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SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Supplement 15

2nd Congress of Slovenian Society for Laboratory Animals

2. Kongres Slovenskega društva za laboratorijske živali

Ljubljana, 19-20 junij 2014

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2. Kongres Slovenskega društva za laboratorijske živali

Ljubljana, Slovenia
19-20 June, 2014

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2nd Congress of Slovenian Society for Laboratory Animals

2. Kongres Slovenskega društva za laboratorijske živali

Proceedings / Zbornik povzetkov

Ljubljana, 19-20 June 2014

Welcome

Dear Colleagues,

On behalf of the Slovenian Society for Laboratory Animals it is a great pleasure to welcome you to the 2nd Congress of Slovenian Society for Laboratory Animals, which addresses fundamental topics in the field of laboratory animal science. Environmental, dietary, microbiological or genetic factors are some of the basic factors that could significantly affect animal welfare as well as the validity of scientific results. It is our moral and professional obligation to follow constantly developing progress in this field. Since it would not be possible to replace all animal experiments in the foreseeable future, ethical considerations and animal welfare should be given a high priority.
Recently, we were faced with the revision of animal protection legislation in EU and consequently in Slovenia in which the public has shown high interest and involvement. Such events on one hand show that the public is very interested in this subject and on the other hand offer the opportunity to open up and speak up to the public about the facts for and against animal experimentation as well as about the current possibilities of alternative methods that are so often mistakenly argued in the public. It is true that after so many years of silence, communication to the public can be very challenging. Therefore, the plenary lecture followed by the round table will address this issue to encourage us to step in this direction.
We believe that this event will stimulate exchange of new research, progress and initiative in your field of work and give you a chance to network with new colleagues.

We would like to thank to all the speakers and all our sponsors that enabled this event.
At the same time we would like to express deepest respect and gratitude to all the animals involved in research.

We look forward to welcoming you to the 2nd Congress of Slovenian Society for Laboratory Animals.

Znan. sod. dr. Martina Perše
President of the Slovenian Society for Laboratory Animals

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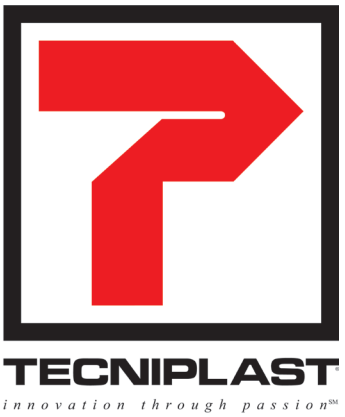
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PROGRAMME / PROGRAM

Thursday, June 19, 2014

8.00 – 9.00 **Congress registration**

9.00 – 9.15 **Congress opening**

Section 1: Microbiology (Chair: Gregor Majdič)

09.15 – 10.00 **Adrian Deeny**, UK

The shock of the new: addressing the challenges of new technologies and microbiological monitoring

10.00 – 10.30 **Biljana Hacin**, Slovenia

Diversity of murine microbiota and its role in investigating probiotic effects of lactic acid bacteria

10.30 – 10.45 **Martina Perše**, Slovenia

Effect of latent infections on the development of experimental inflammatory bowel disease

10.45 – 11.00 **Daša Ševeljević-Jaran**, Croatia

European Society of Laboratory Veterinarians (ESLAV) promotional presentation

11.00 – 11.20 **Vincenzo Spinelli**, Italy (Mikro+Polo)

Environmental and microbiological aspects of IVC system

11.20 – 11.45 **Coffee break and poster session**

Section 2: Methods in transgenesis - genetics (Chair: Simon Horvat)

11.45 – 12.30 **Oskar Ortiz Sanches**, Germany

Direct production of mutants using ZFNs, TALENs or CRISPR/Cas in one-cell embryos

12.30 – 13.00 **Laszlo Hiripi**, Hungary

New generation transgenic techniques in rabbits

13.00 – 13.15 **Duško Lainšček**, Slovenia

A synthetic gene regulatory device for autonomous sensing and suppression of inflammation

13.15 – 13.30 **Urška Kamenšek**, Slovenia

Non-invasive fluorescence imaging for monitoring promoter activity *in vivo*

13.30 – 13.45 **Jasmina Beltram**, Slovenia

Positional cloning of the Chr15 Quantitative Trait Locus *Fob3b2* affecting leanness in mice

13.45 – 15.00 **Lunch and poster session**

Section 3: Models (Chair: Manica Černe)

15.00 – 15.45 **Maja Čemažar**, Slovenia

Severity classification of procedures used in experiments on live animals in cancer research

15.45 – 16.15 **Miroslava Dominis Kramarič**, Croatia

S. pneumoniae induced pneumonia in mice as a reliable model in investigation of new chemical entities

16.15 – 16.30 **Simona Kranjc**, Slovenia

Three-dimensional cellular spheroids in oncology research - a bridge between *in vitro* and *in vivo* studies

16.30 – 16.45 **Valentina Kubale**, Slovenia

Histological adaptations of the gastrointestinal tract of immunocastrated pigs affected by varied dietary net energy content

16.45 – 17.00 **Tatjana Pirman**, Slovenia

Protein digestibility and bioavailability of the F2 homozygous crossing line of the congenic mice for the lean locus *Fob3b2*, fed by high fat diet

17.00 – 17.15 Coffee break and poster session

17.15 – 18.00 Assembly of the Slovenian society for laboratory animals (SloLASA)

18.30 – 21.30 Boat cruise on the river Ljubljanica with dinner (drink included)

Friday, June 20, 2014

8.00 – 9.00 Congress registration

Section 4: Environmental factors (Chair: Valentina Kubale Dvojmoč, Katerina Čeh)

9.00 – 9.45 **Gianpaolo Milite**, Italy

Micro and macro environment in rodents animal facilities

9.45 – 10.15 **Gregor Majdič**, Slovenia

Effect of prenatal stress on sexual and aggressive behavior of adult male mice

10.15 – 10.30 **Tanja Španič**, Slovenia

Does prenatal and early postnatal treatment with testosterone affect aggressive behavior in SF-1 knock-out mice?

10.30 – 10.45 **Vika Smerdu**, Slovenia

Effect of swimming on the muscle fibre type transitions in skeletal muscles of rats with colon carcinoma

10.45 – 11.00 **Katerina Čeh**, Slovenia

Low concentrations of coumaphos do not affect brain development and function

11.00 – 11.30 **Coffee break and poster session**

Section 5: Bioethics of animal research (Chair: Simon Horvat)

11.30 – 12. 15 **Plenary Lecture**

Wendy Jarrett, UK

Helping the public to understand animal research

12.15 – 13.45 **Round table discussion**

Aleš Belič, Friderik Klampfer, Gregor Majdič, Gregor Torkar, Igor Pribac, Manica Černe, Vegan Initiative - Representative, Urh Grošelj, Wendy Jarrett

13.45 – 14.15 **Lunch and poster session**

Section 6: Nutrition (Chair: Tatjana Pirman)

14.15 – 15.00 **Matthew Ricci**, USA

What are you feeding your lab animals? (Remote video conference lecture)

15.00 – 15.30 **Gorazd Drevenšek**, Slovenia

A model of atherosclerosis in guinea pigs induced by atherogenic diet

15.30 – 15.45 **Vida Rezar,** Slovenia

Effects of two levels of dietary hops (*Humulus lupulus*) supplementation on oxidative stress and meat quality in broilers

15.45 – 16.00 **Tina Trebušak**, Slovenia

Differences in fatty acid composition of rabbit's meat after the change of the source of fat in the diet

16.00 – 16.15 **Jelena Savici**, Romania

The outcome of potassium dichromate prepubertal exposure on chromium level and sexual hormon dynamics in male rats

16.15 **Concluding remarks and closing of the congress**

Plenary lecture

Plenarno predavanje

Helping the public to understand animal research

Wendy Jarrett

Understanding Animal Research, Hodgkin Huxley House, 30 Farringdon Lane, London EC1R 3AW,
E-mail: wjarrett@uar.org.uk

Most people can accept the need for well-regulated medical research using animals. However, we cannot assume that the current high acceptance levels will last: While Italy has seen an improvement in public support for animal research, recent opinion polling in the UK shows a slight decline in acceptance and also reveals that many people still do not trust the regulatory system; that they do not always understand why and how animals are used, and that they want to have more information before they form an opinion.

Understanding Animal Research is working to ensure that the UK public really does have all the information it needs to understand animal research. UAR has also led the work to establish a European Animal Research Association, which will facilitate the creation of advocacy and communications groups across Europe.

UAR's main achievement this year is the Concordat on Openness on Animal Research, which it developed with more than 50 stakeholder organisations, from all facets of biomedical research in the UK. The Concordat sets out the principles and practical steps that these organisations should adopt in order to be more open and honest about their work using animals.

If the public is to understand and accept the use of animals in biomedical research, people need to have the full facts. We need to be honest about the limitations of animal models, as well as celebrating the successes they have led to. We need to acknowledge that some animals suffer, while explaining our very high welfare standards. And we need to emphasise the need for more work to find alternatives to animal research.

Round table discussion: Bioethics of animal research

Simon Horvat[1,2] (moderator of the round table)

[1]Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Groblje 3, Domžale, Slovenia; [2]Department of Biotechnology, National Institute of Chemistry Slovenia, Hajdrihova 19, Ljubljana, Slovenia

The roundtable is aimed at illuminating ethical dilemmas behind the use of animals in research and exchanging different standpoints and views in contemporary debates worldwide. With this round table, our society wants to initiate regular critical debates in Slovenia and open the dialogue between the research community and other interested professionals as well as general public. Our goal is to help improve our understanding of the opposing views on issues surrounding the animal research. We are convinced that openness and broader understanding of the humane use of animals in research will have positive effects on regulatory bodies as well as on the quality of the public debate, which so far, in media often has been based on pseudoscience and misconceptions, fuelling the society of fear and anti-science sentiments. We hope to contribute to the informed coverage of the issue of animal research, and bioethics more broadly, and to pave scientific and ethical ground for a responsible public argument.

Topics discussed will involve various aspects of the ethics of using animals in research, including the ethics of not using animals in research and alternatives to animal use in research.

Invited participants are listed below in alphabetical order with their affiliation, education-profession and interest or activities relating to bioethics of animal research:

- ALEŠ BELIČ: University of Ljubljana, Faculty of Electrical Engineering, Ph.D. Electrical Engineering (Modelling of Biological Systems, Biomedical Technics)

- FRIDERIK KLAMPFER: University of Maribor, Faculty of Philosophy, Ph.D. Philosophy (Bioethics)

- GREGOR MAJDIČ: University of Ljubljana, Veterinary Faculty, Ph.D. Physiology (Animal Physiology Researcher)

- GREGOR TORKAR: University of Ljubljana, Faculty of Education, University of Nova Gorica, Faculty School of Environmental Sciences, Ph.D. Protection of Natural Heritage

- IGOR PRIBAC: University of Ljubljana, Faculty of Arts, Ph.D. Philosophy, Practical ethics-Bioethics, representative of »The Civil Initiative for Improvement of the Legal Protection of Animals«

- MANICA ČERNE: Ph.D. Veterinary Sciences (Researcher, Animal Pathology and Toxicology), President of the Slovenian Ethical Committee for Animal Research

- VEGAN INITIATIVE-REPRESENTATIVE

- URH GROŠELJ: University Children's Hospital, UMC Ljubljana (Paediatrics), M.D., Ph.D. (Biomedical Sciences), M.A. (Bioethics), (Paediatrician-Medical Scientist), Member of Slovenian National Medical Ethics Committee.

- WENDY JARRETT: Chief Executive of the Organisation »Understanding Animal Research«, Great Britain

Invited lectures

Vabljena predavanja

The shock of the new: addressing the challenges of new technologies and microbiological monitoring

Adrian Deeny

University College London, United Kingdom, E-mail: a.deeny@ucl.ac.uk

Methods used in microbiological monitoring of laboratory animals are undergoing significant change, not only in terms of laboratory procedures but also in the way samples for testing are taken. The increasing use of individually ventilated cages (IVCs) challenges accepted thinking; sentinel systems have recognised drawbacks, and new laboratory methods have become available, including increased use of PCR and the introduction of dried blood spot (DBS) technology. An understanding of the advantages and disadvantages of conventional and new technologies is crucial to correct interpretation of microbiological monitoring data. Microbiological monitoring must be adapted to new caging technologies to take advantage of these new laboratory methods, while allowing for the possibility that such methods may have yet unestablished shortcomings that could lead to false positive and negative results. Although understanding these new developments may be challenging, they represent a major step forward and provide significant 3Rs advantages through reducing the need to transport and sacrifice animals.

Diversity of murine microbiota and its role in investigating probiotic effects of lactic acid bacteria

Biljana Hacin

National Veterinary Institute, Veterinary Faculty, University of Ljubljana, Pod hrasti 18, SI-5000 Nova Gorica, Slovenia,
E-mail: biljana.hacin@vf.uni-lj.si

It has been only during recent years that the gut microbiota (GM) has received increasing attention as an important factor in the development of inflammatory disease in experimental animal models. Since increased variation in the GM might lead to increased variation in disease parameters, determining and reducing GM variation between laboratory animals may provide more consistent models. Both genetic as well as environmental aspects may influence the microbial composition that may vary between laboratory animal breeding centers and even within an individual breeding center. Variations in the microbial composition of different mouse strains have been observed as well as differences in the GM of individual mice belonging to the same strain and raised in the same breeding center.
In our work we have focused on the probiotic effect of different strains of lactic acid bacteria (LAB) in two animal disease models. We have studied the effect of *Lactobacillus gasseri* K7 on mice infected with *E. coli* O157:H7 and the effect of *Lactobacillus fermentum* BB930 and *Bifidobacterium animalis* subsp. animalis IM386 in a murine model of DSS colitis. The composition of murine microbiota was monitored throughout both experiments and histological slices of different parts of the intestine were evaluated at the end of each study. Variations in the microbial composition of individual mice within each group were observed, however no statistically significant differences were found when comparing the microbial population between groups. As expected, the addition of one LAB strain in the *E. coli* O157:H7 infection model and two LAB strains in the DSS colitis model did not result in major microbial shifts in the murine microbiota.

Effect of latent infections on the development of experimental inflammatory bowel disease

Martina Perše, Anton Cerar

Medical Experimental Centre, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Zaloška 4, Ljubljana, Slovenia,
E-mail: martina.perse@mf.uni-lj.si

Inflammatory bowel disease (IBD) is a complex multifactorial disease. Despite many years of extensive research its pathogenesis is still poorly understood. Numerous animal models of IBD have been developed in the past decades to provide a wide range of options for investigating involvement of various factors into the pathogenesis of IBD. A widely used experimental model of colitis is dextran sulphate sodium (DSS)-induced colitis model, which is known of its simplicity, the high degree of uniformity and reproducibility of the colonic lesions.

Our laboratory has experience with DSS model and is very familiar with clinical and histological features of this model. Colitis is induced by continuous administration of 3% DSS for 5 days. Clinical manifestation of DSS colitis in acute phase include weight loss, diarrhea and occult blood in stools on the 5th day of DSS treatment. In one of our experiments we recently noticed occult blood in stools of mice and even haemorrhage on the 4th day of DSS treatment. Since the observed clinical signs deviated from the usual clinical features of this model, we immediately stopped DSS treatment (one day earlier). In spite of that, mortality was present (which is usually not observed when using this protocol). Seven days after DSS treatment mice developed severe lesions in the colon mucosa, i.e. inflammation and unexpectedly large areas of erosions. Three months later epithelial architecture of colons of these mice did not recover but progressed into chronic inflammation and adenocarcinomas.

The results of microbiological and serological screening have shown that mice were infected with Mouse Hepatitis Virus, Mouse Norovirus, Helicobacter hepaticus and Helicobacter rodentium. Infections were asymptomatic in untreated mice but significatly affected susceptibility and responsiveness to DSS-induced colitis as well as the course and outcome of the disease. The aim of our presentation is to warn to the importance of monitoring of all features of an animal model as well as all the factors that can influence the results. The results demonstrate that certain microorganisms (latent infections) have important role in the pathogenesis and outcome of DSS-induced colitis and may affect validity of the results and reproducibility of DSS model when not monitored.

European Society of Laboratory Veterinarians (ESLAV) promotional presentation

Dasa Ševeljević-Jaran

ESLAV Board ordinary member, ESLAV National Representatives coordinator and New Emerging Coutries coordinator;
E-mail: dasa.seveljevicjaran@glpg.com

The European Society of Laboratory Animal Veterinarians (ESLAV) was created at the 6th FELASA Symposium held in June 1996 in Basel, Switzerland. The Society is registered as a non-profit organization in France.
ESLAV gives veterinarians a forum to discuss issues which concern them, in the field of laboratory animal science (LAS) and by doing so addresses the important issues that are the humane care and use of laboratory animals which is in domaine of equally important general public interest.
The society's objectives are to promote and disseminate expert veterinary knowledge within the field of LAS which is achieved through:

- Organisation of annual scientific meetings always in conjunction with a local LAS organisation. Also the organisation of lectures, discussions and publications (semi-annual Society's magazine ''Briefing'')
- ESLAV sets the right environment and support for the activity of the European College of Laboratory Animal Medicine (ECLAM). The College represents the academic component in our field of laboratory animal medicine.
- The advancement of veterinary knowledge and skills in subjects connected with the breeding, health, welfare and use of laboratory animals
- Collaboration and exchange of information with other Societies (LAVA, FELASA, AAALAC, FVE-EVERI, NC3Rs, AALAS, ACLAM, etc) and allied scientific disciplines. ESLAV and LAVA (Laboratory Animals Veterinary Association, division of the British Veterinary Association) are continuously in the process of building closer links.
- Active encouragement of its Members to provide training for veterinarians practicing or wishing to practice in the field of LAS, both at the under- and postgraduate level
- Representation of the veterinary "voice" at regulatory and governmental bodies. ESLAV representins the veterinary profession specifically in the laboratory animal medicine field at the European decision making bodies. ESLAV at level of European Comission actively participates by representation at Expert Working Group meetings and National Contact Point meetings regarding 2010/63/EU

Direct production of mouse mutants using ZFNs, TALENs or CRISPR/Cas in one-cell embryos

Oskar Ortiz Sanches

Institute of Developmental Genetics, Helmholtz Zentrum München, Germany, E-mail: oskar.ortiz@helmholtz-muenchen.de

Genetically engineered animals models are indispensable tool for the analysis of mammalian gene function in health and disease. Conventional gene targeting, which is performed in embryonic stem (ES) cell cultures, has been used extensively for generating mouse mutants. However, it is a time-consuming and labor-intensive procedure limited to species which established ES cell culture, which restricts the wide application of this approach.
During the last years we assisted to a new age in gene targeting field. Thanks to the engineering of high specific nucleases, like Zinc Finger Nucleases (ZFN), Transcription Activator-Like Effector nucleases (TALENs) other the most recent CRISPR/Cas system, it is possible to overcome the ES cells culture and modify every gene in almost every species. In our group we used successfully these tools for mutagenesis of the mouse genome directly in one-cell embryos, generating knock-in and knockout mice identifying the modified alleles also in their progeny.
As proof of principle we targeted different genes as Rosa26 locus, Rab38 other Fus using targeting vectors other oligodeoxynucleotides (ODNs) and RNA codifying for the nuclease directed injected into the pronucleus. With this approach an investigator could obtain a first founder mutant for genes of choice within 7 weeks after embryo microinjections. After the founders' analysis, CRISPR/Cas system revealed itself as the most powerful and easiest system to use for generating new mouse mutants.

New generation transgenic techniques in rabbits

László Hiripi

Department of Animal Biotechnology, National Agricultural Research and Innovation Centre (NARIC), H-2100 Gödöllő, Szent-Györgyi str. 4., Hungary, E-mail: hiripi@abc.hu

The laboratory rabbit is an ideal model for a variety of acquired and inherited human diseases. Some physiological and disease characteristics in rabbits are more similar to human than rodents. Prenatal development, atherosclerosis or cardiovascular diseases can be investigated more precisely in rabbit models.

Additive transgenesis of normal or mutant human genes have been used to create rabbit models for more than twenty years however with very low efficacy. Our ability to produce transgenic rabbits has been improved dramatically during the last few years as transposon mediated transgenesis has been developed. This simple and efficient non-infectious gene delivery system became a favorite method in biotechnology. We have shown that Sleeping Beauty transposon mediated transgenesis is applicable in rabbits, and at a 15% transgenic founder rate with high germline transmission, this method seems to be far more efficient than any other way of additive transgenesis in rabbit.

There was a long time existing, obvious need for rabbit models that involve not just additive transgenesis but targeted modification, genomic deletions or insertions. Novel transgenic tools such as zinc finger nucleases, TALEN nucelases and CRISP-R system were adapted to the laboratory rabbit in the last two years. All these techniques seem very promising although ZFN technology is less comfortable and flexible while CRISPR shows extreme efficiency than competitor technologies in rabbits.

All these above technologies are available in other livestock animals so new generation of transgenic rabbit and pig models might play a much more significant role in the forthcoming years.

A synthetic gene regulatory device for autonomous sensing and suppression of inflammation

Anže Smole[1], Urban Bezeljak[1], Simon Horvat[1,2], Duško Lainšček[1], Roman Jerala[1,3]

[1]Department of Biotechnology, National Institute of Chemistry Slovenia, Hajdrihova 19, Ljubljana, Slovenia; [2]Chair of Genetics, Animal Biotechnology and Immunology, Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Groblje 3, Domžale, Slovenia; [3]EN-FIST Centre of Excellence, Trg Osvobodilne Fronte 13, Ljubljana, Slovenia, E-mail: roman.jerala@ki.si

Inflammation is a general complex defense response of an organism, activated by the innate immune system and is based upon detecting danger signals, caused by infection by the pathogenic microorganism or by a sterile tissue injury. The activity of a danger signal can result in a local inflammation of the affected tissue. In some cases local inflammation can proceed to systemic form due to the cytokine signaling of the affected tissue cells or via activity of immune cells. In general the inflammation is a beneficial process because the organism fights the infection, however the beneficial inflammation may progress due to the positive feedback loops of cytokines and their receptors. Strong production of pro-inflammatory cytokines, such as TNFα, IL-1β, IL-6 and several others is characteristic for the inflammatory diseases such as inflammatory bowel disease (IBD), rheumatoid arthritis, multiple sclerosis. Currently, the most common therapy of inflammatory diseases is treatment with corticosteroids, immunosuppressive drugs and biological drugs. Corticosteroids have many adverse side effects due to non-specific inhibition of prostaglandin synthesis. Biological drugs are able to specifically and effectively inhibit the activity of pro-inflammatory cytokines, however due to high cost, they are not widely accessible.

A synthetic biology approach enables the design of engineered cells, which could act as a prosthetic artificial implant to treat various conditions. We developed a synthetic gene regulatory device in mammalian cells, which acts as a sensor of the excessive inflammation, triggering the production of anti-inflammatory effectors only as active inflammatory process is detected by the device. We have developed and prepared all the components and tested the function of the whole assembled device. We demonstrated that the synthetic anti-inflammatory device is activated by the inflammatory signal and induces local production and secretion of suppressors of inflammation while it remains silent in the absence of the excessive inflammation. Moreover, the *in vivo* experiments on mice, will be used to demonstrate that such a device could overcome obstacles of the conventional anti-inflammatory therapy.

Non-invasive fluorescence imaging for monitoring promoter activity *in vivo*

Urška Kamenšek[1], Gregor Serša[1], Maja Čemažar[1,2]

[1]Institute of Oncology Ljubljana, Zaloška cesta 2, 1000 Ljubljana, Slovenia; [2]University of Primorska, Faculty of Health Sciences, Polje 42, 6310 Izola, Slovenia, E-mail: ukamensek@onko-i.si

In vivo imaging techniques represent exciting opportunity to conduct noninvasive and longitudinal, over time, studies of dynamic biological processes. In gene therapy experiments non-invasive fluorescence imaging can be employed for following transgene expression and activity of promoters *in vivo*. Sustained and well-defined or controllable expression levels of the transgene are desired characteristics of mammalian gene expression vectors used for research or clinical purposes, thus proper choice of promoter linked to the gene of interest is essential for the success of these vectors. The cytomegalovirus (CMV) promoter is the most commonly used constitutive promoters for this purpose, in spite of reports demonstrating that its transcriptional activity can be changed under specific conditions. Therefore, the aim of our study was to evaluate the role of methylation and upregulation of the CMV promoter *in vivo* using non-invasive fluorescence imaging, which enables long-term follow-up of reporter gene fluorescence in the animals and consequently, the activity of promoters that control reporter gene expression.

Two experimental models in which expression of the reporter gene for green fluorescent protein (GFP) was controlled by CMV promoter were set up using electroporation: stably transfected experimental tumors and transiently transfected muscles. After the treatment with different agents, the activity of the CMV promoter *in vivo* was assessed by non-invasive follow-up of the intensity and duration of GFP expression.

The results of our study demonstrated that the CMV promoter can be altered by different treatments. Observed alterations in the activity of the CMV promoter limit the usefulness of this widely used promoter as a constitutive promoter and highlight the importance of proper choice of promoter linked to the gene of interest for success of gene therapy. Furthermore, we demonstrated that non-invasive fluorescence imaging is an appropriate and convenient method to monitor the activity of the promoter in vivo that also promotes the 3Rs principle. Namely in vivo imaging allows reduction of the number of animals needed to conduct the experiment (without losing information) since there is no need to sacrifice the subject at each time point to obtain the measurements. Additionally, the number of animals can be further reduced due to the fact that every subject can serve as its own control, which is a big plus in a system where inter-individual variations can pamper the results.

Positional cloning of the Chr15 Quantitative Trait Locus *Fob3b2* affecting leanness in mice

Jasmina Beltram[1], Zala Prevoršek[1], Gregor Gorjanc[1], Roderick Carter[2], Nicholas Morton[2], Simon Horvat[1,3]

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Despite rapid spreading of obesity epidemic due to the modern »obesogenic« environment, a relatively large proportion of the human population still remains lean, suggesting genetic resistance to obesity development. Our positional cloning study aimed at identifying a causal gene for the *Fob3b2* QTL that confers anti-obesity effects in a polygenic mouse model. First, fine mapping of the genetic interval of *Fob3b2* QTL by using F2 crosses of congenic lines, interval-specific haplotyping and comparative mapping confined the causal leanness effect region to a small Lean-line segment (~2 Mbp) on mouse Chr 15. Additionally, to examine genotype-diet interactions the segregated F2 population was treated with the high fat diet (HFD). Statistically significant differences ($p < 0.0001$) in body mass and various fatness traits were obtained between the FF (homozygotes for Fat alleles) and LL (homozygotes for Lean alleles) F_2 genotypes, of which more pronounced effects, were obtained in females suggesting a gene-sex interaction. Furthermore, F_2 animals homozygous for the Lean-line segment exhibited improved glucose tolerance and insulin sensitivity. Gene expression and functional analyses of the ~20 positional lean gene candidates identified the nuclear-encoded mitochondrial thiosulfate sulfur-transferase (*Tst*, rhodanese) as the only upregulated adipose-specific gene mapping to the *Fob3b2* interval. An SNP in the 3'UTR potentially affecting a miRNA binding site was revealed by sequencing of transcribed regions. Comparative sequencing of the whole *Tst* locus in F and L lines including upstream and downstream regions is also under way to identify other potentially casual SNPs in non-coding segments of the gene. Allelic mRNA expression imbalance test was performed on F2 heterozygotes in the *Fob3b2* segments, and showed significantly higher levels of the Lean-line *Tst* allele in the adipose tissue. Our genetic, transgenic and functional analyses in mice as well as results from our collaborative human genetics group strongly suggest that we identified a novel gain-of function adipose-derived lean gene potentially applicable for treatment of obesity and related metabolic disorders.

Severity classification of procedures used in experiments on live animals in cancer research

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Animal experiments are of great importance for the cancer research. The use of laboratory animals in oncology is carried out in the exploration of carcinogenesis (study of the biological mechanisms that lead to cancer), identification of potential carcinogenic agents in the environment and testing of new therapies on the experimental tumor models. Even though many *in vitro* and also molecular and biochemical tests were developed, animal testing is still needed for the launch of a clinical trials. These studies involve the conformation of the therapy efficacy on a specific tumor model; they give the insight into the drug metabolism and its normal tissue toxicity and serve as the basis for the determination of maximum tolerated dose in the phase I clinical trial. According to the European Directive 2010/63 EU, as in other field of research, also in oncology the 3Rs principle (reduction, refinement and replacement) is strictly followed. One of the prerequisites for animal experiments in cancer research is to determine the expected severity of procedures used. The severity of the effects on the animals will be dependent on the models and the purpose of the study. Furthermore, the assessment of severity within a procedure should be a continuous process beginning with initial study design (project planning), monitoring of the animals on day-to-day basis and at the completion of the experiments to assess »actual« severity level. All these will provide opportunity for further refinements for future projects.
Although new techniques, especially non-invasive *in vivo* monitoring, and material conditions enable implementation of 3Rs, one should always take into account that experimental animals with cancer demand specific animal observation, monitoring and care. During the project planning, it is important to decide on appropriate strain and get the knowledge of strain specific severity assessment. Then, the decision of monitoring tools, frequency and type of monitoring should be implemented together with the appropriate staff training and statistical planning. Only personnel with all necessary skill should be involved in the study. Appropriate documentation, consistency in observations, good communication between the personnel involved and on-going review of assessment of the procedures should be guaranteed during the project. After the project, analysis and feedback should be performed including the assessment and scoring of the actual severity with the aim to implement 3R's into future studies. Publication of the results is also of crucial importance. Severity of the procedures is classified as mild, moderate and severe. Examples of severity classification in certain procedures involved in cancer research will be presented.

S. pneumoniae induced pneumonia in mice as a reliable model in investigation of new chemical entities

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Objective: Characterization of the inflammatory and antioxidant enzyme responses to live and heat-killed *S. pneumoniae* as potential model for drug testing.
Materials & treatment: Male C57Bl6J mice received intranasally, single inoculations with either live or heat killed *S. pneumoniae* B0541.
Methods: CFUs; histological assessment of lungs; chemokines and cytokines; antioxidant enzymes, SAA in lung tissue and/or serum were assessed at different time points.
Results: Heat-killed *S. pneumoniae* resulted in a weak lung neutrophil infiltrate, associated with serum and/or lung tissue increases of CXCL1 and 2, TNFα, IL-6, GM-CSF, with later increase in CCL2 and IL-1β. Live bacteria induced more profound acute pulmonary inflammation with perivascular/peribronchial neutrophil infiltration and chemokine/cytokine elevations. Live *S. pneumoniae* induced a delayed rise in CXCL2 and CCL2, and increases in TNFα, IL-1β and IL-6, followed with mononuclear infiltration of lungs. A decrease in GPx and an increase in SOD were observed. A sustained increase in serum SAA was detected.
Conclusions: Comparing the overall responses to i.n. inoculation of live *S. pneumoniae* with those to single inoculation with heat-killed bacteria, it can be concluded that early inflammatory changes observed in both conditions are qualitatively similar, probably reflecting TLR-2 mediated responses leading to neutrophil infiltration. In response to live bacteria, neutrophil infiltration is greater, accompanied by changes in antioxidant enzymes. Delayed inflammation to live bacteria involves sequential cytokine/chemokine production and little change in tissue antioxidant enzymes. The findings indicate that the use of heat-killed bacteria is sufficient to study early effects of drugs on the innate immune response, as these will remain applicable also to the early response to live bacteria. For study of later responses and the involvement of monocytes and antioxidant mechanisms, live bacteria are essential.

Three-dimensional cellular spheroids in oncology research - a bridge between *in vitro* and *in vivo* studies

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In research of biological properties of cancer disease in organisms it is important to simulate the *in vivo* condition. Most cells in the organisms are organised in three-dimensional structures, where complex cell to cell interactions are established. Such intercellular interactions cannot be reproduced in two-dimensional cell culture monolayers and consequently reliable data for the translation in *in vivo* research cannot be obtained. On the other hand, studying intercellular interactions *in vivo* is still rather complex, and expensive. To bridge the gap, multicellular spheroids (3D cell culture model) have been designed as an *in vitro* model system to study cellular interactions in tissues (1, 2, 3). Since they are physiologically more relevant in terms of secretion of extracellular matrix, intercellular communications and signalling, regulation of cell proliferation and growth, the expression of growth factors and mimicking the structure of tissue with microenvironment, they are popular models in tissue engineering, drug development, toxicological test and in research of cancer stem cells as well as microenvironmental conditions on tumour malignancy, where the goal is to better understand tumour progression and metastasis. A number of approaches enable to provide multicellular spheroids as single culture or co-cultures of different types of cells and serve as good models for the transfer of findings and better planning of the research on animals (4, 5). Therefore, developing 3D cell culture models by retaining the *in vivo* phenotype of cells also complies with the ethical principles of animal research (3 R's: Reduction, Refinement, and Replacement) (6).
Research work in our lab, which deals mainly with preclinical studies on the development of new and combined cancer treatment, is currently focused on electrogenetherapy with antitumour and antiangiogenic effect on different types of spheroids, as well as on studying the role of reactive tumour stroma on migration and invasion of tumour cells. This type of research represent a substantial improvement of *in vitro* research, since it authentically explain the basic mechanisms of action of the tested therapies, which can be transferred furthermore to the level of research in an animal model.

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Histological adaptations of the gastrointestinal tract of immunocastrated pigs affected by varied dietary net energy content

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Introduction: Immunocastration represents an alternative to the surgical castration. Studies focused mostly on growth performance, boar taint elimination and physiological and morphological changes in immunocastrated pigs. Results of recently published meta-analysis (1) have shown that immunocastration improves fattening results. However after effective immunisation pigs considerably raise food consumption, which decreases effect of fattening compared to entire males (EM). In this case measures such as feed restriction could be applied. Energy restriction can be achieved by increasing the percentage of fibres in the diet, which can be expected to have an effect on intestinal morphology and function. The present study aimed to evaluate intestinal morphology of IM under different energy restricted diets compared to *ad libitum* fed IM and EM.
Methods: The study was conducted on 26 EM and 18 IC allocated in four treatment groups. Pigs received feed differing in NE content (EM and HNE: 11.6 MJ/kg DM, MNE: 11.1 MJ/kg DM and LNE: 10.5 MJ/kg DM) from the age of 84 days until slaughter at 172 days. Differences in NE of feed were achieved through addition of dietary fiber. Duodenum, jejunum, ileum, caecum, colon ascendens and colon descendens were sampled and stained with HE and PCNA antibodies. Measurements of intestinal villi and mucosa depth were measured in 20 microscopic fields/sample. Cell proliferation was estimated by counting PCNA positive cells. Experiment was performed in INRA Saint-Gilles facilities, France in accordance with French laws on animal experimentation (agreement of E. Labussière: n°35-110).
Results: No significant differences among treatment groups ($p<0,001$) were observed in the duodenum and caecum. In comparison to HNE the LNE diet exhibit increased cell proliferation accompanied by villous height in ileum and jejunum and crypt depth in ileum, jejunum, colon ascenders and colon descendens. No significant differences were observed between IM and EM.
Conclusions: Study indicates that intestinal morphology is altered by the diet applied. LNE and higher dietary fiber were associated with increased rate of cell proliferation, villi size and crypt depth.

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Protein digestibility and bioavailability of the F2 homozygous crossing line of the congenic mice for the lean locus *Fob3b2*, fed by high fat diet

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The aim of the study was to determine whether there are differences in protein digestibility and bioavailabillity among F2 homozygotes from a cross of congenic lines with different genetic variant for the lean locus *Fob3b2*. Both lines originate from outbred mice selected for a high proportion of fat (fat line, F), and differ genetically only for ~ 2Mbp-long section on chromosome 15 (locus Fob3b2). This locus increases leanness the *Fob3b2* allele originates from the lean line. We tested the hypothesis if the observed phenotypic differences in fatness/leanness could be the result of different protein digestibility and bioavailability. In the experiment was included 11 F2 homozygous mice, which had a section of *Fob3b2* from the fat line (M2-F2 FF) and 11 F2 homozygous mice, which had a section of *Fob3b2* from lean line (M2-F2, LL). Animals were housed in individual metabolic cages, which permitted the collection of faeces and urine separately. The experiment was divided into two parts, 7 days of pre-experimental period, during which animals get used to a new environment and 5 days when the consumed diet was weighted and collected and excreted faeces, urine and body weight weighted. In the diet, faeces and urine, the nitrogen contents were determined by the Kjeldahl method and the apparent protein digestibility and bioavailability were calculated. The average body mass of the mice was similar during the experiment. In both groups, animals consumed the same amount of diet, on average 3.0 g/day and excreted on average 0.5 g of faeces/day, which means that the apparent protein digestibility was in both groups the same, on average 93.8% and 94.0% in the M2-F2 FF and M2-F2 LL lines, respectively. Excretion of urine was on average 2.6 g/day and 2.3 g/day in M2-F2 FF and M2-F2 LL lines, respectively, so the average apparent protein bioavailability was also similar, 65.3% ± 9.6% and 65.1% ± 6.8% in M2-F2 FF and M2-F2 LL lines, respectively. According to our results, the observed phenotypic differences among homozygous F2, M2-F2 FF and M2-F2 LL lines are not due to differences in protein digestibility or bioavailability and the effect on leanness must be the consequence of the activity of *Fob3b2* locus on the cell-tissue or systemic level.

Micro and macro environment in rodents animal facilities

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There are a number of physical parameters that can be measured in rooms and cages housing rodents, all of them capable to interfere with the physiology of the animals. The micro-environment is the result of the interaction between animals, cage layout and its mode of operation, but for many years, working with open cages, the microenvironment was rarely monitored and because the continuity between the cage enclosure with the room the macro and micro environment were considered “one unit”. Today a peculiar situation is represented by Individually Ventilated Caging systems (IVCs) a true enclosure isolating very small groups of animals between them and from the room environment (macro environment)

The number of information we have on IVCs microenvironment and their positive, peculiar characteristics is disclosing a new world of knowledge, we can play with them thanks the technological control available and positively interfere with the welfare of the laboratory animals and of the workers taking care of them. The monitoring of physical parameters in a laboratory animals facility take into consideration at least: light intensity, sound, temperature and Relative Humidity. Today exactly the same parameters are monitored inside the Ventilated cages.

In addition more strictly primary enclosure (cage) related parameters are considered during environmental monitoring and the list account for: NH_3, CO_2 and O_2, due to their potential impact on animal welfare.

A detailed review of IVCs microenvironment and its impact on animal welfare will be given during the talk.

Effect of prenatal stress on sexual and aggressive behavior of adult male mice

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During prenatal development, a very important and vulnerable period of life, different external and internal factors may influence fetus development and have long lasting effects on its behavior in adult life. Prenatal stress in mice (maternal stress during gestation) has been shown to impact different behaviors of the offspring of stressed mothers in both sexes such as lateralization, sexual, aggressive, maternal and depressive-like behavior. In the present study the effect of prenatal stress (caused by injection of mothers by syringe) on male sexual and aggressive behavior in adult male mice was examined. Female C57BL/6J wild type mice were mated and checked daily for vaginal plugs. Pregnant females were housed individually and divided into three different subgroups. In the first group pregnant mice received injection through the skin on days 13, 14 and 15 of gestation, in the second group on days 17 and 18 of gestation and the third group served as a control group and was left undisturbed during pregnancy. Injections were made subcutaneously without any substance applications on the neck of the mouse. Adult male offspring of injected mothers were tested in behavioral tests of sexual and intermale aggressive behavior. The results of male sexual behavior tests indicate that male offspring from the first group were more sexually active (larger number of mounts, mounts with thrusts, intromissions and ejaculations) than males from the second group, suggesting that stress in late gestation influence development of capacity to display sexual behavior in adult life. Results from aggressive behavior tests show that male offspring from the first group were more aggressive (more attacks and bites) than males from the second group, which mostly performed aggressive grooming but very little attacks and bites. These data therefore suggests that prenatal stress due to injection of the pregnant mice has an impact on development of capacity to display proper male sexual and aggressive behavior in adult life of their male offspring, and that there might be different sensitivity to stress during different gestational periods. The results of this study show the importance of proper care for mice during gestational periods.

Does prenatal and early postnatal treatment with testosterone affect aggressive behavior in SF-1 knock-out mice?

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Aggressive behavior involves a range of various behavioral patterns and is multidimensional in terms of its origins, motivations, expressions, and functions. It is observed in many animal species, such as insects, fish, lizards, frogs, and most mammals including humans. Several parts of the brain are involved in the complexity of aggression, such as the hypothalamus, prefrontal cortex, dorsal raphe nucleus, nucleus accumbens, and the olfactory system. In rodent models it is usually classified as offensive or defensive and it is well established that testosterone is needed for full expression of intermale aggressive behavior.

In the present study, the influence of neonatal treatment with testosterone propionate (TP) on aggressive behavior in agonadal SF-1 knock-out (KO) mice was examined. Tested mice were divided into four groups: 1. TP treatment through mothers on E13 and E16; 2. TP treatment on postnatal days P1, 3 and 5; 3. TP treatment pre- and postnatally (E13, E16, P1, P3, P5); 4. Control group without TP. During testing in adulthood, they were all on TP implants for at least six days before testing. Behavioral tests for offensive aggressive behavior were performed with each mouse three times on three consecutive days.

Preliminary results are revealing that only prenatal treatment on E13 and E16 is sufficient to induce aggressive behavior in SF-1 KO mice in adulthood, suggesting that prenatal organizational effects of testosterone are sufficient for development of male typical aggressive behavior in adult mice.

Effect of swimming on the muscle fibre type transitions in skeletal muscles of rats with colon carcinoma

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The characteristics of skeletal muscles and muscle fibre types are substantially determined by myosin heavy chain (MyHC) isoforms, whose expression can be modulated by altered physiological demands. We studied the effect of swimming on fibre type transitions using a comparative analysis of MyHC expression pattern in soleus (SOL), extensor digitorum longus (EDL), gastrocnemius medialis (GM) and lateralis (GL) muscles of rat swimmers and non-swimmers with chemically induced colon carcinoma. Though it has been postulated that endurance training induces transitions from fast to slower fibre types, we found transitions in such direction only in muscles with predominance of fast fibre types except EDL in which only elevated shares of hybrid fibres were noticed. On the contrary, in slow muscles with predominance of slower fibre types (SOL, deep portion of GL) the transitions from slower towards faster fibre types were induced by swimming. The extent of transitions differed among the muscles and was obviously related to the basic or initial muscle fibre type composition and probably to its function as well.

Low concentrations of coumaphos do not affect brain development and function

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Coumaphos is an organophosphate insecticide, used for the treatment of varosis (caused by *Varroa Jacobsoni*) in beekeeping. Organophosphates (OP) with their irreversible inhibition of enzyme acetylcholinesterase as an important component of cholinergic part of the nervous system are also very harmful for humans. Besides acute toxicity of organophosphates, potentional harmful effects of prolonged exposure to low concentrations of some OP during development and in adulthood could result in an abnormal activity of brain function and consecutively contribute to the development of various mental disorders. In our study, we have examined the effects of low doses of coumaphos (1 mg/kg and 0,1 mg/kg) on brain development and function in balb/c mouse strain. The first group of mice was exposed to coumaphos throguh mothers before birth until weaning and the second group was exposed in adulthood. Adult mice were examined by standard behavioural tests as models for different neurophsyciatric disorders in humans. Anxiety related behaviours were tested with elevated plus maze test (EPM), marble burying test and open field test (OFT). Social behaviour and social memory disorders, which are one of the signs for autism by humans, were tested with social recognition test. Depression related behaviours were tested with forced swim test (FST). Results did not reveal any statistically significant differences between two groups exoposed to coumaphos and control group. In EPM, OFT and social recognition test there were statistically significant differences between sexes, as expected. Results of our study therefore suggest that prolonged neonatal exposure or adult exposure to low doses of coumaphos does not have harmful effects on brain development and/or function in mice.

What are you feeding your lab animals?

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The choice of what diet to feed your laboratory animals is very important and will have real and measurable effects on the animal's phenotype and therefore experimental data. Despite this, many researchers pay little attention to the diet even though they go to great efforts to control the temperature, humidity and assay reagents in the same experiment. Crucial to the ability to make a decision about what to feed is to understand the diet choices. There are essentially two categories of lab animal diets: grain-based (GB) chows and purified ingredient diets. GB chows, the most commonly used type of lab animal diet, are made with unrefined plant or animal ingredients such as ground corn, ground wheat, soybean meal, alfalfa meal and fish/porcine meal. Each of these ingredients contains multiple nutrients and non-nutrients, and can vary due to season and harvest location. As a result, the GB chow itself can vary from batch to batch and importantly, can expose the animals to fluctuating levels of nutrients and non-nutrients such as phytoestrogens and/or heavy metals, both of which can affect experimental data. Purified ingredient diets are made with refined ingredients that contain one main nutrient each and are commonly phytoestrogen-free. Because the ingredients are refined, purified diets contain minimal non-nutrients, have little batch to batch variation and are easier to control than GB chows. This presentation will discuss the differences in diet types using specific examples and data, with the goal of broadening researchers' knowledge about this important experimental factor.

A model of atherosclerosis in guinea pigs induced by atherogenic diet

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Introduction: Atherosclerosis is a chronic process that results in impairment of functional (endothelial dysfunction) and structural (plaque formation) arterial wall properties and progressively leads to cardiovascular events, such as myocardial infarction, critical limb ischemia and stroke. Therefore, the model enabling detailed studying of the atherogenesis and possible sites of intervention is needed. There are several animal models of atherosclerosis with different types of plaque induction: chemical, mechanical or combined. Guinea pigs were shown to be the most appropriate animal model for the study of atherosclerosis, since they have similar distribution of cholesterol and plasma lipoproteins to humans. The aim of the present study was to establish a simple and effective model of atherosclerosis in guinea pigs by proatherogenic diet for studying possible anti-atherogenic potency of drugs.
Methods: Sixteen Dunkin-Hartley guinea pigs were randomly assigned to control group (fed with standard diet- Altromin No. 3123) or to atherogenic group (fed with atherogenic diet). The latter were 8 weeks fed with atherogenic diet composed of 77 % standard diet, 1 % cholesterol, 8 % yolk powder, 5 % lard and 9 % fructose. After 8 weeks the animals were sacrificed, blood samples collected, abdominal aortas excised (for atherosclerotic plaque area determination) and thoracic aortas isolated (for endothelial function testing).
Results: Atherogenic diet significantly induced the formation of atherosclerotic plaques in abdominal aorta to 6.95 ± 0.5 % compared to control group, where the plaque area was 0.19 ± 0.02 % ($P<0.001$). Endothelium-dependent relaxation of thoracic aorta was also significantly impaired in atherogenic group compared to control group (48.2 ± 3.6 % versus 78.8 ± 2.3 %, respectively; $P<0.001$). Atherogenic diet significantly increased the concentration of total cholesterol, LDL cholesterol and triglycerides.
Conclusion: We established a simple and effective guinea pig model of atherosclerosis by feeding the animals with atherogenic diet for eight weeks. Both functional (thoracic aorta endothelium dysfunction) and structural (plaques in abdominal aorta) arterial wall changes were present, confirming the effectiveness of this model. It is useful for studying the atherosclerotic process itself and also possible interventions with pharmacological agents.

Effects of two levels of dietary hops (*Humulus lupulus*) supplementation on oxidative stress and meat quality in broilers

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Slovenian hop growing is one of the main branches in Slovenian agriculture. With ban of antibiotics in animal nutrition in EU the economics of animal production reduced and for this reason effective substitutes for it were used. Because of the needs for supplying safe food and saving the sustainable Slovenian hop growing, we did the experiment on growing chickens with the intention of research the antioxidative effect of hops and their influence on animal health, performance and meat quality. Into the nutritional study 84 broiler chickens Ross 308 were included. Animals were divided into three groups. The diets were supplemented with 7,5 % of linseed oil, which is high in n-3 PUFA. The experimental diets where as follows: 1. diet-no additives (CONT), 2. HOPS_0.9: CONT + 0.9 g hops/kg diet and 3. HOPS_3.6: CONT + 3.6 g hops/kg diet. We evaluated performance, feed consumption, animal health, oxidative stress and meat quality and oxidative stability of chicken meat. Oxidative stress *in vivo* was studied by measuring the DNA damage of blood cells, measuring malondialdehyde (MDA) in plasma, and analysing the antioxidant capacity of the lipid (ACL) and water (ACW)-soluble compounds. The hop supplementation did not influenced on chicken performance. The supplement of 3.6 g hop/kg induces MDA formation and decreased DNA fragmentation, ACL content were significantly lower in the group supplemented 3.6 g hops/kg in comparison to the group CONT. Supplementation of hop showed a tendency towards improving weight of breast muscle in the group HOPS_0.9. Further studies are suggested to confirm the results of the present study.

Differences in fatty acid composition of rabbit's meat after the change of the source of fat in the diet

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The aim of the research was to determine the changes of the fatty acids composition of rabbit's meat, if palm fat, as the source of fat in rabbit diet, was changed with linseed oil. Palm fat contains 99.5% of saturated fatty acids (SFA). Linseed oil has 71% of polyunsaturated fatty acids (PUFA), and within that 52% of α-linolenic acid (n-3 PUFA). 24 SIKA rabbits (12 males and 12 females), randomly stratified by gender and weight into two (control and experimental) homogenous group, were used in the study. The only difference in the composition of diets was source of fat, 6% of palm fat in the control group was replace with 6% linseed oil in experimental group. After 22 days of experimental period, the animals were killed and the samples of the back muscle and the hind leg muscle were weighed and stored in -70 °C till the analysis of the fatty acids by gas chromatography were performed. The fatty acid composition of the meat was changed, there were high levels of unsaturated fatty acid (74% and 73% in and hind leg muscle and back muscle, respectively) and also PUFA (48% and 45% in the hind leg muscle and back muscle, respectively), mainly on account of n-3 PUFA (17% in both muscles). Those values were in the control group significantly ($P < 0.001$) lower, 62% and 61% of unsaturated fatty acids and 35% and 34% of PUFA in the hind leg muscle and in back muscle, respectively. The biggest difference was in the levels of n-3 PUFA, 4.5% and 4.0% in the hind leg muscle and back muscle, respectively. However, high level of unsaturated fatty acids leads to a reduction of oxidative stability of meat, which could be prevented by the addition of antioxidants in the diet.

The outcome of potassium dichromate prepubertal exposure on chromium level and sexual hormon dynamics in male rats

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Hexavalent chromium has serious toxic effects on humans and animals, because it enters rapidly into cells, using sulphate transport system, and produce ROS.
The present study has the purpose to understand the effect of chromium cumulative and differentiate exposure on some male reproductive biomarkers: chromium level in sexual organs, testosterone and LH seric level.
The study was carried out initially on 8 white Wistar female rats fed on standardized normal diet and water *ad libitum*. Treatment schedule was selected to determine the effect of relative low chromium levels, doses indicated by EPA to be LOAEL for male reproductive function. Female rats were mated and after weaning 28 male pups were distributed in four groups: three experimental, exposed via drinking water from weaning until sexual maturity, E_1 - 25 ppm Cr VI (LOAEL); E_2 - 50 ppm Cr VI; E_3 - 75 ppm Cr VI; and one control group which received tap water.
At sexual maturity all individuals were sacrificed following protocols and ethical procedures. Sexual organs (testes, epididymis, seminal vesicles, prostate and bulbo-urethral glands) were used for chromium level determination - atomic absorption spectrometry (AAS – 6650 Shimadzu). Blood samples were used for testosterone and LH level determination by Tody Laboratories Bucharest (ISO 17025) - chemiluminicence method.
Results showed that: chromium concentration in sexual organs increased significantly in exposed groups comparative to control, being directly significantly correlated to exposure level (except in testicles when doubling the dose). Testosterone seric level was lower in exposed individuals comparative to control ones, significantly only at 75 ppm Cr VI, and directly correlated to exposure level. All the values were within the physiological limit (between 2-3 ng/ml). LH seric level increased in exposed groups, significantly only in E3, directly correlated to chromium level. Physiological limit hasn't been exceeded (0.5 ng/ml).

Poster presentations

Posterji

Cisplatin induced acute kidney injury mice model

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Acute kidney injury (AKI) is characterized by a rapid loss of kidney function resulting in rapid fall in glomerular filtration rate (GFR) and failure to maintain electrolyte, fluid and acid-base balance in the body, which over a period of hours or days lead into a high mortality rate.

Numerous animal models of kidney injury have been developed to evolve or evaluate potential therapeutic agents or therapeutic strategies to successfully cure or prevent high mortality caused by AKI. To test new strategies of AKI therapy, cisplatin induced animal model was established in our laboratory for the first time. Cisplatin is an effective chemotherapeutic agent used in the treatment of a wide variety of malignancies, but its use in clinical practice is limited due to its nephrotoxicity that is dose dependent and cumulative and may result in AKI in man. Although cisplatin induced mice model is one of very frequently used animal models of AKI, there are numerous protocols, which suggest high precaution when establishing this model.

Our aim was to find the appropriate mode and dose of cisplatin application to establish the model of AKI. The dosage of 17 mg/kg administered intraperitoneally (ip) was based on previous PubMed literature search and after preliminary experiment. Experiment was performed using 11- to 12-wk-old male BALB/c mice. According to the literature search we used relatively low dose of cisplatin to induce AKI. However, results showed that the administration of cisplatin at a dosage of 17 mg/kg led to high mortality. Interestingly, 48 hours after cisplatin application mice showed no clinical signs of illness only a slight drop of body weight. Functional deficits developed progressively 48 hour after cisplatin induction, reaching the peak after 96 hours, when mice showed ataxia with loss of coordination, tremor and rotating body movements after upholding their tails (very likely due to ototoxicity caused by cisplatin). At that time also gross pancytopenia due to severe myelosuppression was detected and mice developed renal failure documented by significant increase of urea and creatinine in the serum. At autopsy mice showed gastrointestinal damage with enlarged stomachs, which were full of consumed food, and almost empty small and large intestine. We regularly observed gastric hemorrhages and gastrointestinal bleeding presenting as melena. Kidneys were macroscopically pale, spleen and thymus markedly atrophic. Histological analysis revealed extensive tubular cell necrosis, loss of brush border, tubular dilatation and accumulation of PAS-positive material in the tubular lumen.

Results show that cisplatin at a dosage of 17 mg/kg causes not only severe renal tubular damage in BALB/c mice, but also produces systemic toxicity with gastrointestinal, neurologic, ototoxic and myelotoxic effects leading to almost exclusive lethality in 120 hours after administration.

Effect of probiotic strains *Lactobacillus fermentum* L930BB and *Bifidobacterium animalis* subsp. animalis IM386 on DSS colitis in mice

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Inflammatory bowel disease (IBD) is a complex multifactorial diseases that affects the quality of human life or may even progress to colorectal cancer. Despite many years of extensive research pathogenesis of IBD is still poorly understood. Today, it is believed that the interaction between microbiota and colon mucosa may be the cause of IBD. Treatment of IBD is symptomatic and often associated with adverse side effects. Therefore, other strategies of treatment are under intense investigation. Probiotics have shown to have a significant effect on the intestinal microbiota and more importantly to amelliorate the symptoms of various gastrointestinal diseases including IBD.

In our study we evaluated the effects of a probiotic preparation with *Lactobacillus fermentum* L930BB and *Bifidobacterium animalis* subsp. *animalis* IM386 on dextran sodium sulphate (DSS) induced colitis in mice. In order to prove the safety and efficacy of the selected probiotic strains we conducted a study with 7 groups of C57BL/6JOlaHsd female mice (n=10): three control groups, in which we evaluated the effects of probiotic or cyclosporin A (CsA) on the healthy colon mucosa, and four DSS treated groups for the evaluation of the effects of CsA or probiotic on mice with colitis. Selected strains were tested in two regimes: probiotic treatment of the first group started 7 days before induction, while in another group probiotics were applied simultaneously with the induction of colitis. We evaluated the effects of tested agents based on clinical, pathological and histological parameters in the late acute phase of DSS-induced colitis in female mice.

Our results show that selected probiotic strains had no adverse effects on the colonic mucosa of healthy animals. Treatment with probiotics (when animals were treated with probiotics 7 days before colitis induction) even reduced the body weight loss of animals with induced colitis. Additionally, decreased extent of colonic lesions and the depth of inflammation in the colon wall was observed in this group of animals.

Frog physiology practicum on 111, Eternal road

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Isolated tissues and organs of amphibians represent an essential object in animal physiology education for the students of experimental life sciences. Here we present the essential components of physiology practical at the Department of Biology, which is based on the use of isolated frog organs. We discuss the functional properties of frog preparations which yield them irreplacable in the practice. We also present a novel preparation of frog skin which allows for a graphic and convincing demonstration of the regulation of blood flow in the peripheral circulatory system.

Software management for laboratory animals in user's organization

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Based on legislation, Animal protection act (ZZZiv-UPB3, Official Gazette RS, no. 38/2013 and Rules on animal testing (Official Journal RS, no.37/2013), which is in accordance with Directive 2010/63/EU of the European Parliament, the breeding, supplier and user organizations for dealing with experimental animals, are obliged to ensure traceability from their birth or arrival till their sale or death. With the intention to precisely manage the prescribed data of experimental animals, custom software for the management and implementation of the experiments on animals in our user's organization was developed. The program was designed using Microsoft Access. This software allows managing and tracking of: the supply of animals, the quarantine, the integration of animals in the experiments and execution of the experiments, and the euthanasia and the removal of animal bodies. Therefore, animals that arrive in our organization receive the computer generated traceability code according to their purpose (type of license), the so called speaking code, which accompanies the animals to their final destiny. Animals, which have completed the period of quarantine, can be divided into the groups of the corresponding experiments. The software enables us to describe all procedures that were performed on used experimental animals. The final fate of the individual animal is monitored and recorded chronologically. In addition, the software allows us to search for the required data according to different criteria, and construction protocols, records and reports required by our legislation. The benefits of the software for electronically managing experimental animals are: accurately recording the status of animals in all stages of the trial, simple and fast creating of the protocols, records and reports, daily insight into the implementation of the experiments, fast checking of the use of animals under the current license and the software can be used by multiple users at the same time. In the future, the upgrade and operation of the software on the data server, Microsoft SQL Server Express, is planned.

Cholesterol is involved in regulation of dynamics and extent of Ca^{2+} dependent exocytosis

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How cholesterol, a key membrane constituent, affects membrane surface area dynamics in secretory cells is unclear. Using methyl-β-cyclodextrin (MβCD) to deplete cholesterol, we imaged melanotrophs from male Wistar rats in real-time and monitored membrane capacitance (C_m), fluctuations of which reflect exocytosis and endocytosis. Treatment with MβCD reduced cellular cholesterol and caused a dose-dependent attenuation of the Ca^{2+}-evoked increase in Cm (EC_{50} = 5.3 mM) vs. untreated cells. Cytosol dialysis of MβCD enhanced the attenuation of Cm increase (EC_{50} = 3.3 mM), suggesting cholesterol depletion at intracellular membrane sites was involved in attenuating exocytosis. Acute extracellular application of MβCD resulted in an immediate C_m decline, which correlated well with the cellular surface area decrease, indicating the involvement of cholesterol in the regulation of membrane surface area dynamics. This decline in C_m was three-fold slower than MβCD-mediated fluorescent cholesterol decay, implying that the exocytosis is a strong physiological means for plasma membrane cholesterol replenishment. MβCD had no effect on the specific C_m and the blockade of endocytosis by Dyngo 4a, confirmed by inhibition of dextran uptake, also had no effect on the time-course of MβCD-induced C_m decline. Thus acute exposure to MβCD evokes a C_m decline linked to the removal of membrane cholesterol, which cannot be compensated for by exocytosis. Furthermore, by using high-resolution cell-attached membrane capacitance measurements, we have monitored unitary exocytic events in cholesterol-depleted membranes of rat pituitary lactotrophs. We show that the frequency of these events is attenuated, providing evidence at the single vesicle level that cholesterol directly influences the merger of the vesicle and the plasma membranes. Together these results suggest that cholesterol is required at prefusion and fusion steps of regulated exocytosis.

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Astrocytic vesicle mobility in physiologic and pathologic conditions

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The view of how astrocytes, which are a type of glial cells, contribute to the functioning of the central nervous system has changed significantly in the last decade. Astrocytes are no longer considered subservient to neurons, and are, instead, now understood to play an active role in brain signaling. This role was long considered to be exclusively present in neurons. The intercellular communication of astrocytes with neurons and other non-neuronal cells involves the exchange of molecules by exocytotic and endocytotic processes through the trafficking of intracellular vesicles. Vesicles are delivered to and are removed from the site of exocytosis by an amazingly complex set of processes that we have only started to learn about recently. Recent studies of single vesicle mobility in astrocytes have prompted new views of how astrocytes contribute to information processing in nervous tissue. Here, we assessed the trafficking of several types of membrane-bound vesicles that are specifically involved in the processes of (i) intercellular communication by gliotransmitters (glutamate, adenosine 5'-triphosphate, atrial natriuretic peptide), (ii) plasma membrane exchange of transporters and receptors (EAAT2, MHC-II), and (iii) the involvement of vesicle mobility carrying aquaporins (specifically AQP4) in water homeostasis. The properties of vesicle traffic in astrocytes are discussed in respect to networking with neighboring cells in physiologic and pathologic conditions, such as amyotrophic lateral sclerosis, multiple sclerosis, and states in which astrocytes contribute to neuroinflammatory conditions.

Titles of Papers:

1. Potokar M et al., Glia, 2013, vol. 61, iss. 6, pgs. 917-28.
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Astrocyte-derived neurotrophin-3 mediates the neuroprotective effect of resveratrol: involvement of estrogen receptors

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Astrocytes actively control neuronal activity and synaptic transmission and by producing various neurotrophic factors represent an important local cellular source of trophic support in the normal and diseased brain. By expressing estrogen receptors (ER) ERα and ERβ they may play role in the estrogenic effects. Resveratrol is a phytoestrogen in grapes and red wine with a pronounced neuroprotective activity that result from activation of numerous cell-signaling pathways. The present study examined the regulatory effect and underlying mechanisms of resveratrol-induced synthesis of neurotrophin-3 (NT-3) in cultured rat astrocytes. Resveratrol was able to potently and transiently increase NT-3 mRNA, NT-3 protein content and NT-3 secretion. Its stimulation was mimicked by 17ß-estradiol and attenuated by nonselective ER antagonist, ICI 182,780. The ER dependency was confirmed by partial inhibition of resveratrol effect by ER antagonists selective for ERα (MPP) and for ERβ (PHTPP) and complete block by a combination of both antagonists. The effect of resveratrol appeared to involve mediation by PLC/PKC signal transduction route, CaMK II and MAP kinase activity as well as phosphotidylinositol 3-kinase (PI3K)/Akt signaling pathway, because pharmacological inhibition of these pathways prevented resveratrol-induced NT-3 production. These results indicate that the examined neuroprotective effect of resveratrol on astrocytes is mediated by the estrogen receptors, suggesting that estrogenic effects must be considered in the complex polypharmacology of resveratrol.

Determination of end diastolic pressure volume relationship by measuring pressure-volume loops of the left ventricle in rats

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The aim of our study was to determine the end diastolic pressure volume relationship (EDPVR) of the left ventricle (LV) in rats.
Male, Wistar rats (n=10, 240-270g) were used in these experiments, anesthetized with a mixture of ketamine (100 mg/kg) and Xylazinhydrochlorid (0,65mL/kg). The trachea was exposed, cut and connected to the rodent ventilator (Harvard, model 683) for artificial ventilation with tidal volume of 2.5 ml at 95 cycles/min. The chest was opened through a midline incision and the pericardium was dissected to expose the heart. Two micromanometer (Millar 2F, Millar instruments, Houston, Tex.) were inserted, first into the right carotid artery to monitor arterial pressure and second directly into the LV through the apex to measure the LV pressure. Six ultrasonic crystals (1 mm, Sonometrics, London, Otario) were attached to the LV epicardium using cyanocryate adhesive (Vetbond, 3M, Animal Care Product) to measure the changes in LV short and long axis. The descending aorta was occluded transiently to increase afterload and to shift the LV pressure-volume loops toward smaller and greater LV volumes.
After a brief period of stabilization, arterial pressure, LV pressure and LV dimensions were recorded simultaneously at sampling rate of 2000 Hz at baseline and during aorta occlusion. The ventilator was stopped during data acquisition to eliminate effect of positive pressure ventilation.
All data were stored on a computer for off-line analysis by use of commercialy available software (Sonolab; Sonometric Co., London Ontario, Canada). The volume of the LV was calculated using the two axes ellipsoid heart model and the rate of LV pressure increase (dP/dt) was determined. The LV end diastolic pressure (LVEDP) was defined as the pressure in the LV just prior to the dP/dt rising above 100 mmHg/s. Pairs of LVEDP and corresponding LV volume were determined and represented as EDPVR.

Effects of ibogaine on glial-derived neurotrophic factor signalling in rat brain

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Ibogaine is a Tabernanthe iboga root bark-derived alkaloid with a centuries-long history of use in Bwiti initation rituals and ethno-medicine of the equatorial Africa. In human case series, ibogaine was shown to facilitate addiction withdrawal. In animal models of addiction, a single systemic dose of ibogaine (or its metabolite, noribogaine) was shown to cause reduced self-administration of alcohol and opiates and this effect was recently ascribed to increased dopaminergic trophic factor (glial-derived neurotrophic factor, GDNF) synthesis and signalling, leading to neuroplastic changes in the brain reward network. Beyond addiction, possible increased signalling via the GDNF system would be therapeutically relevant for neurodegenerative conditions like parkinsonism, which have been shown to respond favorably to GDNF.

The aim of our study is to test the hypothesis that exposure to ibogaine leads to measurable changes in GDNF via its receptor complex (GFRa1/Ret) signalling. We are looking at primary cell cultures of rat astrocytes and neurons (incubated with various concentrations of ibogaine), and at rat brain tissue sections collected in experiments employing systemic (intraperitoneal) ibogaine injection to determine transcriptional and translational changes in the GDNF signalling system under ibogaine influence.

The experimental work is under way and the preliminary results will be presented.

Chronic treatment with LEK8829 for further characterisation of its putative antipsychotic and antiparkinsonic propreties

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LEK-8829 has D2 antagonistic and D1 agonistic properties on dopaminergic transmission and thus paradoxically posses both antipsychotic and antiparkinsonic potential. Prolonged treatment with antypsichotic drugs, such as D2 antagonist haloperidol often produces unwanted inhibitory psychomotor effects, while excessive stimulation of dopaminergic transmission by indirectly acting dopaminergic agonists, such as amphetamine or D2 agonist bromocriptine may induce unwanted stimulatory psychomotor effects. It has been thus proposed that the unusual antagonistic/agonistic dopaminergic profile of LEK-8829 may confer a lower propensity of LEK-8829 for the induction of above mentioned unwanted psychomotor effects. The aim of our study was for the first time to evaluate the effects of prolonged pretreatment with LEK-8829 on the expression acutely evoked psychomotor behavioral parameters. We used rats with intact dopaminergic transmission and compared the effects of daily treatment for 21 days with LEK-8829 or with antipsychotic haloperidol and reserpinized rats with impaired dopaminergic transmission and compared the effects of daily treatment for 10 days with LEK-8829 or with antiparkinsonic bromoergocriptine. We found that in rats with intact dopaminergic transmission, LEK-8829, as opposed to haloperidol, significantly increased its potency for the induction of catalepsy and did not diminish its potency for the inhibition of amphetamine-stimulated open field behavior. In rats with impaired dopaminergic transmission, LEK-8829, as opposed to bromoergocriptine, induced a significant, D1 receptor mediated psychomotoric sensitization, as determined by progressively enhanced locomotor and stereotyped behavior of rats in the open field test that could be prevented by D1 receptor antagonist SCH23390. We conclude that depending on the intact or impaired status of dopaminergic transmission, prolonged daily treatment with LEK-8829 could either enhance its inhibitory effects on D2 receptor mediated behavior or its stimulatory effects on D1 receptor mediated mediated behavior, respectively.

Role of endothelin B receptors in bone modelling during orthodontic tooth movement in rats deficient for gene for ET_B receptor

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Bone modelling is the key factor in orthodontic tooth movement (OTM). Many local and systemic mediators are released when force is applied. Among them are also cytokines which play an important role in the mechanism of OTM. Endothelins are the most potent endogenous vasoconstrictors which comprise a group of three isopeptides. They act through two endothelin receptor subtypes: endothelin A (ET_A) and endothelin B receptor (ET_B). ET-1 was shown to increase osteoclastic resorption in the linear phase of OTM in rats via ET_A receptors. The role of ET_B receptoris is not known yet. There are no specific ET_B antagonists which can be used for "in vivo" studies, so the animals deficient for gene for ET_B receptors were used. The aim of the present study was to determine the role of ET_B receptors in bone modelling in OTM. The study was performed on male Wistar rats (N=39) which were divided into 4 groups: group I and III consisted of Wistar rats deficient for gene for ET_B receptors with DßH gene inclusion (KOETB rats). In the group II and IV the animals with the gene for ET_B receptors and DßH gene inclusion were included (KOBWT rats). The animals of group I and II were applied an appliance consisted of super elastic closed coil spring between the upper left second molar and upper incisors. On a day 0, 7, 14, 28, and 35 the distance between the most mesial point of the upper left first molar and the most palatal point of the ipsilateral incisor at the gingival level was measured by digitronic caliper. Application of the appliances and measurements were done under general anesthesia (ketamine 50 mg/kg; medetomidine 67 µg/kg; thiopental 3 mg/kg; all i.p.). After 5 weeks the animals were sacrificed and samples of the maxilla containing all three molars were taken for bone histological analysis. The amount of tooth movement in KOETB animals was significantly less on day 7*, 14*, 28** and 35** (* $p<0.01$; ** $p<0.05$) when compared to KOBWT group. Alveolar bone volume covered by osteoblasts on pressure side was not significantly different in KOETB animals (4.7%± 1.2) compared to KOBWT animals (5.0%±1.6). Alveolar bone volume covered by osteoclasts was significantly higher in KOETB animals (5.0%±1.6) then in KOBWT animals (0.63± 0.10) ($p<0.05$). According to the results ET_A and ET_B receptors are included in bone modelling during OTM in rats. Wistar animals deficient in gene for ET_B receptor showed some particularities during breeding – their growth and development were diminished. When they survived their reactions were close to normal – so there must be some parallel systems which take the role of ET_B receptor. Rats deficient for gene for ET_B receptor found to be an appropriate model for studying the role of ET_B receptors in different physiological and pathological processes.

Acute hyperglycemia affects the activity of endothelial nitric oxide synthase by inducing changes in its fosforylation

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Diabetes is known to induce endothelial dysfunction of micro- and macrocirculation in different animal species as well as in humans. It has been shown that even transient increases in plasma glucose concentrations impair the endothelium-dependent vasodilation presumably due to altered activity of endothelial nitric oxide synthase (eNOS). Besides from cytosolic Ca^{2+}, one of the main regulator of eNOS activity is the fosforylation of the specific aminoacid residues: Ser1177 and Thr459. Their fosforylation is regulated reciprocally: while the Ser1177 fosforylation increases eNOS activity, the Thr 459 fosforylation reduces it. Thus, the aim of our study was to assess the effect of acute hyperglycemia on endothelium-dependent, NO-mediated vasodilatation in rat aorta and to elucidate possible involvement of alterations of eNOS fosforylation on the two specific sites. In isolated, precontracted, endothelium-intact rat aortic rings, incubated with diclofenac (0,01 mM) to block cyclooxygenase, 30 minutes exposure to high glucose concentration (30 mM) caused a rightward shift in the concentration-relaxation curve to acetylcholine (ACh), as compared to 5 mM -glucose solution or 30 mM-mannitol solution (pD2=7,16±0,07 for 5mM glucose and 6,61±0,14 for 30 mM glucose solution, $p \leq 0,01$, one-way ANOVA). In the culture of freshly isolated aortic endothelial cells, hyperglycemia increased the fosforylation of the Ser1177 residue and decreased the fosforylation of the Thr 459 residue, as assessed by Western blot analysis, pointing to alterations of eNOS activity. The effect of Ca^{2+} increase could be excluded as the culture was treated with EDTA. The results show that acute hyperglycemia diminishes the endothelium-dependent, NO- mediated relaxation in rat aorta. There is indirect evidence that diminished relaxation might be the consequence of altered eNOS activity due to alterations in eNOS fosforylation.