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Recent doctoral theses (biochemistry, biology and biophysics) in Lithuania

Prepared by Indrė LIPATOVA

ANALYSIS OF LIGAND BINDING TO RECOMBINANT HUMAN CARBONIC ANHYDRASES I, II, VII, IX AND XIII

Lina Baranauskienė

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The dissertation defended:
15 March 2013

Carbonic anhydrases (CAs) are metalloenzymes that catalyze the conversion between carbon dioxide and bicarbonate. Their inhibition can be applied for treatment of different diseases, such as glaucoma, cancer, obesity, epilepsy, osteoporosis, etc. There are nearly 30 small molecule ligands that are used as drugs for carbonic anhydrase related diseases. In this work interaction between recombinant human carbonic anhydrases I, II, VII, IX, XIII and sulfonamide ligands was analysed. Stability of selected carbonic anhydrases was evaluated in different experimental conditions. Oligomeric structure of anticancer target CA IX was determined. Using carbonic anhydrases as model proteins, the application range of thermal shift assay was extended. Binding parameters of 40 new compounds to human carbonic anhydrases were measured. The binding thermodynamics of sulfonamide ligands to CA XIII was analyzed and intrinsic binding parameters, independent of the experimental conditions and linked protonation reactions, were determined.

INVESTIGATION OF PROPERTIES OF LIPID LIQUID CRYSTALLINE DRUG CARRIERS AND THEIR INTERACTIONS WITH MODEL CELL MEMBRANES

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The dissertation defended:
6 June 2013

The aim of this work was to study structural features of lipid liquid crystalline (LC) drug carriers based on soya phosphatidylcholine (SPC), glycerol dioleate (GDO) and water and their interactions with different surfaces. In this thesis lipid SPC/GDO LC phases and dispersed nanoparticles (LCNPs) were described. The phase behaviour was studied at the limited and full hydration conditions. The link between LC structure and the function of drug release was presented. The interactions with different model surfaces were designed. It was found that by changing the ratio of SPC and GDO it is possible to control and vary the sustained release of the encapsulated material. This work revealed that LCNPs of SPC/GDO mixtures have low haemolytic activity, and more likely than others investigated LCNPs fit for an intravenous drug delivery. For the first time the adsorption properties of these nanoparticles on different modified silica surfaces were investigated. By changing the surface properties it is possible to control the adsorption. *In vitro* studies of LCNPs enable to prognosticate their behaviour *in vivo*. These results will decide on further use of the studied lipid mixtures as release systems of bioactive materials. It will be easier to programme and construct the drug carrier of desirable properties.

INVESTIGATION OF TETRAMETHYLPYRAZINE DEGRADATION IN RHODOCOCCUS SP. TMP1 BACTERIA

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The dissertation defended:
28 June 2013

The catabolism of alkylpyrazines is poorly described. The pathways for the degradation of di- and tri-substituted pyrazines have been proposed, but these related routes consistently include a hydroxylation step that cannot be performed on tetramethylpyrazine. Here for the first time we describe the catabolic pathway of tetramethylpyrazine in tetramethylpyrazine-degrading Rhodococcus jostii TMP1 strain. MS/MS analysis of the protein primarily upregulated by tetramethylpyrazine led to the identification of the gene locus encoding proteins required for the initial steps of tetrametylpyrazine degradation and for the regulation of this locus. Tetramethylpyrazine degradation starts with oxidative ring cleavage catalysed by monooxygenase TpdAB, which produces (Z)-N,N'-(but-2-ene-2,3-diyl)diacetamide. This compound is further hydrolysed by amidase TpdC to N-(3-oxobutan-2-yl)acetamide. TpdE was confirmed to be an aminoalcohol dehydrogenase yielding N-(3-hydroxybutan-2-yl)acetamide. By determining intermediates, enzymes involved and genes responsible for tetramethylpyrazine degradation we provide the first validated pathway for pyrazine degradation. We also report that Rhodococcus jostii TMP1 is capable of modifying various alkylpyrazines and alkylpyridines and can be employed for the bioconversion of 2,4,6-trimethylpyridine and 2,4,6-trimethylpyridin-3-ol biosynthesis.

TRANSFERABLE ANTIBIOTIC RESISTANCE DETERMINANTS IN GRAM NEGATIVE BACTERIAL PATHOGENS

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The dissertation defended:
6 September 2013

The prevalence and nature of elements responsible for antibiotic resistance-associated genes transmission among Gram negative bacteria in clinical and agricultural environments in Lithuania are largely unknown. Herein, molecular epidemiology study of 859 Gram negative bacterial isolates from Lithuanian hospitals and farms mostly focusing on the characteristics of antibiotic resistance-associated transferable elements, integrons and plasmids, is presented. Three novel gene cassette arrays of class 1 integron were elucidated, among them gene coding for a new OXA-type β-lactamase, OXA-205, was determined. Certain integron types between isolates from different sources suggested the possibility for horizontal transfer of antibiotic resistance genes. The analysis of genotyping results revealed that majority of *A. baumannii* isolates from all hospitals belonged to European clonal lineages I and II. Clonal spread of ECII strains with a newly observed plasmid pAB120 carrying two copies of OXA-72 carbapenemase coding gene was largely responsible for a dramatic increase in the rate of carbapenem resistant *A. baumannii* in the country tertiary care hospitals. The abundance of XerC/XerD recognition sites in pAB120 suggested that Xer recombination might play an important role in the rearrangement of plasmids via common genetic platforms. The genetic data obtained in this work could serve as a basis for the further monitoring of the dissemination of antibiotic resistance elements.

INVESTIGATION OF DIFFERENTIATED MUSCLE-DERIVED STEM CELL DEATH AND SURVIVAL SIGNALLING MECHANISMS

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The dissertation defended:
27 September 2013

The present study demonstrates that adult muscle-derived stem cells with unlimited proliferative potential *in vitro* are able to differentiate into myogenic, adipogenic, osteogenic and neurogenic lineages. Differences in susceptibility to apoptotic stimuli depending on cell differentiation status and direction were shown in muscle-derived stem cells. The results prove that protein kinase Akt and ERK, p38, MAPK promote survival of differentiated cells during apoptotic treatments. The role of JNK kinase in apoptosis may depend on cell differentiation stage and type of apoptotic stimuli. We have proved that JNK has a potential to regulate pro- and antiapoptotic Bcl-2 family protein level in these cells. We have also shown the ability of JNK to regulate pro-survival Akt kinase phosphorylation level in differentiated cells. In conclusion, our results show that modulation of Akt and MAP kinases activity in accordance with muscle stem cell differentiation stage and apoptotic treatment can enhance the survival of cells during chemotherapeutic treatment and improve the effectiveness of stem cell therapy.

OXIDOREDUCTASES IN BIOELECTROCHEMICAL SYSTEMS: INVESTIGATION AND APPLICATION

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The dissertation defended:
27 September 2013

The aim of this work was to study the action of pyrroloquinoline quinone (PQQ) dependent oxidoreductases in homogeneous and heterogeneous ambiences and to create new bioelectrocatalytic systems based on these enzymes. Bioelectrochemical systems with PQQ dependent alcohol (sADH and mADH), glucose (GDH) and fructose (FDH) dehydrogenases were constructed by using new electrode materials, enzyme immobilization techniques and electron transfer (ET) mediators. Enzymes and systems were studied by different electrochemical methods and atomic force microscopy. pKa values and ET pathways in bioelectrochemical systems were determined for sADH and mADH. The main characteristics of systems and influence of heterogeneous ambience to the specificity of the enzymes were determined. The GDH immobilization method, which ensures enzyme activity up to 9 months, was created. The direct ET from reduced enzymes active sites to poly(N-(N',N'-diethyldithiocarbamoylethylamidoethyl) aniline) and graphite oxidation products was revealed for the first time. It was observed that 2-(3-nitro(phenyl)amino)-ciclohexa-2,5-dien-1,4-dione is the most effective mediator for FDH. The ability of bioamperometric systems with FDH to oxidize D(-)tagatose was determined for the first time. It was shown that bioamperometric systems based on PQQ dependent enzymes can be applied for detection of alcohols, carbohydrates and carbon monoxide.

METHYLTRANSFERASES AS TOOLS FOR SEQUENCE-SPECIFIC LABELING OF RNA AND DNA

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The dissertation defended:
5 December 2013

Investigation of RNA and DNA function often requires sequence-specific incorporation of various reporter and affinity probes. This can be achieved using AdoMet-dependent methyltransferases (MTases) as they can be active with synthetic AdoMet analogues equipped with transferable chains larger than the methyl group. These chains usually carry reactive groups that can be further chemically appended with required reporters. For this, azide-alkyne 1,3-cycloaddition (AAC), also called "click", reaction is particularly attractive. This work shows that the HhaI cytosine-5 DNA MTase (variant Q82A/Y254S/N204A) catalyzes an efficient sequence-specific transfer of hex-2-ynyl side chains containing terminal alkyne or azide groups from synthetic cofactor analogues to DNA. Both the enzymatic transfer and subsequent "click" coupling of a fluorophore can be performed even in cell lysates. For RNA labeling, the activity of an archaeal RNA 2'-O-MTase C/D ribonucleoprotein complex (RNP) with synthetic cofactors was investigated. It was shown that synthetically reprogrammed guide RNA sequences can be used to direct the C/D RNP-dependent transfer of a prop-2-ynyl group to predetermined nucleotides in substrate RNAs. Followed by AAC this can be used for programmable sequence-specific labeling of a variety of RNA substrates *in vitro*. These new possibilities for specific labeling of nucleic acids can be adopted in biochemistry, biomedical, nanotechnology, etc. research.

INVESTIGATION OF THE IMPACT OF ENDORIBONUCLEASES ON BACTERIOPHAGE T4 EARLY TRANSCRIPTS

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The dissertation defended:
17 December 2013

Phage T4 has developed a complex mechanism of ribonucleases control which needs yet to be investigated. Apart from the impact of endoribonuclease RegB, no other effects of *Escherichia coli*- or phage-encoded proteins are known to be involved in the degradation of early mRNAs. This study has aimed to identify *E. coli* endoribonucleases that are involved in secondary processing in RegB-cleaved T4 mRNAs and to determine what phage T4-encoded factors affect the activity of these enzymes. We have shown that the endonucleolytic events at secondary sites of RegB-processed transcripts involve RNases G and E. The RNase G appears to be the main ribonuclease that cleaves all known secondary targets. Moreover, the revealed targets are the first RNase G targets identified in the bacteriophage T4 mRNA. This study has revealed that RNase G can be covalently modified during the infection cycle of bacteriophage T4. However, such modifications do not affect its activity related to the origin of secondary cuts in RegB-processed T4 mRNA. Another important finding is that T4K10 phage encodes defective polynucleotidkinase (PNK). In this study, we have shown that the G14D mutation of phage T4K10 PNK impairs 5'-kinase activity *in vivo*, as well as *in vitro*, and leads to the diminished processing of RegB-cleaved transcripts. This study has revealed that both the T4 RNase RegB- and PNK-mediated activity of the *E. coli* RNases E and G are designed to accelerate degradation of phage T4 early transcripts.

A STUDY OF TUMOR SUPPRESSOR GENE EXPRESSION AND PROMOTER METHYLATION FOR THE IDENTIFICATION OF PROGNOSTIC MARKERS IN GLIOBLASTOMA

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The dissertation defended:
16 April 2013

Glioblastoma (GBM) is the most common primary brain tumor in adults. This study attempted to identify the genes potentially regulated by promoter methylation in GBM. Therefore, we analyzed the expression of COX7A1, SPINT1, AREG, NPTX2, and KRT81 in glioblastomas and human brain tissue and investigated if there were any associations between the expression and methylation of these genes. Moreover, we aimed to determine the methylation frequency of 11 genes (AREG, CASP8, CD81, DcR1, DR4, GATA4, GATA6, hMLH1, NPTX2, TES, and TFPI2) promoters in 100 patients with glioblastoma multiforme and to evaluate the associations between patients' clinical characteristics and prognostic value. The methylation status of the following 4 gene promoters was significantly related to patients' survival after surgery: AREG, CASP8, GATA6 and TFPI2. Identification of the methylation status of these genes could be one of the objective criteria in the prognosis of disease course in patients with glioblastoma and could supplement the list of already known epigenetic markers. The methylation status of a combination of 6 genes (AREG, CASP8, DR4, GATA4, GATA6, and TFPI2) was found to be a more accurate independent prognostic factor associated with patients' survival after surgery when compared with the methylation status of individual genes. The molecular classification of GBM according to the methylation profile of a combination of these 6 genes could help clinicians tailor an appropriate treatment strategy.

MUSCLE DAMAGE DEPENDENCE ON TRAINING LOAD PROGRESSION STRATEGY, SPORTS SPECIALIZATION AND GENOTYPE

Audrius Sniečkus

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The dissertation defended:
14 May 2013

The aim of the research was to determine the effects of muscle damage evoking eccentric exercise depending on the training load progression strategy, sports specialization, and ACTN3 genotype. We have established that two different strategies of exercise load progression, while maintaining identical total training volume and the same training frequency, results in divergent dynamics of muscle damage and end-point adaptation of muscular function. Namely, rapid stepwise increase in training stimulus periodically induces more pronounced muscle damage and is superior in improving muscle function in comparison to the training programme of continuous progression of the same load over the same time period. We have also confirmed that while sport training renders muscle tissue more resistant to eccentric exercise induced damage, its type is of minor significance in this respect at best. Specifically, for the first time we have shown that endurance cyclist (whose training is almost exclusively composed of concentric muscle contractions) and distance runners (inevitably integral and major part of whose training involves eccentric contractions of the same muscles as in cycling) are equally superior in resisting eccentric exercise induced damage. Contrary to the results of several other studies, we have shown that XX rather than RR genotype of the ACTN3 provides a better resistance to exercise induced muscle damage. Although the effects of the ACTN3 genotype on the deterioration of muscle function in response to drop jump bout were moderate and manifested primarily immediately after the cessation of the exercise, we have also shown that the recovery was faster in XX homozygotes. According to the results of our research, it could be advised to avoid application of the continuous progression of exercise loads and rather to try a strategy of more sporadic (intermittently jerky) load increments. The latter is intended to invoke repeated muscle damage and therefore a superior adaptation in long-term. High-level distance runners and road cyclists as well as ACTN3 XX genotype subjects have better resilience to exercise bouts inflicting muscle damage, which presumably allows them to favour more frequent training sessions in maximizing the long-term adaptation.

DEVELOPMENT OF A MULTIPLE-RUN HIGH-RESOLUTION MELTING ASSAY FOR *SALMONELLA* SPP. GENOTYPING

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The dissertation defended:
31 May 2013

The aim of the recent study was to develop a new High-Resolution DNA melting (HRM) based rapid and reliable *Salmonella* spp. typing method that can overcome the disadvantages associated with the other typing techniques. At the beginning of procedure development proper seven targets for the HRM analysis were selected. The target amplification and the HRM analysis were optimized. It was not possible to include all tested *Salmonella* spp. strains in the single run. Consequently, the difference analysis was performed for the HRM data collected outside of run files in the same MS Excel data table. Prior to the data collection, the essential variation or the error among the runs was observed. The reference sample for the HRM inter-run error evaluation was constructed. The calculations for the error minimization between the runs were applied. The fluorescence level of the resulted inter-run normalized HRM data also has to be normalized. All 134 *Salmonella* spp. samples of 20 serotypes were successfully genotyped using the selected targets and the suggested procedures for the multiple-run HRM data. The 45 of unique genotypes were obtained for the tested strains. The developed technique is a quick and robust multiple run HRM based *Salmonella* spp. genotyping method. It allows to obtain a high typeability, reproducibility, discriminatory power and can be adopted for routing genotyping of *Salmonella* spp.

MECHANISMS OF THE REGULATION OF GAP JUNCTION CONDUCTANCE

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The dissertation defended:
7 June 2013

Gap junctions (GJ) are intercellular channels, which provide a direct pathway for electrical and metabolic cell-to-cell communication. GJ play an important role in cell proliferation, differentiation, migration, and coordination. The family of connexin (Cx) genes consists of 21 member in the human genome, and a variety of diseases is associated with Cx mutations. Electrical conductance (gj) of gap junctions is regulated by transjunctional voltage, intracellular pH (pHi), intracellular divalent cation concentration ([Ca2+]i and [Mg2+]i), phosphorylation and different chemical compounds. In this work we demonstrate for the first time that the potency of widely used GJ inhibitors such as alkanols, forane and mefloquine in GJ channel uncoupling is pHi- and Cx type-dependent. This type of modulation may occur through the formation of hydrogen bonds between uncouplers and histidines of Cx45 protein. We determined gj-pHi dependence of Cx36, Cx40, Cx43, Cx45 and Cx47 GJ channels, and evaluated the influence of [Mg2+]i on the conductance of Cx43 and Cx45 GJs and on the sensitivity of Cx36 GJs to pHi. The investigation of the Cx45 and Cx36 mutants provided with new knowledge on the structural elements, which may be responsible for the sensitivity of Cxs to pHi.

DYNAMICS OF MOTOR CONTROL RECOVERY AFTER KNEE ANTERIOR CRUCIATE LIGAMENT RECONSTRUCTION

Vilma Jurevičienė

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The dissertation defended:
28 June 2013

The aim of the research was to establish the changes in motor control after undergoing knee anterior cruciate ligament reconstruction with the application of conventional rehabilitation. After a conducted reconstruction of the knee joint anterior cruciate ligament with the application of conventional rehabilitation, the dynamics of movement control recovery was established which had not been investigated by other scholars, namely, the knee joint ACL reconstruction and the conventional rehabilitation increase the accuracy of the joint position sense which, 9 weeks after the surgery, reaches the pre-surgery levels, and 13, 17 and 21 weeks after the surgery, gradually increases in accuracy but does not fully recover yet. During the research, the accuracy of leg movements was evaluated by applying the original stable knee flexion test which allowed the identification of the most accuracy movements of a leg and the shortest duration of a movement. The accuracy of leg movements 5, 9 and 13 weeks after the surgery decreases while 17 and 21 weeks after the surgery it is comparable to pre-surgery levels, yet it does not reach the levels of the healthy leg. When the knee extensor muscles are at long length, 13 weeks after the surgery, the accuracy of the force decreases and the variability rate increases; besides, the accuracy of force is restored earlier (17 weeks after the surgery) than its variability rate (21 weeks after the surgery). The awareness of the dynamics of the movement control restoration after the performed knee joint anterior cruciate ligament reconstruction expands the knowledge of physiotherapy, rehabilitation and the physiology of nerves and muscles. The knowledge in the above-mentioned fields may be applied when developing rehabilitation programs or treating patients diagnosed with the tear of knee joint anterior cruciate ligament that underwent reconstructive surgery.

EVALUATION OF THE EFFECTS OF LEAD, NICKEL AND ZINC IONS ON THE LIVER OF LABORATORY MICE

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The dissertation defended:
28 August 2013

Heavy metals are among the most toxic and harmful environmental factors. In the body, they promote the formation of active oxygen species, causing the damage to the cell membranes, nucleic acid and protein structure. This study was conducted to evaluate the toxic effects of Pb and Ni on antioxidant defence and protein synthesis (translation) systems in liver cells and to determine the possible protective effect of Zn against Pb and Ni toxicity. We established the complex effect of these metal ions on the content of an oxidative stress marker reduced glutathione, lipid peroxidation as well as on total protein, metallothionein and heme synthesis. It was determined that Zn ions are able to protect cells from the toxic effects of Pb and Ni ions exposure, but the protective effect was ambiguous. The Zn ions protected cells from Pb ions induced oxidative damage in the early stages of intoxication. The protective effect against Ni ions toxicity, on the contrary, was observed only after two weeks of intoxication. This study provides deeper understanding of Zn ability to protect the organism from toxic effects of Pb and Ni.

REGULATION OF CONNEXIN 36 GAP JUNCTION CHANNELS BY LIPOPHILIC COMPOUNDS AND INTRACELLULAR MAGNESIUM IONS

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The dissertation defended:
30 August 2013

Regulation of gap junctions (GJs) is important in normal and pathological conditions. In this study we show for the first time that short carbon chain n-alkanols (SCCAs; pentanol–heptanol) stimulate intercellular communication while long carbon chain n-alkanols (LCCAs; octanol–decanol) block it. SCCAs increase junctional conductance of Cx36 GJ channels by interfering with endogenous arachidonic acid (AA) dependent inhibition, increasing the open probability and a fraction of functional channels. We also evaluated the role of endogenous AA using other activators of Cx36 GJs coupling – bovine serum albumin (BSA) and inhibitor of phospholipase A2 isoforms methyl arachidonyl fluorophosphonate (MAFP). The observed regulation of Cx36 by SCCAs and AA in HeLa cells was the same as in β cells from mouse pancreas, natively producing Cx36. Furthermore, we demonstrate that junctional conductance of Cx36 and Cx47 is regulated by [Mg2+]i in a concentration-dependent way in HeLa cells. [Mg2+]i affects the channel open probability and the number of functional channels, however, it has no impact on single channel conductance. We found that cysteines in the TM2 and TM4 domains are involved in binding of SCCAs and isoflurane and in regulation of gating properties of Cx36. Also, TM2 domain and CL loop could be potential structural elements forming binding cavity/ies for LCCAs.

THE RELATION BETWEEN FOOT ARCH STABILITY, AND MECHANICAL AND PHYSIOLOGICAL PROPERTIES OF THE FOOT

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The dissertation defended:
13 September 2013

The aim of the research is to determine the relation between foot arch stability and the mechanical and physiological properties of the foot.

The research of foot arch height assessment methods showed that feet distribution according to medial longitudinal arch type depends on arch height determination methods provided by F. Forriol, L. T. Staheli, H. H. Clarke and D. S. Williams. The same feet evaluated using four methods showed different distribution according to normal, low and high arch type. A very strong ($r = 0.901$; $p = 0.000$) relation was determined between Chipaux-Smirak and Staheli foot indexes; however, the distribution of feet according to the type of medial longitudinal foot arch is diverse. According to Chipaux-Smirak index 81 percent of analysed feet were with low arch; meanwhile according to Staheli index 87 percent of the feet were with normal arch. Further research is necessary in order to create adaptive foot arch classification scale.

It was found that foot stiffness depends on foot arch type. The stiffness of low foot arch (45 N/mm) was smaller compared to that of normal (111 N/mm) and high (165 N/mm) foot arch. The plantar fascia of feet with normal arch was determined to be of higher elasticity compared to fascia of feet with low arch; while stiffness of plantar fascia was found to be independent of foot arch height. Both elasticity and stiffness of Achilles tendon do not vary in feet according to different types of arch. *In vitro* stiffness of feet with and without soft tissue depends on relative deformation level of feet under compression. The increase of relative deformation caused increase of progressive stiffness, and compression speed influenced foot stiffness. After speeding the compression from 100 up to 500 mm/min, the stiffness of feet with soft tissue decreased; whereas stiffness of feet without the soft tissue significantly increased at this speed of compression.

It was determined that body stability depends on stability of foot arch. The foot rotation around sagittal axis increases the transverse (left-and-right) sways of body centre of pressure.

A relation was determined between physiological and mechanical properties of a foot. The body centre of pressure (COP) and electrical muscle activity depend on elasticity and stiffness of plantar fascia and Achilles tendon. More elastic plantar fascia means lower COP variability and trajectory length. When subject's plantar fascia was stiffer and Achilles tendon more elastic the body centre of pressure sways back-and-forth were reduced. The electrical activity of abductor hallucis muscle increased when plantar fascia was stiffer. The body centre of pressure sways side-to-side were smaller when Achilles tendon stiffness decreased.

STROKE EFFECTS ON ACCURACY AND STABILITY CONTROL OF ISOMETRIC CONTRACTIONS AND MOVEMENTS OF ARM AND LEG MUSCLES

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The dissertation defended:
20 September 2013

The aim of the research was to determine the effect of ischemic stroke on the control of accuracy and stability of isometric contractions and movements of leg and arm muscles in the postrehabilitation period. The research evaluated the changes in accuracy and stability of the isometric contraction (20% of maximal voluntary strength) of forearm flexor muscles of both arms and knee extensor muscles of both legs in case of a different length of muscles in the patients after ischemic stroke. The effect of visual feedback on accuracy and stability of isometric contractions performed by arms and legs was determined in case of a different muscle length. It was also determined how dynamic parameters of arm and leg movements, such as reaction time and movement speed, changed when tasks of different complexity were performed. It was determined how the angular velocity influenced maximal voluntary torque values of the forearm flexor and extensor as well as knee flexor and extensor muscles. The research also analyzed the accuracy and stability of the isometric contraction of forearm flexor and knee extensor muscles as well as the dynamic movement and voluntary activation after an ischemic stroke.

THE RESIDUAL EFFECT OF ECCENTRIC CONCENTRIC PRIOR EXERCISE ON PULMONARY GAS EXCHANGE AND MUSCLE ELECTRICAL ACTIVITY DURING CYCLING OF DIFFERENT INTENSITY

Neringa Baranauskienė

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The dissertation defended:
30 September 2013

The aim of the research was to determine the acute and residual effect of preceding eccentric concentric exercise on pulmonary gas exchange and muscle electrical activity during different intensity load on cycle ergometer. The prior eccentric concentric exercise has a significant effect on pulmonary ventilation and heart rate during moderate but not heavy constant cycling load 45 minutes after preceding eccentric concentric exercise. There was no effect on theses indices 24 hours after eccentric concentric exercise during moderate and heavy constant cycling load. The EMG amplitude only of m. Vastus lateralis increased 24 hours after eccentric concentric exercise during moderate intensity constant cycling. The prior eccentric concentric exercise has neither acute nor residual effects on aerobic work efficiency during different intensity constant cycling. During increasing ramp cycling load aerobic work efficiency was unaffected by eccentric concentric exercise but it increased pulmonary ventilation, heart rate and EMG amplitude of m. Vastus lateralis, the mean frequency of EMG power spectrum of m. Vastus medialis decreased 24 hour after eccentric concentric exercise. The pulmonary ventilation increased and the EMG of m. Vastus medialis of the right leg, which was affected by preceding eccentric exercise, did not change, but EMG amplitude of m. Vastus lateralis of unaffected leg decreased during constant cycling load one hour after preceding eccentric exercise. There was no effect on these indices 24 hours after preceding eccentric exercise during heavy constant cycling load.

THE EFFECT OF HORSEBACK RIDING AND EXERCISES ON A THERAPEUTIC BALL ON GROSS MOTOR FUNCTION, SYMMETRY OF MUSCLE ACTIVITY AND PSYCHO-EMOTIONAL STATE OF HEALTHY CHILDREN AND CHILDREN WITH CEREBRAL PALSY

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The dissertation defended:
30 September 2013

This doctoral thesis contributes to necessary further focused study of rehabilitation methods' effectiveness for children with cerebral palsy (CP) in Lithuania. The conducted research revealed the differences in effectiveness of various rehabilitation methods applied for children with cerebral palsy. This research is the first to analyze activeness symmetries of gluteus medius muscle and lumbar erector spinae muscle of healthy children and children with CP on various surfaces. The myotonometry method applied in this study showed that exercises on a therapeutic ball decreased the asymmetry of passive mechanical properties of gluteus medius muscle and lumbar erector spinae muscle. These results reveal that exercises on a therapeutic ball can be successfully applied in rehabilitation by physical therapists when seeking to decrease the asymmetry of passive mechanical properties of gluteus medius muscle and lumbar erector spinae muscle of healthy children and children with cerebral palsy. Horseback riding muscle activeness symmetry of beginner and advanced horsemen (both healthy and with CP) was evaluated during the research. Also, the horseback riding influence on gross motor function of beginner and advanced horsemen with CP was evaluated. The study showed that ten horseback riding practices do not have relevant influence on gross motor function and its scale change of beginner horsemen with CP. Therefore a greater number of exercises is likely to be purposeful when seeking more relevant results in gross motor change. Further research is necessary since the lack of horseback riding effectiveness on gross motor of children with cerebral palsy is still evident.

CHANGES OF THE IMMUNE SYSTEM IN THE PATHOGENESIS OF PRIMARY SJÖGREN'S SYNDROME

Gintaras Sūdžius

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The dissertation defended:
19 December 2013

There are many studies done to determine factors what can cause susceptibility to Sjögren's syndrome. Despite intensive research of the immune system, the model of the Sjögren's syndrome pathogenesis is not completely clear. Lymphopenia is a common symptom found in the pSS patients. Numerous studies are performed in order to determine the causes of lymphopenia, but there is a lack of detailed studies to reveal which cell population counts increase or decrease. Scarce studies are done to associate the changes in the immune cell population and the expression of humoral factors in the peripheral blood of pSS patients. The aim of dissertation work was to investigate the changes of the components of the systemic immune response in the peripheral blood of patients with primary Sjögren's syndrome (pSS) with different manifestations of the disease. In this study, a comprehensive analysis of B, NK and T cell populations and humoral factors in the peripheral blood of pSS patients was performed. For the first time the expression of CD57 and CD27 markers on CD8+ T cell population was analyzed. For the first time Th17/Th1-like cells in the peripheral blood of pSS patients were identified and imbalance in the distribution of T helper cell population was revealed. It can be explained by the significant increase of Th17/Th1-like lymphocyte population. The levels of IL-27 and IL-35 in sera of pSS patients were measured (not in the model system) for the first time as well. Primary Sjögren's syndrome patients with extra-glandular manifestations have higher imbalance of B cell homeostasis than patients with only glandular manifestations. This is caused by an imbalance in the expression of BAFF, IgG, κ and λ FLC and C4d in serum.

FLUORESCENCE SPECTROSCOPY AND IMAGING STUDIES OF FUNCTIONALLY DIFFERENT HUMAN HEART TISSUES

Jonas Venius

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Rhythmical contraction of the heart is controlled by the cardiac conduction system (CCS). However, this highly important system visually could not be distinguished from the surrounding heart tissues – myocardium (MC) and connective tissue (CT); therefore during surgical procedures CCS could be damaged. The reliable method for CCS identification either *in vivo* or *ex vivo* does not exist therefore there is a definite need for developing a CCS imaging method. Fluorescence spectroscopy studies of cardiac tissues revealed that most distinct spectral differences between CCS and the surrounding tissues were observed in 400–550 nm region under excitation from 330–380 nm region. The visualization method, based on the intensity ratios calculated for two excitation wavelengths, has been established. The calculated ratio R = I(330)/I(380) is different for CCS, CT and MC tissues, therefore, the method may be used for identification of CCS. Time resolved fluorescence spectroscopy revealed no significant difference in composition and lifetimes between CCS and MC. On the other hand, the lifetimes and the relative spectral composition of CT differed significantly from those of CCS. Reflection confocal microscopy allows visualizing MC, CT, Purkinje cells and CCS bundles because of different reflection properties of tissue components and their specific distribution inside the tissue. The results of *in vivo* performed procedure revealed that the distribution of fluorescence intensities are similar to those observed during *ex vivo* experiments, therefore the established CCS identification method, based on intensity ratios, is suitable for cardiac investigations *in vivo*.

THE ORGANISATION PRINCIPLES OF SPINAL NEURAL NETWORK: TEMPORAL INTEGRATION OF SOMATOSENSORY INPUT AND DISTRIBUTION OF NETWORK ACTIVITY

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Spinal cord integrates somatosensory information and generates coordinated motor responses. Temporal integration can be used for discrimination of important stimuli from noise. Here it is shown that temporal integration of somatosensory inputs in sub second time scale is possible without changes of intrinsic properties of motoneurons. The activity of premotor neurons increases during temporal integration and can be a mechanism for short-term information storage in spinal cord. Suppression of motor activity after painful somatosensory stimulus is called cutaneous silent period. This motor suppression is well described in humans and used for diagnostics. However, it is not known if the suppression of motor activity is due to inhibition of motoneurons or reduction of excitatory drive from premotor neurons. Here it is shown that motoneurons are inhibited during cutaneous silent period. Neural networks of spinal cord not only process somatosensory information but also generate locomotion and reflexes. It is accepted that neural networks controlling front and hind limb movements are located in cervical and lumbar enlargements respectfully. Here it is shown that thoracic segments of spinal cord also contribute to hind limb movements. It means that neural network which generates movements is much more widely distributed than previously thought.

INVESTIGATION OF QUANTUM DOTS MIGRATION IN THE ORGANISM USING OPTICAL METHODS

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Nanoparticles (NP) are already in the composition of commercial products. New methods for medical diagnostics and therapy based on NP are developed. However the mechanisms of NP penetration through protective human barriers, biodistribution in the body, clearance properties and long-term accumulation risk remain undiscovered. This knowledge is needed to optimize biomedical applications of NP and to estimate nanotoxicological effects.

This thesis investigates the migration of semiconductor NP – CdSe/ZnS-mPEG quantum dots (QD) in the tissues of experimental animals *in vivo* by means of optical methods. The diffusion of QD in extracellular matrix, accumulation in different cell types, and penetration through the barriers of vessel walls, skin and placenta are analyzed. The main results show that QD migration pathways in the body are distinct from the conventional organic drugs. QD are not transferred through the wall of most blood vessels and do not extravasate into the tissues. It can be used for imaging of blood vessels, angiogenesis and vessel damage research. It is shown that QD diffusion in the tissues is limited by dense tissue fiber layers, e. g. basement membrane, and it retains QD from passage to epidermis, hair follicles, dermal glands, nerves and muscle cells. These results can be used to explore the mechanisms of biological barriers, contribute to the estimation of QD safety and expand the application areas of QD in biomedicine.