per day during the experiment and 15 mg/kg doxorubicin ip on the 14th day). Three days after DOX-treatment rats were sacrificed, in vivo hemodynamic was measured by echocardiography, while ex vivo cardiodynamic parameters of isolated rat hearts were monitored on Langendorff apparatus. Systemic oxidative stress parameters were determined in blood and histopathological examination of the heart was performed.
DATS treatment led to a minor increase in the ejection fraction in the DOX group, while the levels of free radicals were significantly decreased. Histopathological examination corroborated these findings by demonstrating significant and severe structural injury in the cardiac tissue of DOX rats.
Our study demonstrated that DATS can be an important cardioprotective agent against doxorubicin-cardiotoxicity through modulation of oxidative stress and the possibility to improve myocardial performance and morphometric structure of rats` hearts.

Keywords: Cardioprotective agents; Cardiotoxic agents; Pharmaceutical potential; Oxidative stress

LIRAGLUTIDE PRETREATMENT SIGNIFICANTLY ATTENUATES ISOPRENALINE-INDUCED MYOCARDIAL INJURY IN RATS

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Liraglutide (LIR) is an agonist of the glucagon-like peptide-1 receptor (GLP-1). Besides its primary effect in glucoregulation, LIR has been shown to have cardioprotective, antioxidative and antiinflammatory properties. The aim of the study was to investigate the effects of LIR in the isoprenaline (ISO) model of myocardial injury (MI).
ISO-induced MI in Wistar rats was induced by two subcutaneous applications of ISO 85 mg/kg of body weight on two consecutive days, 24 h apart. The experimental animals were divided into 4 groups: control (C) group (receiving saline for 10 days + saline on days 9 and 10), I group (saline for 10 days + ISO on days 9 and 10), L group (LIR for 10 days + saline on days 9 and 10) and L+I group (LIR for 10 days + ISO on days 9 and 10). The parameters of MI and oxidative stress were evaluated histologically, immunohistochemically and biochemically.
Pre-treatment with LIR significantly attenuated cardiotoxicity and oxidative stress markers induced by ISO. The histopathological findings showed significant level of MI after exposure to ISO that was significantly reduced in the group pretreated with LIR. Decreased expression of cleaved caspase-3 was also found in this group, as well as the decreased concentration of high-sensitive troponin I (hsTnI) which was reduced up to 3.35 times.
The results of the present study suggest that pretreatment with LIR significantly attenuates ISO-induced MI in rats.

Keywords: Myocardial injury, liraglutide, cardioprotection

POSTER PRESENTATIONS

Poster Session II

September 30, 2022

16.15-18-30

METHANE-ENRICHED CUSTODIOL PRESERVATION SOLUTION IMPROVES GRAFT FUNCTION IN EXPERIMENTAL MODEL OF HETEROTOPIC HEART TRANSPLANTATION

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The key goal of cold storage is to maintain cell viability for a prolonged time during solid organ transplantation. Methane (CH_4) has been recognized as novel therapeutic gas exerting anti-inflammatory effects in ischemia-reperfusion (IR) injuries. We aimed to investigate whether cold storage of donor hearts in CH_4-enriched Custodiol preservation solution could protect against IR and preserve myocardial function in a rat model of heterotopic heart transplantation (HTX).
The hearts of donor Lewis rats were stored for 60 minutes in cold histidine-tryptophanketoglutarate (Custodiol [CS]) or CH4-saturated CS solution (CS-CH4) (n = 12 each). Standard heterotopic HTX was performed, and 60 minutes later, the left ventricular (LV) pressure-volume relationships LV systolic pressure (LVSP), systolic pressure increment (dP/dtmax), diastolic pressure decrement, and coronary blood flow (CBF) were measured. Tissue samples were taken to detect proinflammatory parameters, structural damage (by light microscopy), endoplasmic reticulum (ER) stress, and apoptosis markers, whereas mitochondrial functional changes were analyzed by high-resolution respirometry.
LV contractility, active relaxation and CBF values were significantly ($p < 0.05$) improved in CS-CH_4 grafts as compared to the CS group. CS-CH_4 storage significantly reduced the transcription of pro-apoptotic proteins and Bcl2/Bax ratios as compared to CS grafts. Increased mitochondrial oxidative phosphorylation, reduced leak respiration and cytochrome c release were demonstrated in response to CS-CH_4 preservation.
The addition of CH4 during 1 hour of cold storage improved early in vitro graft function and reduced mitochondrial dysfunction and activation of inflammation. Evidence shows that CH4 reduced ER stress−linked proapoptotic signaling.

Keywords: Cardioprotection, Heart transplantation, experimental cardiac surgery, ischemia/reperfusion injury, graft preservation

THE EFFECT OF REMOTE ISCHEMIC PRECONDITIONING ON THE RESISTANCE OF THE HEART AGAINST ISCHEMIA-REPERFUSION INJURY IN AGING RATS. STUDY OF MOLECULAR MECHANISMS

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The effect of age on reduced tolerance to ischemia-reperfusion (I/R) injury and adaptative mechanisms has been demonstrated in several studies in human and animal hearts. One of the most studied forms of cardioprotection is remote-ischemic preconditioning (RIPC), mainly for its possible clinical use. Positive effect of RIPC has already been found in elderly patients. However, little is known about its effect and molecular basis in elderly animals.

Our work focuses on clarifying the effect of RIPC on the resistance of heart against I/R injury and identifying proteins involved in protective pathways in aging 13 months old rats. In Langendorff-perfused hearts exposed to 30-min I/120-min R without or with prior RIPC. RIPC (3 cycles, 5-min I/5-min R) was applied on the hind limb of anesthetized rats. We measured infarct size (IS), susceptibility to ventricular arrhythmias and recovery of contractile function (LVDP). In parallel groups, LV tissue was sampled for the detection of protein levels of RISK and pro/anti-apoptotic pathways.

Application of RIPC caused decrease in myocardial IS and LVDP recovery was also improved in RIPC group after I/R. Positive effect of RIPC was associated with increased phosphorylation of GSK3β and expression of eNOS, and apoptotic activity of myocardial cells was decreased (Bax/Bcl-2). However, increasing age is likely to have caused a premature increase in Akt phosphorylation. As a result, RIPC provided protection of the heart against I/R injury in 13 months old rats. Therefore, even at this age, RIPC appears to be still an effective and clinically easy-to-use form of cardioprotection.

Keywords: remote preconditioning, aging, molecular mechanisms

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POTENTIAL PROTECTIVE ROLE OF *XANTHOPARMELIA STENOPHYLLA* LICHEN ACETONIC EXTRACT IN DOXORUBICIN-INDUCED CARDIOTOXICITY IN RATS

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Lichens are a unique symbiotic community of fungi (mycobiont) and algae (photobiont). Although lichens are used in traditional medicine and the effects of some of the secondary metabolites have been studied, there is insufficient data on the cardioprotective effects of lichens. The aim of this study was to evaluate the effect of acetonic extract of *Xanthoparmelia stenophylla* lichen on doxorubicin-induced cardiotoxicity in rats.
The lichen sample was collected on Stara Planina. Acetone extract of lichen *Xanthoparmelia stenophylla* (XSA) was prepared by cold maceration. The study was conducted on 40 male Wistar albino rats. The extract was administered orally at a dose of 125 mg/kg for 28 days. After 28 days, doxorubicin was administered intraperitoneally at a cumulative dose of 15 mg/kg. Three days after doxorubicin administration, hearts were isolated and subjected to *ex vivo* examination on a Langendorff apparatus. Blood and coronary venous effluent samples were also collected in order to determine the markers of oxidative stress by spectrophotometric method.
Administration of XSA at a dose of 125 mg/kg for 28 days leads to the preservation of cardiac function in a model of doxorubicin-induced cardiotoxicity. Also, a reduction in cardiac oxidative stress can be observed in treated animals compared to the animals not treated with XSA.
Our results showed that XSA exhibits cardioprotective and antioxidant activity, which indicates the potential that XSA or some of its ingredients can potentially be used as cardioprotective agents.

Keywords: *Xanthoparmelia stenophylla*; Lichen, Doxorubicin; Heart; Oxidative stress; Rats.

ABT-333 (DASABUVIR) PROLONGS THE ACTION POTENTIAL OF CANINE LEFT VENTRICULAR CELLS

Zsigmond Máté Kovács[1,2], Csaba Bálint Dienes[1,2], József Óvári[1,2], János Magyar[1,5], Tamás Bányász[1], Péter P. Nánási[1,3], Balázs Horváth[1,6], Norbert Szentandrássy[1,4]

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ABT-333 is an antiviral agent used against hepatitis C. The molecule, similarly to several IKr inhibitors, has a methanesulfonamide group. The arrhythmia inducing effect of ABT-333 has been previously reported. In clinical practice, ABT-333 produced this property when its plasma concentration was increased due to inhibition of its degrading enzyme.
Our goal was to investigate the acute effects of ABT-333 on the cells enzymatically isolated from canine heart left ventricles, which is a good electrophysiological model of the human heart.
Action potentials were recorded using a sharp microelectrode technique. We first applied ABT-333 in 1 μM for 15 min, followed by a 20 min washout. In further experiments we used increasing concentrations (1, 3, 10 and 30 μM, 5-5 min) in a cumulative manner.
1 μM ABT-333 reversibly increased AP duration with approximately 8%. When used in increasing concentrations, the drug also increased the action potential duration in a dose-dependent and reversible manner. The elongation was 7, 21, 37, and 50%, respectively. In addition, early afterdepolarizations occurred in some cells in the presence of higher ABT-333 concentrations (10 and 30 μM). ABT-333 reduced the maximal rate of the early repolarization phase of the action potential as well, but this effect was only partially reversible.
In light of our results, it is likely that the effect of ABT-333 on action potential is achieved primarily through the inhibition of potassium currents, mainly IKr. Slowing down early repolarization is likely due to the inhibition of transient outward potassium current.

Keywords: ABT-333, Dasabuvir, action-potential, canine, cardiomyocyte

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A NOVEL TKS4-KO HUMAN STEM CELL-BASED FTHS MODEL SYSTEM

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Frank-Ter Haar syndrome (FTHS) is a rare inherited developmental disease caused by the mutation of the tyrosine kinase substrate with four SH3 domains (Tks4) scaffold protein gene (SH3PXD2b). FTHS leads to cardiovascular disorders, facial and skeletal abnormalities, decreased adiposity and developmental delay in patients. Pluripotent stem cells (PSCs) can be used as in vitro human disease models. This cell type can be maintained in culture under laboratory conditions for an unlimited period of time, the resulting cell lines are able to differentiate into any cell type and show a more accurate match to human tissue than animal models. In this study, human PSCs were used for the modeling of this genetic disease to study the effect of the absence of the Tks4 protein in different cell types created by using CRISPR/Cas9 system to knock out the SH3PXD2b gene, resulting in homo- and heterozygous Tks4-KO HUES9 human embryonic stem cell lines. Gene knockout caused no change in pluripotency, therefore other cell types relevant to FTHS were examined. The differentiation of HUES9 cell lines into mesenchymal stem cells (MSCs), furthermore MSC-derived adipocytes and osteocytes, as well as future cardiomyocyte differentiation gives an opportunity to examine the influence of the absence of the Tks4 protein on cell lineage differentiation and maturation.

Keywords: Frank-Ter Haar syndrome; Tks4; human pluripotent stem cells; mesenchymal stem cells, cardiomyocytes.

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FRONTIERS IN VASCULAR DEVELOPMENT: THE POTENTIAL ROLE OF THYMOSIN BETA-4 IN COCHLEAR VASCULOGENESIS

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The auditory system is an imperious sensory organ possessing a significant role in communication and three-dimensional orientation. Based on the WHO's data, 5% of the world's population suffer from hearing loss. To achieve proper auditory regeneration in adults, it is crucial to understand the development and the molecular role players of the ear. In this study we investigated the relevance of vascularization and the molecular regulatory components of embryonic inner ear development in mice. E10.0-E14.0 C57BL6 mouse embryos were harvested in 4% PFA. In addition to performing in situ hybridization utilizing Thymosin beta-4 (TB4) specific mRNA probes, samples were equally processed to acquire paraffin embedded sections. To investigate morphology, half of the slides were stained with hematoxylin-eosin. The remaining sections were processed for immunohistochemistry against TB4. Signals were detected via confocal microscopy and analyzed using ImageJ software.
In situ hybridization and immunodetection of TB4 indicated significant presence for the peptide during embryonic ear development. Distinctively, we found strong signals in the spiral vascular components of the developing embryonic inner ear at various time points.
Our research is the first in describing a role for TB4 throughout cochlear vascular development. Since the peptide is capable of reminding the adult heart on its embryonic program regarding vascular re-growth, we predict it will perform similarly in the adult ear. Our observations provide novel findings highlighting the complex and specific processes of cochlear spiral vascularization, implying the potential for TB4 in treating hearing loss in the future.

Keywords: Inner ear, Embryonic development, Vasculogenesis, Thymosin beta-4

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THYMOSIN BETA-4 ALTERS MIR-139-5p EXPRESSION IN THE HYPOXIC MAMMALIAN HEART

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Although myocardial infarction (MI) occurs approximately every forty seconds in the U.S. alone, medical research still lacks the key to fully support post-hypoxic myocardial regeneration. Thymosin beta-4 (TB4), a 43 amino acid long secreted peptide, was proven to possess a beneficial impact regarding myocardial cell survival, coronary re-growth and progenitor cell activation following myocardial infarction (MI) in adult mammals. The primary aim of our study was to identify novel molecular contributors responsible for the impact of TB4 in the hypoxic heart.
16-week-old male C57BL6 mice underwent permanent left anterior descendent (LAD) coronary ligation and received TB4 or PBS treatment. miRNA profiling, real-time qPCR, western blot and immunohistochemical analyses were performed in search of target proteins, to confirm results and to unveil the potential mechanisms.
miRNA microarray results revealed a significant increase in mmu-mir-139-5p expression. We identified ROCK1 as a potential target protein aligned to mmu-mir-139-5p. Western blot analyses confirmed significant ROCK1 protein downregulation among infarcted adult mouse hearts 24 hours following ligation. Immunohistochemical studies utilizing ROCK1 specific antibody imply hypoxic myocardial and remote vascular endothelial cells are primarily responsible for the distinct alterations observed in protein levels.
Our results demonstrate downregulation of ROCK1 protein in the hypoxic mammalian myocardium in response to systemic TB4 treatment in vivo. We hypothesize these alterations are primarily due to elevated mmu-mir-139-5p expression. Given the beneficial effects of ROCK1 inhibition in various cardiac pathologies, we propose a potential utilization for TB4 as a ROCK1 inhibitor in the future.

Keywords: Cardiac regeneration, miRNA-139-5p, Thymosin beta-4, ROCK1

OTKA-K108550, GINOP-2.3.2-15-2016-00047 and 2020-4.1.1-TKP2020 Thematic Excellence Program 2020 - National Excellence Sub-program

IDENTIFICATION OF PSYCHOLOGICAL STRESS INITIATED MOLECULES IN CARDIOVASCULAR IMPAIRMENT PREVENTION

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Intense psychological stress initiates numerous molecular and biochemical alterations in the human body. Stress factors impact a wide variety of pathways to sustain/preserve homeostasis. However, the activation and/or inhibition of specific genes which control optimal gene expression and the biochemical pathways associated with these regulations require further in-depth investigations. The primary goal of our research was to identify novel protective molecules and pathways capable of enhancing adult organ regeneration and repair.
Plasma samples were collected from armed forces cadets who participated in higly controlled and supervised stress situations prior to and immediatly following the training session. First, we performed 2D gelelectrophoresis following silver stain detection. Candidates were further investigated by MALDI-TOF analysis. Secondly, we performed LC-MSMS analyses following trypsin digestion and SP3 protocol purification.
We identified more than 200 protein targets to be altered in the screens. Supported by findings of others, Apolipoprotein A-I and Alpha-1 antitrypsin were respectively detected as significant with both methods. Moreover, our results indicated a significant decrease in plasma SPARC/SPARCL1 levels following thirty minutes of psychological stress. As recent findings indicate, elevation of these proteins was associated with maladaptive right ventricular remodelling in mice. The relationship between SPARC/SPARCL1 and stress requires further investigations in humans.
In our research we identified numerous potential marker molecules associated with short term psychological stress response of the human body. We genuinely believe, future therapeutic utilization or inhibition of the detected molecules can equally prevent irreversible tissue damage and support organ regeneration and repair in adults.

Keywords: Psychological stress, Proteomics, Adaptation, Regeneration

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INFLUENCE OF OXIME K870 AND OBIDOXIME ON SURVIVAL AND CARDIORESPIRATORY PARAMETERS IN RATS POISONED WITH PARAOXON

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Paraoxon, an organophosphorus compound is an irreversible inhibitor of acetylcholinesterase. The aim of the study was to examine the effect of antidotes (obidoxime and oxime K870) on survival and cardiorespiratory parameters in rats poisoned with paraoxon.
Paraoxon (0.25 mg/kg subcutaneously) was administered to Wistar albino rats, then 1 minute later N-butyl scopolamine (63.36 mg/kg intramuscularly) and in 0.9% NaCl, obidoxime (22 mg/kg) or oxime K870 (35 mg/kg) were injected intramuscularly. The rat's blood pressure was measured non-invasively and the ECG was recorded. The transducer measured spontaneous contractions of the diaphragm. Arterial blood from the femoral artery was taken for gas analyses.
An average survival time for rats treated with saline (unprotected rats) was 55.8 min. Rats treated with obidoxime lived significantly longer (144.2 min) and with oxime K870 217.6 min, significantly longer compared to both saline and obidoxime. In unprotected rats heart rate increased from the average 285 bpm baseline value to average 480 bpm 10 minutes after paraoxon administration. Significantly lower increase in heart rate was noted in obidoxime and oxime K870 protected rats (420 and 395 bpm, respectively). Ventricular tachycardia was noticed in 90% of rats prior to arrest. Transitory bradycardia was noticed in 15% of oxime-protected rats during first hour after paraoxon administration. In unprotected rats, blood pressure (BP) increased 10 minutes after paraoxon administration (from mean BP: 83 mmHg up to 159 mmHg), hypertension lasted for 15 minutes, then slowly decreased to baseline values, hypotension (39 mmHg) and immeasurable values. Significantly lower increase in blood pressure was noted in obidoxime and oxime K870 protected rats (129 mmHg and 121 mmHg, respectively). Respiratory rate in unprotected rats showed bradypnoea (30-40% of baseline values) 15 minutes after paraoxon administration while rats treated with oximes showed slight oscillations in respiratory rate (10-20% of baseline values). Severe acidosis in unprotected rats occurred as early as 15 min compared to 45 min in protected rats.
Oximes significantly prolonged survival and improved cardiorespiratory parameters in rats poisoned with paraoxon, with better antidotal potential of oxime K870 compared to obidoxime.

Keywords: Acetylcholinesterase, Organophosphorus compounds, Oxime, Muscarinic effects, Nicotinic effects.

ASSOCIATION BETWEEN PERIAPICAL LESIONS AND MYOCARDIUM FUNCTION IN HYPERTENSION CONDITIONS

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The study was labeled to investigate the possible association between periapical lesions (PA) and myocardium function in hypertensive rats.
Forty-eight normotensive Wistar albino and spontaneously hypertensive rats were divided into four groups: control (C), normotensive with PA (PA), spontaneously hypertensive (SHR), and SHR with PA (SHR+PA). PA has been induced on the first right molar lower jaw by exposing the pulp to the oral environment for 4 weeks. The animals were sacrificed by cervical dislocation, whilst hearts were isolated and perfused according to the Langendorff technique. Biomarkers of oxidative stress were determined in myocardium tissue homogenate. The hemimandibles were analyzed pathohistologicaly. The PA extension of inflammatory infiltrate was significantly higher in the SHR+AP compared to the AP group ($p < 0.01$). The levels of the maximum left ventricular pressure development rate of the SHR+AP group were significantly higher compared to the AP and C groups, and of the SHR group compared with the C group ($p < 0.05$). The levels of the minimum left ventricular pressure development rate of the SHR + AP group were significantly lower compared to the AP, SHR, and C groups, and of the SHR group compared to the C group ($p < 0.05$). The activities of SOD in the homogenate of heart tissue were significantly lower in the PA group in comparison with the C group ($p < 0.05$).
PA was associated with impaired cardiodynamics and disturbed cardiac oxidative stress in hypertensive conditions. Hypertension was correlated with increased PA inflammatory infiltrate extension compared with normotensive conditions.

Keywords: Periapical lesions, Hypertension; Oxidative stress, Isolated rat heart.

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

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Hesperetin is the main flavonoid in oranges. Flavonoids are known to reduce cardiovascular mortality, however, their effects on cardiac electrophysiology may have both antiarrhythmic and proarrhythmic consequences as they can attenuate the repolarization reserve.
The present work aimed to study the additive inhibitory effect of Hesperetin on the cardiac action potential repolarization with normal and attenuated repolarization reserve conditions. Hesperetin's effect on transmembrane I_{Ks} and I_{Kr} was also investigated.
Action potentials were recorded using conventional microelectrode techniques. To attenuate the repolarization reserve, Dofetilide 0.1 µM (I_{Kr} blocker) and Veratrine 50 µg (late Na^+ channel activator) were added to the tissue bath. Transmembrane I_{Ks} and I_{Kr} were measured in rabbit myocytes using the whole-cell configuration of the patch-clamp technique.
Hesperetin 10 µM alone has no notable effect on action potential duration (APD), however, during the impaired repolarization reserve, 10 µM Hesperetin caused a significant prolongation of the steady APD (from 466±18 ms to 512±23 ms (n=14), $p<0.05$). In agreement with APD data, a moderate but statistically significant inhibitory effect of 10 µM Hesperetin was observed on the transmembrane I_{Ks} ((n=6), $p<0.05$), without influencing I_{Kr}.
Hesperetin alone has no or negligible effect on APD, therefore, the proarrhythmic risk is low among healthy individuals. However, if the repolarization reserve has been attenuated due to certain pathological conditions such as heart failure or some variable abnormalities such as adverse drug effects, genetic mutations, a high amount of orange juice consumption might lead to moderately increased risk of arrhythmia due to inhibition of I_{Ks} and lengthening of cardiac repolarization.

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PULSE WAVE ANALYSIS IN AMATEUR CYCLISTS: WHY WE SHOULD FIRST BIKE (AND THEN EAT ALL THE S'MORES)

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Pulse wave velocity (PWV) is the most studied technique to assess arterial stiffness in population-based studies. The present pilot study was purported to compare the pulse wave analysis results in amateur cyclists and medical students using the Mobil-O-Graph equipment. The following parameters were measured in each participant: pulse wave velocity, augmentation index and pressure, peripheral and central blood pressure and vascular age. The two groups were matched for age and body mass index. The values of PWV (5.63±0.53 vs. 5.09±0.48 m/s, p=0.033), augmentation pressure (AP: 10.62±5.92 vs. 4.63±3.17 mmHg, p=0.011) and diastolic blood pressure (DBP: 75±6.74 vs. 68±6.64 mmHg, p=0.039) were significantly higher in the first group as compared to the second. However, the augmentation index was increased in 37.5% of the cyclists *vs* 45.45% of the students when considering the normal values for age. PWV was normal for age in all the participants of the first group and increased for age in 27% of the members of the second group. Early vascular aging was detected in 37.5% of the amateur cyclists and 63.63% of the medical students. In conclusion, increased arterial stiffness and early vascular aging are more common in medical students as compared to amateur cyclists. Larger studies are needed to confirm the results of this pilot study.

Keywords: cyclists, pulse wave velocity, augmentation index, arterial stiffness, vascular age

SACUBITRIL/VALSARTAN PROVIDES CARDIOPROTECTION AGAINST ISCHEMIA/REPERFUSION INJURY IN EXPERIMENTAL METABOLIC SYNDROME

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This study was designed to investigate the ameliorative effect of synergism between sacubitril, as an inhibitor of natriuretic peptide-degrading enzyme and valsartan, an angiotensin II type 1 receptor blocker, on cardiac function in rats with metabolic syndrome exposed to ex vivo-induced ischemia/reperfusion injury (I/R). Adult-male Wistar albino rats (n=40) were equally categorized into healthy control group (CTRL), rats with metabolic syndrome (MetS), healthy rats treated with sacubitril/valsartan (Sac/Val) and rats with metabolic syndrome treated with sacubitril/valsartan (MetS + Sac/Val). Animals from Sac/Val groups received oral suspension of these drugs every day in dosage of 68 mg/kg during 4 weeks. Cardiac function and dimension of the left ventricle (LV) were evaluated via echocardiograph. Moreover, blood pressure and heart rate were estimated. By completing experimental protocol, animals from all groups were sacrificed and following cardiodynamic parameters were collected: maximum rate of pressure development in the left ventricle (dp/dt max), minimum rate of pressure development in the left ventricle (dp/dt min), systolic left ventricular pressure (SLVP), diastolic left ventricular pressure (DLVP), heart rate (HR) and coronary flow (CF). Our results demonstrated for the first time that combined sacubitril/valsartan administration preserved cardiac contractility, systolic and diastolic function of the MetS hearts exposed to I/R injury. Moreover, it was observed that examined drugs decreased blood pressure in diabetic rats while no significant differences in the heart rate values were observed. Obtained results indicate promising potential of combined sacubitril/valsartan use for the management of metabolic syndrome.

Keywords: sacubitril; valsartan; ischemia/reperfusion injury; metabolic syndrome; rat.

THE ROLE OF INSULIN TREATMENT IN CONTROLLING OXIDATIVE STRESS IN BLOOD OF DIABETIC RATS

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An increase in oxidative stress is associated with hyperglycemia, development, and progression of diabetes complications. This condition can lead to lipid peroxidation in muscle cell membranes, which contributes to the development of insulin resistance. The purpose of the present study was to verify the action of insulin combined with HBO treatment in oxidative stress control of streptozotocin-diabetic rats. The rats were subjected to 24-hour starvation and given an intraperitoneal injection of streptozotocin (STZ, 60 mg/kg body weight, dissolved in 0.01 M sodium citrate buffer, pH 4.5) to induce diabetes. A total of 48 male Wistar rats were randomly divided into 4 groups: 1) Control group, no diabetic induction without HBO treatment; 2) HBO group, exposed to 100% oxygen at 2.8 ATA (atmosphere absolute) for 1 h once daily, for 5 days (two weeks); 3) DM group, diabetes induced by streptozotocin (STZ) injection; and 4) DM + HBO group, received both STZ injection and HBO exposure; 5) DM+INS group, NPH insulin 5U/day, 6) DM+HBO+INS, received both NPH insulin and HBO exposure for 2 weeks. The body weight, glycemic control, and parameters of oxidative stress were evaluated. DM+INS reduced the oxidative stress levels and the activity of catalase and superoxide dismutase in blood when compared to DM+INS+HBO rats. In conclusion, our results reveal that dual therapy with HBO and insulin promotes more benefits to oxidative stress control in blood of hypoinsulinemic rats than insulinotherapy alone.

Keywords: diabetes mellitus type 1, streptozotocin, hyperbaric oxygen therapy, neutral protamine hagedorn (NPH) insulin

PROTEOMIC ANALYSIS OF EXERCISE-INDUCED HYPERTROPHY REVEALS SEX-RELATED MITOCHONDRIAL DIFFERENCES MEDIATED BY AMPK

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Regular physical activity results in characteristic structural and functional changes in the heart. The extent of exercise-induced left ventricular (LV) hypertrophy and functional changes show significant differences between men and women, the molecular background of which is not fully elucidated. The aim of this study was to provide a proteomic characterization of long-term exercise-induced LV myocardial hypertrophy in a rat model, focusing on sex-related differences.
Our male and female rats were divided into trained and control groups. In the trained groups, athlete's heart was induced by a 12-week swimming protocol. Myocardial hypertrophy was confirmed by echocardiography and functional adaptation by pressure-volume analysis. Proteomic measurements based on liquid chromatograph-coupled mass spectrometry were performed on proteins isolated from our LV myocardial samples.
Echocardiography showed significant LV hypertrophy in both sexes, which was more pronounced in female animals. LV contractility increased to the same extent in both sexes. Relative expression of 3074 proteins were determined by proteomics. There was a significant change in expression of 229 proteins in males and 599 in females compared to the level of same-sex controls. Based on our gene ontological analysis, physiological LV remodeling in females is characterized by increased expression of proteins in mitochondrial function and remodelling associated with increased expression of AMPK-SIRT3, whereas in males, proteins that bind to the actin cytoskeleton is primarily increased.
Our data suggests that physiological LV hypertrophy resulting from regular, balanced exercise is associated with sex-specific changes in the myocardial proteome and the AMPK-mediated mitochondrial distinctions might be in the background.

Keywords: athlete's heart, proteomic analysis, sex differences

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AORTIC PRESSURE POSITIVELY AFFECTS LEFT VENTRICULAR PRESSURE IN ISOLATED, LANGENDORFF-PERFUSED GUINEA PIG AND RAT HEARTS

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The Langendorff-perfused isolated heart is a commonly used cardiovascular experimental model. Previously, we proved that, contrary to popular belief, the left ventricle is loaded and ejects in this model, similarly to physiological conditions. However, it is unclear how aortic (column) pressure affects left ventricular pressure and ejection.
We recorded the left ventricular pressure, aortic pressure and aortic flow in Langendorff-perfused, isolated guinea pig (n=33) and rat (n=29) hearts. The hearts were randomly assigned into one of three predetermined aortic pressure groups: 60, 70, or 80 mmHg.
Elevating aortic pressure increased left ventricular pressures (max. left ventricular pressure in guinea pig hearts [mean±SE]: 71.0±1.4 mmHg, 87.8±1.1 mmHg, 97.9±1.5 mmHg in 60, 70, and 80 mmHg group, respectively, $p<0.05$; max. left ventricular pressure in rat hearts: 71.8±2.1 mmHg, 79.0±2.7 mmHg, 91.7±3.5 mmHg in 60, 70, and 80 mmHg group, respectively, $p<0.05$). In guinea pig hearts, elevating aortic pressure increased stroke volume and cardiac output (stroke volume: 6.6±2.1 µl, 21.0±4.6 µl, 29.4±8.1 µl in 60, 70, and 80 mmHg group, respectively, $p<0.05$; cardiac output: 1.39±0.44 ml/min, 4.04±0.79 ml/min, 6.19±1.53 ml/min in 60, 70, and 80 mmHg group, respectively, $p<0.05$). On the contrary, aortic pressure did not affect stroke volume or cardiac output in rat hearts.
In Langendorff-perfused guinea pig and rat hearts, aortic pressure positively affects left ventricular pressure. In guinea pig hearts, elevating aortic pressure increases stroke volume and cardiac output, whereas aortic pressure does not affect these parameters in rat hearts indicating an interspecies difference.

Keywords: Langendorff-perfused hearts, aortic pressure, left ventricular pressure, stroke volume, cardiac output.

ENDOTHELIUM-INDEPENDENT EFFECT OF PINACIDIL ON SAPHENOUS VEIN OBTAINED FROM PATIENTS WITH/WITHOUT TYPE 2 DIABETES MELLITUS THROUGH VOLTAGE-GATED POTASSIUM CHANNELS

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Type 2 diabetes mellitus (T2DM) is one of the major risk factors for cardiovascular complications. Reduced relaxation of blood vessels from diabetic patients could be result of different expression and/or function of smooth muscle potassium (K) channels. Thus, the objective of our study was to investigate differences in the involvement of voltage-gated K (K_v) channels in the effect of pinacidil on human saphenous veins (HSV) obtained from patients with/without T2DM.
Rings of HSV from bypass surgery, without endothelium, were mounted in organ bath system and isometric tension was being recorded. The relaxation of HSV, precontracted with phenylephrine, was produced by pinacidil, a potassium channel opener.
Pinacidil produced concentration-dependent relaxation of HSV from patients with/without T2DM. 4-aminopiridine (4-AP, 1mM and 3mM), non-selective blocker of K_v channels did not antagonize pinacidil effects on HSV from patients with T2DM. However, 4-AP antagonized pinacidil effects on HSV from patients without T2DM ($P < 0.05$, for both concentrations). Margatoxin, specific blocker of Kv1 channels did not antagonize pinacidil effects on HSV from patients with/without T2DM.
Pinacidil produces comparable relaxation of HSV in patients with/without T2DM. 4-AP-sensitive Kv channels are probably involved in pinacidil-induced relaxation of HSV from patients without T2DM. However, in patients with T2DM, 4-AP-sensitive Kv channels did not participate in pinacidil effect on HSV. According to the results obtained with margatoxin, it seems that Kv1 channels are not included in pinacidil effects on HSV of patients with/without T2DM. It seems, that presence of T2DM influences strongly function and/or expression of vascular Kv channels.

Keywords: potassium channels, saphenous vein, type 2 diabetes mellitus, pinacidil, bypass grafts

EFFECTS OF THE NOVEL MYOSIN ACTIVATOR DANICAMTIV ON THE CONTRACTILITY AND CA2+ TRANSIENTS OF ISOLATED LEFT VENTRICULAR CARDIOMYOCYTES

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Introduction: Advanced stage heart failure (HFrEF) has a poor prognosis, and is difficult to treat. Myosin activators have been developed to improve systolic function. The present study is focused on danicamtiv, a new myosin activator.
Aim: The aim was to investigate the effects of danicamtiv and its concentration-dependency on systolic and diastolic function.
Methods: Enzymatically isolated left ventricular myocytes were loaded with Fura-2 AM calcium sensitive fluorescent dye. Contractions and relaxations were monitored at room temperature while treating with ranging danicamtiv concentrations (10 nM - 2 µM). Cell contraction was induced by field excitation and the shortening of the sarcomere length and the changes in intracellular Ca^{2+} concentration were recorded parallelly.
Results: In the presence of 0.5 Hz stimulation and 2 µM danicamtiv, both the contraction duration (2.0±0.7 s vs. 0.8±0.2 s, mean±SEM, $P<0.05$) and the systolic ejection time were prolonged (1.6±0.6 s vs. 0.6±0.1 s, $P<0.05$), while the kinetics of contraction and relaxation were both decelerated (0.21±0.19 µm/s vs. 0.94±0.49 µm/s and 0.22±0.18 µm/s vs. 1.30±0.75 µm/s, $P<0.05$, respectively) (n=18). Treatment with 2 µM danicamtiv showed a positive ionotropic effect, a shortening could be observed in diastolic (1.59±0.13 µm vs. 1.90±0.03 µm $P<0.05$, n=18), as well as in systolic sarcomere lengths (1.46±0.08 µm vs. 1.68±0.0 µm $P<0.05$, n=18). Danicamtiv treatment was not associated with an increase in intracellular Ca^{2+} concentration, regardless of the frequency of stimulation.
Conclusion: The results suggest that the positive inotropic effect of danicamtiv is accompanied by a significant reduction in resting sarcomere length of isolated myocardial cells and a deceleration of relaxation kinetics, which may impair diastolic function. All these may limit the clinical efficacy of the agent.

Keywords: positive inotropy, HFrEF, danicamtiv, myosin activator

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CARDIOPROTECTIVE EFFECTS OF GLP-1 RECEPTOR AGONISTS ON ISCHEMIA/REPERFUSION INJURY IN ISOLATED HEARTS OF RATS WITH METABOLIC SYNDROME

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Metabolic syndrome (MetS) represents a cluster of medical conditions, including central obesity, hypertension, dyslipidemia, and insulin resistance, associated with increased risk of cardiovascular morbidity and mortality. Liraglutide, exenatide and dulaglutide belong to a group of drugs called glucagon-like peptide-1 (GLP-1) receptor agonists. Although they are used in clinical practice, their effects in various types of disorders and organs are still the subject of research. The aim of this study was to assess and compare the effects of three GLP-1 receptor agonists, liraglutide, exenatide and dulaglutide on cardiodynamic parameters during ischemia and reperfusion in isolated hearts of rats with metabolic syndrome.
The experiments was conducted on 32 male Wistar Albino rats (8 rats per each group): healthy untreated rats, untreated rats with MetS, rats with MetS treated with liraglutide, exenatide and dulaglutide, respectively. MetS was induced by the use of a high-fat diet for 4 weeks and low doses of streptozotocin (30 mg/kg), and the use of the drugs lasted 6 weeks from the confirmation of hyperglycemia. At the end of the experiment the animals were sacrificed, the hearts were perfused according to the Langendorff technique.
All applied drugs exerted cardioprotective effects. The most pronounced cardioprotective effect of liraglutide was observed in the parameters of myocardial contractility (dp/dt max and dp/dt min) and systolic left ventricular pressure (SLVP). Both liraglutide and dulaglutide showed similar protective effect in maintaining heart rate and coronary flow during the reperfusion period.
GLP-1 receptor agonists, as a novel therapeutic approach, have the potential as cardioprotective agents in ischemia/reperfusion injury.

Keywords: Liraglutide; Exenatide; Dulaglutide; Heart Function; GLP-1 Receptor Agonists.

EFFECTS OF FOUR WEEKS TREADMILL TRAINING ON CARDIAC TISSUE LACTATE DEHYDROGENASE ISOFORMS ACTIVITIES AND HEPATORENAL BIOMARKERS IN EXPERIMENTALLY INDUCED HYPERHOMOCYSTEINEMIA IN RATS

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The aim of this study was to investigate the effects of experimentally induced hyperhomocysteinemia independently and also in the condition of aerobic treadmill training on hepatorenal biomarkers in sera, and on lactate dehydrogenase (LDH) isoforms activities in cardiac tissue of rats.
Male *Wistar albino* rats were divided into four investigated groups (10 per group): C: saline (0.9% NaCl 0.2 mL/day s.c.); H: homocysteine (0.45 μmol/g b.w./day s.c.); CPA: saline (0.9% NaCl 0.2 mL/day s.c.) and a program of aerobic training on a treadmill; and HPA: homocysteine (0.45 μmol/g b.w./day s.c.) and a program of aerobic training on a treadmill. Injection of substances was applied 2 times a day at intervals of 8h during the first two weeks of experimental protocol. After four weeks samples of blood and cardiac tissue were taken for analysis.
Homocysteine level in sera was significantly higher in the HPA group compared to the CPA group ($p < 0.01$). Glucose, proteins, albumin, aspartate aminotransferase, alanine aminotransferase, urea, creatinine and amylase levels in sera were all higher in both active groups compared with the sedentary group: CPA ($p < 0.01$ vs. C; $p < 0.01$ vs. H) and HPA ($p < 0.01$ vs. C; $p < 0.01$ vs. H). Total activity of LDH was increased in the HPA group in comparison to all other groups ($p < 0.01$ vs. C; $p < 0.01$ vs. H; $p < 0.01$ vs. CPA). In HPA group, relative activities of LDH isoforms were significantly higher compared to the C and H group: LDH 1 ($p < 0.01$ vs. C; $p < 0.01$ vs. H), LDH 2 ($p < 0.01$ vs. C; $p < 0.01$ vs. H) and LDH 4 ($p < 0.01$ vs. C; $p < 0.01$ vs. H).
Experimentally induced hyperhomocysteinemia under the condition of aerobic treadmill training can lead to increased concentrations of hepatorenal biomarkers in sera and increased activity of LDH in cardiac tissue of rats.

Keywords: Exercise; Heart; Homocysteine; Lactate dehydrogenase; Rat

MONOAMINE OXIDASE CONTRIBUTION TO VALVULAR HEART DISEASE: MORE THAN MEETS THE EYE

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A growing body of research showed that oxidative stress has an important causative role in the pathophysiology of valvular heart disease complicated with fibrocalcification. The identified local enzymatic sources of reactive oxygen species (ROS) are: NADPH isoform 2 (NOX2) and uncoupled NOS. Monoamine oxidases (MAOs) with 2 isoforms, A and B, are flavoenzymes located at the outer mitochondrial membrane which constantly generate hydrogen peroxide (H_2O_2) as a by-product of their activity of oxidative deamination of biogenic amines and neurotransmitters. Whether MAOs are mediators in oxidative stress in the pathogenesis of valvular disease it is not known and was investigated here in patients with severe mitral regurgitation (due to valve degeneration and chordae rupture). Samples of mitral valve (n = 30) were harvested during the valvular replacement procedures and used for ROS assessment (immune-fluorescence, spectrophotometry/FOX assay) and MAO-A and B gene and protein expression measurement (qPCR and immune-fluorescence). We report here that human mitral valve contain both MAO isoforms involved in catecholamine degradation. Ex vivo incubation of the mitral valve samples with AII (100 nM, 12 h) induced MAO-A and B expression and resulted in increased H_2O_2 formation. MAO-related oxidative stress was mitigated by MAO inhibition with the MAO-A inhibitor, clorgyline (10 microM) and the MAO-B inhibitor, selegyline (10 microM) and also by the AII receptor type 1 antagonist, irbersartan (10 microM). In conclusion, MAO is expressed in human mitral valves, can be induced by AII stimulation and thus may contribute via H_2O_2 generation to the pathophysiology of valvular heart disease.

Keywords*:* valvular heart disease, oxidative stress, monoamine oxidase, angiotensin 2

IMPACT OF CARDIOVASCULAR DRUGS ON THE DIAGNOSIS OF A MAINLY EXTRACARDIAC DISEASE, SARCOIDOSIS

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Introduction: Sarcoidosis is a granulomatous inflammatory disease, which mainly affects the lungs and extracardiac tissues. Determination of the usually elevated angiotensin-converting enzyme (ACE) activity is important in establishing the diagnosis and in assessing the success of treatment. However, ACE inhibitor drugs (ACEI) can significantly reduce ACE activity, influencing diagnostic and treatment decisions.
Aim: The aim of the study was to set up a measurement method that can detect the presence of any ACEI in the sample, and to investigate to what extent the requested ACE activity measurements were influenced by taken medication, and how this affected clinical decision making.
Methods: In this study, the results were analysed of patients who had diagnostic ACE activity measurements between 2014 and 2021 in Debrecen, Hungary. Serum ACE activity was measured in 4-, 35-, 400-fold dilutions using a fluorescent kinetic method.
Results: A total of 1853 diagnostic measurements were performed during the study period, of which 30 patients' results were not evaluated due to missing data. In 302 (17%) cases ACEI effect (>80%) could be observed, resulting a significant decrease in serum ACE activity compared to patients not taking ACEI (median [interquartile range], respectively: 4.42 [2.93-6.75] U/L; 11.32 [8.79-13.92] U/L; $p<0.01$). Eighty-three percent of patients with results below the reference range (RR) would fall at least within the normal range, while 43% of patients with results in the RR would have a value above RR if they were not taking ACEI. Thus, sarcoidosis in at least 61 patients may not have been detected in time or at all due to ACEI treatment during the study period. Physicians associated low ACE activity with ACEI treatment in only 3 cases.
Conclusion: With the adjusted method, the misleading presence of ACEI can be highlighted to the physician, helping to ensure proper interpretation of results and decision making. This can significantly reduce the time and cost, as well as increase the efficiency of establishing the diagnosis.

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DIET-INDUCED HYPERCHOLESTEROLEMIA LEADS TO CARDIAC DYSFUNCTION AND ALTERATIONS IN THE MYOCARDIAL PROTEOME

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High blood cholesterol is a major risk factor for coronary heart disease. Moreover, direct effects on the myocardium also contribute to the adverse effects of hypercholesterolemia. In the present study, we aimed to investigate the global proteome changes in the left ventricle of hypercholesterolemic rats in order to clarify the underlying protein expression changes associated with the direct cardiac effects of hypercholesterolemia. Male Wistar rats were fed with a laboratory rodent chow supplemented with 2% cholesterol and 0.25% sodium-cholate hydrate for 8 weeks to induce hypercholesterolemia. Proteomic characterization of left ventricular samples from normo- and hypercholesterolemic animals was performed with liquid chromatography-mass spectrometry analysis. The significantly altered proteins from the proteomic data were subjected to gene ontology and protein interaction analyses. Additionally, similar expression patterns were explored through the whole unfiltered proteome data. Elevated circulating cholesterol level was accompanied by mild diastolic dysfunction in cholesterol-fed animals. Proteomic characterization of left ventricular samples revealed altered level of 45 proteins due to hypercholesterolemia. Based on our gene ontology and protein interaction analysis results, hypercholesterolemia was associated with disturbed expression of cytoskeletal and contractile proteins. Beta-actin was downregulated in the hypercholesterolemic myocardium and established a prominent hub of the revealed network. Deeper GSEA analysis of the unfiltered dataset revealed concordant downregulated expression patterns in proteins associated with the arrangement of contractile and cytoskeletal system, and in protein subunits of the mitochondrial respiratory chain system. We conclude that disturbed expression of proteins associated with the contractile apparatus as well as with the mitochondrial respiratory chain due to hypercholesterolemia may play a role in the cardiac diastolic dysfunction in the myocardium of cholesterol-fed animals.

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THE EFFFECTS OF ADRENERGIC AND CHOLINERGIC DRUGS ON DOFETILIDE-INDUCED ARRHYTHMIAS IN CONSCIOUS RABBITS

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The mechanisms of arrhythmias associated with acquired long QT syndrome are not fully understood. We tested the effects of anaesthesia as well as adrenergic and cholinergic drugs on QTc prolongation and proarrhythmic effects of dofetilide in conscious rabbits. Dofetilide was infused over 5 min at 20 min intervals (5, 10, 35, 100 and 350 µg/kg, i.v.) after a 60-min control period (n=9-11/group). Dofetilide prolonged the QTc interval (Bazett) in a dose-dependent manner with a peak effect of 38±4 %, which was fully abolished following chloralose-urethane anaesthesia. Phenylephrine (Phe) at low or high dose rates (2 and 8 µg/kg/min, i.v.) induced small (5.1±1.2%) and large (25.9±2.3%) increases in blood pressure and a consequent mild (-11.1±2.8%) and profound (-30.3±4.6%) bradycardia, respectively. Phe dose-dependently attenuated dofetilide-induced QTc lengthening. The low dose of Phe decreased but the high dose of Phe markedly increased the number of “torsade de pointes” (TdP) polymorphic ventricular tachycardia episodes (243 vs.152 and 759, respectively, $p<0.05$). Atropine (40 µg/kg bolus and 20 µg/kg/h, i.v.) caused a small tachycardia, and decreased the number of TdP episodes (243 vs. 165, $p<0.05$). Isoproterenol (Iso, 1 µg/kg/min, i.v.) increased HR and diminished dofetilide-induced QTc prolongation, but metoprolol (Meto, 1 mg/kg bolus + 1 mg/kg/h, i.v.) slightly decreased HR. Iso induced small but Meto induced marked reductions in the number of TdP episodes.
In conclusion, the dofetilide-induced QTc prolongation and proarrhythmic effects are modulated in a complex manner by agents affecting adrenergic or cholinergic receptors in the conscious rabbit.

POTENTIAL ELECTROPHYSIOLOGICAL IMPACTS OF SUPRA-PHYSIOLOGICAL TESTOSTERONE LEVEL: INVESTIGATION OF THE EFFECTS OF TESTOSTERONE-UNDECANOATE ADMINISTRATION CHRONICALLY IN A LARGE ANIMAL MODEL

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Despite the legally stipulated availability of various androgen anabolic steroids (AAS), abuse of these products causes a major public health problem among young adults. Chronic use of AASs may result in structural and functional remodeling of the heart. The aim of our study was to investigate the potential electrophysiological effects of chronic administration of testosterone-undecanoate in a large animal model in *in vivo* and *in vitro* studies.
Eight male beagle dogs were randomized into control (’Cont’) and treated (’Tr’) groups (n = 4; n = 4). The latter group received 15 mg/kg testosterone-undecanoate weekly for 3 months. Blood samples were taken for monitoring testosterone levels. ECG and ECHO procedures were performed. Ventricular myocytes were enzymatically dissociated via retrograde perfusion. The transmembrane ionic currents were recorded using the whole-cell configuration of the patch-clamp technique and the action potential duration (APD) was measured by the perforated patch-clamp technique.
Testosterone level was significantly higher in the ‘Tr’ group compared to the ‘Cont’ group (47.02 nm/L vs. 15.23 nm/L; p = 0.0002). The APD of isolated left ventricular myocytes was seemingly shorter in the ‘Tr’ group. In terms of different potassium currents, the amplitude of the slow delayed rectifier potassium current was increased in the ’Tr’ group.
The repolarization of canine ventricular myocardium was significantly modified by constantly high doses of testosterone. Although the physiological level of testosterone provided many benefits to health, the supra-physiological level induced potential cellular and structural changes may lead to life-threatening arrhythmias under certain circumstances.

Keywords: testosterone, electrophysiology, patch-clamp, hypertrophy, fibrosis

EFFECTS OF FOUR WEEKS TREADMILL TRAINING ON CARDIAC TISSUE MALATE DEHYDROGENASE AND MATRIX METALLOPROTEINASE ENZYMES ACTIVITIES IN EXPERIMENTAL HYPERHOMOCYSTEINEMIA IN RATS

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The aim of this study was to investigate the effects of experimentally induced hyperhomocysteinemia alone and under the condition of aerobic physical activity on, high-sensitive troponin T concentration in sera, cardiac tissue malate dehydrogenase and matrix metalloproteinase enzymes activities.
Male *Wistar albino* rats were divided into four groups (n = 10, per group): C: 0.9% NaCl 0.2 mL/day subcutaneous injection (s.c.); H: homocysteine 0.45 μmol/g b.w./day s.c.; CPA saline (0.9% NaCl 0.2 mL/day s.c.) and a program of physical activity on a treadmill; and HPA homocysteine (0.45 μmol/g b.w./day s.c.) and a program of physical activity on a treadmill. Subcutaneous injection of substances was applied 2 times a day at intervals of 8h during the first two weeks of experimental protocol. After four weeks samples of blood and tissue were taken for further analysis. High-sensitive troponin T concentration was increased in HPA in comparison to C ($p < 0.01$) and H ($p < 0.05$) group. Total activity of MDH was highest in HPA group but without reaching statistical significance. Three isoforms of malate dehydrogenase were expressed in rat cardiac tissue: peroxysomal (pMDH), mitochondrial (mMDH), and citosolyc (cMDH) isoform. Increased activity of mMDH was detected in HPA group compared to CPA group ($p < 0.01$). Individual application of Hcy did not lead to these changes. Physical activity led to activation of MMP-2 isoform and to increased activity of the MMP-9 isoform in both Hcy-treated ($p < 0.01$ vs. C; $p < 0.01$ vs H) and control rats ($p < 0.01$ vs. C; $p < 0.01$ vs H). Experimentally induced hyperhomocysteinemia under the condition of aerobic physical activity can lead to increased concentrations of high-sensitive troponin T in sera, mitochondrial isoform of malate dehydrogenase and MMP-9 isoform of matrix metalloproteinase in cardiac tissue of rats.

Keywords: Exercise; Heart; Homocysteine; Malate dehydrogenase; Rat

PUNICA GRANATUM PEEL EXTRACT SIGNIFICANTLY ATTENUATES THE ISOPRENALINE-INDUCED MYOCARDIAL INJURY IN RATS

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Pomegranate peel extract (PoPEx) is a rich source of bioactive polyphenols with beneficial anti-inflammatory and antioxidative effects. In the present study, isoprenaline-induced myocardial injury (MI) model was used to explore the cardioprotective and antioxidative effects of PoPEx. The aim of this study was to investigate the effects of PoPEx pretreatment on isoprenaline-induced MI.
Wistar rats were used in this experiment. The MI was induced by injecting 85 mg/kg of isoprenaline, on two consecutive days. In order to alleviate the effects of isoprenaline, the PoPEx 100 mg/kg was administered by gavage as a pretreatment for 14 days. The experimental animals were divided into 4 groups: I group (saline for 14 days + ISO on days 13 and 14), P group (PoPEx for 14 days + saline on days 13 and 14) and P+I group (PoPEx for 14 days + ISO on days 13 and 14), while in the control (C) group both, PoPEx and isoprenalin were replaced by saline. The biochemical parameters of MI, as well as markers of oxidative stress, and histological analysis were evaluated.
Isoprenaline-induced MI was demonstrated by increased levels of high-sensitivity troponin I (hsTnl), lipids, AST, homocysteine and decreased level of LDH. Pretreatment with PoPEx significantly attenuated the ISO induced changes in lipid status, as well as the levels of glucose, homocysteine, high sensitive troponin I (hsTnl), AST, ALT and LDH. Additionally, the pretreatment with PoPEx significantly ameliorated the changes in oxidative stress markers in cardiac tissue homogenates such as superoxide dismutase, glutathione and catalase. The histopathologic analysis confirmed the cardioprotective effects of PoPEx pretreatment in ISO-induced MI.
Pretreatment with PoPEx showed significant cardioprotective effects by attenuating humoral and morphological signs of MI induced by ISO.

Keywords: Pomegranate peel extract, isoprenaline, myocardial injury, cardioprotection

LIRAGLUTIDE ATTENUATES ISOPRENALINE-INDUCED MYOCARDIAL INJURY AND APOPTOSIS IN RATS BY MODULATING WNT/β-CATENIN SIGNALLING PATHWAY

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β-adrenergic receptor stimulation with isoprenaline (ISO) induces substantial oxidative stress in the heart, leading to the infarct-like necrotic damage. During myocardial injury (MI) reactive oxygen species are generated, which causes cardiomyocyte apoptosis. The up-regulation of canonical Wnt/β-catenin pathway induces cardiac hypertrophy and fibrosis in the isoprenaline (ISO)-induced model of MI. The aim of this study was to clarify the role of cardioprotective effects of liraglutide (LIR), an antidiabetic glucagon-like peptide-1 (GLP-1) receptor agonist on ISO-induced myocardial pathological manifestations.

The rats in this study were randomly allocated to four groups: control (C) group, I group (physiological saline + ISO injection for two days at a dose of 85 mg/kg), L group (LIR for 10 days + physiological saline) and L+I group (LIR for 10 days + ISO on days 9 and 10). In order to produce an infarct-like necrotic damage, ISO injections were given to Wistar albino rats for two days. The expression of β-catenin, cyclin D1 and cleaved caspase-3 (a key apoptotic protein) was monitored using immunohistochemical detection.

The results of the present study suggest that pre-treatment with LIR significantly ameliorated ISO-induced MI in rats. It also decreased apoptotic marker expression, which subsequently resulted in the significant decrease in the expression levels of Wnt/β-catenin signalling pathway-associated molecules such as β-catenin and cyclin D1.

Our findings revealed that Wnt/β-catenin signalling pathway is a potential molecular target for LIR, what might be promising in the prevention of MI and apoptosis in cardiomyocytes.

Keywords: Liraglutide, Cardioprotection, Wnt/β-catenin, Cleaved caspase-3, Isoprenaline induced myocardial injury

NOVEL BIOMARKERS OF BRAIN INJURY FOR EARLY OUTCOME PREDICTION IN CARDIAC ARREST SURVIVORS

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Early determination of neurological prognosis in cardiac arrest survivors remains challenging. The aim of our study was to compare prognostic values of guidelines- recommended neuron-specific enolase (NSE) with novel biomarkers serum tau protein (Tau) and neurofilament light chain (Nfl) at different timepoints after cardiac arrest.
Eligible subjects for this single-center prospective study were out-of-hospital cardiac arrest survivors. NSE, Tau and Nfl levels were measured at 24 hrs (D1), 48 hrs (D2), 72 hrs (D3), and 96 hrs (D4) after hospital admission. Prognostic values of NSE, Tau and Nfl for the prediction of poor outcomes were determined using ROC analysis. Poor outcome was defined as Modified Rankin Scale (mRS) 4-6.
A total of 43 cardiac arrest survivors were enrolled. The area under the ROC curve (AUC) for NSE was 0.776 at D1, 0.911 at D2, 0.982 at D3, and 1.0 at D4 (all $P<0.001$). The AUC for Tau was 0.823 at D1, 0.893 at D2, 0.938 at D3, and 0.980 at D4 (all $P<0.001$). The AUC for Nfl was 0.614, $P=0.232$ at D1, 0.782, $P=0.001$ at D2, 0.969, $P<0.001$ at D3, and 0.990, $P<0.001$ at D4. Tau has the highest sensitivity for the prediction of poor prognosis with 100% specificity at D1 or D2 and NSE at D3 or D4.
Our results indicate that the novel biomarkers Tau and Nfl have comparable predictive value for clinical outcomes as NSE at 48 to 96 hrs after cardiac arrest. Predictive value at 24 hrs was highest with Tau.

Keywords: Cardiac arrest, Outcome prediction, Neuron-specific enolase, Serum tau protein, Neurofilament light chain.

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THE CARDIOPROTECTIVE EFFICACY OF DRUGS TARGETING ADRENERGIC RECEPTOR PATHWAYS WHEN ADDED PRIOR TO CARDIOPLEGIC ISCHAEMIC ARREST

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Pre-conditioning of perfused heart with drugs targeting α and β adrenergic receptors has been shown to be cardioprotective against global ischaemic and reperfusion injury. These drugs include Isoprenaline (β-receptor agonist), A61603 (α1A-receptor agonist) and the cAMP permeable analogue 8-Br-cAMP-AM (8-Br). All these interventions produce an inotropic response in the heart which may underly the mechanism of protection that involves glycogen store depletion and inhibition of the mitochondrial permeability transition pore. However, whether these interventions are also efficacious when used during cardioplegic arrest is not presently known. This study aims to address this issue. Adult male Wistar rat hearts were arrested with cardioplegia and subjected to 45-minutes ischemia followed by 2 hours reperfusion on a Langendorff setup. Hearts were pre-treated with either isoprenaline (100nM), 8-Br (10uM), or A61603 (10nM). To assess cardioprotection, haemodynamic function, lactate dehydrogenase (LDH) release and infarct size were measured. Onset of ischemic contracture occurred earlier following isoprenaline and 8-Br treatment compared to cardioplegia alone. Pre-conditioning with 8-Br improved functional recovery and reduced LDH release and infarct size. Isoprenaline also exhibited some protection against IRI with a reduction in infarct size. Treatment with A61603 did not provide any protection for the heart. This work shows that activation of α1A-receptor is only protective when used during normothermic ischaemia but not when used in conjunction with cardioplegia. It also suggests that cAMP analogues confer strong protection when used during cardioplegic arrest and provide an excellent tool for intervention given the known changes in β-adrenergic receptors sensitivity in disease states.