SESSION 12

Specificity of tissue and organ metabolism

Organized by S. Angielski, J. Rogulski
Renal proximal tubules secrete diverse organic anions and cations including widely prescribed drugs. In addition they are capable to reabsorb some organic anions and cations. Recently a family of transport proteins (SLC22) has been identified containing polyspecific transporters that participate in these functions. The SLC22 family comprises organic anion transporters (OATs and URAT1), organic cation transporters (OCTs) and zwitterion/cation transporters (OCTNs). Transporters of the SLC22 family function in different ways; 1) as uniporters which mediate facilitated diffusion in either direction (OCTs), 2) as anion exchangers (OAT1, OAT3 and URAT1), and 3) as Na+/L-carnitine cotransporter (OCTN2). In human, renal excretion of organic anions is mediated by the basolateral transporters OAT1, OAT2 and OAT3 in combination with luminal anion transporters including OAT4 and URAT1. Renal excretion of organic cations is performed by the basolateral transporter OCT2 together with a not yet identified luminal cation/proton antiporter and OCTN1. Mutagenesis experiments and functional studies strongly suggest that polyspecific transporters of the SLC22 family contain large substrate binding pockets that allow high affinity binding of structurally different compounds. These binding pockets may be exposed to extracellular or intracellular; both conformations exhibit different inhibitor sensitivities. Polymorphisms in transporters of the SLC22 family have been detected that lead to inactivation and/or changed substrate specificity. It is challenge to characterize the interaction of anionic and cation drugs with the human transporters in order to avoid adverse drug reactions that are due to drug-drug interactions or to mutations in individual transporters.

Diabetes is a chronic disease, but its incidence worldwide in the past twenty years has almost reached epidemic proportions. Current treatment strategies are clearly limited in terms of their effectiveness and new hypoglycemic drugs are required. In addition to vanadium (V), recently tungstate (W), molybdate (Mo) and selenate (Se) have been identified as insulin-mimetic compounds when given orally to diabetic animals. In contrast to V, W and Mo actions were less pronounced. In addition, the influence of latter compounds on the rate of gluconeogenesis was less pronounced in diabetic rabbits than in control ones. Both W and Mo increased lactate production for about 20%, indicating their stimulatory action on renal glycolysis. In view of W- and Mo-induced changes in intracellular gluconeogenic intermediates and gluconeogenic enzyme activities, it is likely that W and Mo might decrease flux through glucose-6-phosphatase. In addition, W affected also the mitochondrial phosphoenolpyruvate carboxykinase activity. Furthermore, V markedly diminished the mitochondrial membrane potential ($\Delta$φ), while W and Mo actions were less pronounced. The effects of the transient metals on $\Delta$φ were partially attenuated following preincubation of mitochondria with either N-acetylcyesteine or melatonin, suggesting that V, W and Mo induce ROS generation. In view of these data a potential therapeutic application of V, W- and Mo-compounds needs a careful evaluation.
Hypermetabolism is a systemic response to a severe underlying infection or trauma. It is characterized by an increased metabolic rate, severe muscle wasting and a negative nitrogen balance. Elevations in the serum concentrations of epinephrine and cortisol are accompanied by elevation in corticotropin, GH and glucagon levels. Change in GH/IGF-1 axis are permissive to protein catabolism. All these changes as well as increased insulin level with accompanied tissues insulin resistance are responsible for the hyperglycemia. Cytokine expressions and other inflammatory mediators are believed to be of importance. Enhanced gluconeogenesis in the liver and increased lipid peroxidation are evident. Enhanced rate of muscle protein breakdown are responsible for hyperaminoacidemia followed by liver uptake of amino-acids, sufficiently large to accounts for severalfold increase in urea excretion. Hypercatabolism of skeletal muscle protein appears to be mainly due to hyperactivation of the ATP-ubiquitin — proteosome dependent proteolytic system at the level of gen transcription. Augmented transcription of gens encoding proteins of this pathway result in severalfold increase in the poliubiquitin mRNA, ubiquitinylating enzymes mRNA and multiple subunits of proteosome mRNA. These changes are limited to the muscle and correlate directly with muscle proteolysis. Amino-acids transfer form the periphery to the liver is of importance for survival. There is increased liver synthesis of acute-phase proteins, such as protease inhibitors, components of complement cascad and others important for tissue healing and immunologic defenses. Reversal of the metabolic disturbances by exogenous proteins is not possible. We still cannot control the catabolic stimulus of auto-cannibalism of skeletal muscle mass, which if prolonged delays recovery and results in multi-organ failure.

Mucopolysaccharidoses are rare genetic diseases from the group of lysosomal storage disorders caused by deficiency of enzymes involved in degradation of mucopolysaccharides (glycosaminoglycans). Within each mucopolysaccharidosis, there is a continuous spectrum of clinical features from the very severe to the more mildly affected individuals. Surprisingly, in most cases, it is not possible to predict severity and clinical progress (i.e. the natural history) of the disease on the basis of detection of particular mutations or residual activity of the deficient enzyme. In this article, the reasons for such an unexpected difficulty are discussed. Moreover, it is proposed that apart from measurement of activity of the enzyme involved in degradation of glycosaminoglycans, the efficiency of synthesis of these compounds should also be estimated. If the hypothesis presented in this article is true, the ratio of the synthesis of glycosaminoglycans to the residual activity of the deficient enzyme should be of considerable prognostic value. Moreover, it is suggested that inhibitors of enzymes involved in synthesis of glycosaminoglycans might be potential anti-mucopolysaccharidosis drugs that could facilitate enzyme replacement therapy.
Method: Study group consists of 26 alcohol dependent women. Mean age of subjects was 39 yrs (std 7 yrs), mean age of onset was 36 yrs (std 6 yrs), mean duration of illness was 6 yrs (std 4 yrs).

Control group consists of 10 healthy women.

The concentration of CDT was assessed using immunoturbidimetric method using %CDT TIA by Bio-Rad. Referential values was <2.6% CDT.

Activity of following enzymes: GGT, AST, ALT was completed using tests Roche, normal values: GGT 9 — 39 U/l; AST <31 U/l; ALT <32 U/l.

Results: Study group before treatment had statistically significant higher CDT level both than in healthy controls. (3.34 ± 3.43% vs. 0.86 ± 0.47%, p=0.034) and in study group after hospitalization (3.34 ± 3.43% vs. 1.81 ± 1.54%, p=0.046).

The concentration of CDT after treatment normalized. There was no differences of CDT concentration in alcohol dependent women with high and normal level of GGT, AST, ALT (GGT: 2.35 ± 1.55% vs. 4.50 ± 4.61%, p=0.088; AST: 3.40 ± 1.56% vs. 3.28 ± 4.70, p=0.077; ALT: 2.55 ± 1.60% vs. 3.84 ± 4.18, = 0.168).

Conclusions: 1. There was statistically significant higher concentration of CDT in alcohol dependent women before treatment than both in healthy controls and alcohol dependent women after treatment. 2. Our results suggests that CDT can be a useful factor of alcohol dependence in patients with hepatic disorders.

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**The influence of exercise on concentration E-cadherin in serum and urine**

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E-cadherin is a 120 kDa transmembrane glycoprotein expressed mainly on the surface of epithelial cells. The best characterised function of E-cadherin is homotypic, calcium-dependent cell-cell adhesion. Soluble forms of E-cadherin, 80–84 kDa was found to be circulating in biological fluids of healthy persons and those with systemic inflammatory response syndrome and multiorgan dysfunction syndrome. Physical exercise is one of the factors disturbing organism homeostasis which can be seen in the urine composition owing to the changes in the hemodynamic and metabolic processes in the kidneys. The aim of the study was the evaluation of the influence of physical exercise on the blood serum and urine E-cadherin concentration.

The group under study comprised 10 students in the second year of the University of Physical Education in Wrocław who do not take sports professionally.

Students performed the progressive test on the cycloergometer Monark 839 E (Sweden). The blood and urine collected three times in test: A — pre-exercise, B — immediately after the exercise, C — after 24 hours.

The concentration E-cadherin was determined by ELISA method with the Human E-cadherin EIA kit (TaKaRa, Japan). We detected 7.2-time increase of E-cadherin concentration in urine B—a as well as 18-fold fall of this parameter in urine C—2 4h after effort. The profile of changes of E-cadherin in serum is different. The highest concentration was observed in serum A and after effort undergoes reduction about 25% and in serum C 3-fold in relation to preexercise value. The correlation was not found between concentrations in serum and urine. It cans this to provide about origin of this protein in urine from renal structures. The increase of expression of this protein in urine immediately after effort can be related with structural changes of permeability membrane of nephron.

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**The role of melatonin in the regulation of glucose metabolism in primary cultures of rabbit kidney-cortex tubules**

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Melatonin (N-acetyl-5-methoxytryptamine) — the pineal gland hormone — causes a broad spectrum of metabolic and physiological effects. As a lipophilic and hydrophilic compound the hormone freely diffuses through the cellular membrane, binds to the target proteins in cytosol and directly neutralizes a number of both reactive oxygen and nitrogen species. Moreover, the effects of melatonin can be also mediated via binding to the plasma membrane or nuclear receptors. Numerous data suggest that daily supplementation with this hormone
may be beneficial for treatment of diabetes. However, the role of melatonin in glucose homeostasis is poorly understood. Under normal conditions glucose blood level is modulated by circadian rhythm and there are no convincing data in terms of relations between melatonin action, glucose metabolism and diabetes.

The aim of this investigation was to elucidate the mechanisms responsible for melatonin-induced changes in glucose metabolism in the presence and absence of insulin in the primary cultures of renal tubules growing in the defined medium supplemented with transferrin, hydrocortisone, selenite and gluconogenic substrates.

In the presence of insulin 100 nM melatonin stimulated both glucose and lactate synthesis, while in the medium devoided of insulin the effects of the hormone on glucose metabolism were significantly decreased. Melatonin-induced increase in glucose and lactate synthesis was accompanied by enhancement of alanine and glycerol consumptions, elevation of intracellular GSH/GSSG ratio and a decrease in intracellular cAMP level. Attenuation of stimulatory effect of melatonin on glucose formation following luzindole administration indicate that the action of this hormone is due to stimulation of renal tubules ML1 membrane receptors. As concluded from a decline of intracellular fructose-1,6-bisphosphate content accompanied by a significant rise in fructose-6-phosphate level, melatonin might result in acceleration of flux through fructose-1,6-bisphosphatase probably due to decrease in the phosphorylated form of this enzyme. In view of these observations melatonin might be considered as an important factor regulating glucose metabolism in kidney-cortex tubules.

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**Activity of lactate dehydrogenase in blood serum of women with cancerous diseases of reproductive organs**

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Lactate dehydrogenase (EC.1.1.1.27) occurs in cells of different organs and tissues showing varying activity characteristic for particular organs or tissues. The activity of this enzyme changes in different pathological states and the enzyme is transferred into blood. Therefore, the enzyme is used, more, or less successfully, as a marker in diagnoses of various diseases.

The aim of the research was to determine lactate dehydrogenase in blood serum of patients with various diseases of reproductive organs, to determine the interrelation between dehydrogenase activity and patient’s age.

The research material was blood serum from peripheral blood of women suffering from Cancer endometrium, Myomata uteri, Tumor ovarium. In each case 25 samples were taken for analysis. Serum of healthy persons served as the control. The results of the experiment showed the lowest LDH activity in Cancer ovarium, and the highest one in Sterilites primaria. In other diseases low activity of LDH was determined and the data did not have any statistical significance. The results of research show that LDH analysis has little diagnostic value in diagnosing diseases of female reproductive organs.

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**Influence of alcohol abuse on homocysteine metabolism**

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Metabolism of homocysteine is accomplished in the remethylation cycle where vitamin B12 and folic acid are essential coenzymes. Markedly elevated homocysteine concentrations have been observed in patients with nutritional deficiencies of vitamin B12 and folate. Hyperhomocysteinemia in alcohol abusers may result
from malnutrition and disorder of intestine absorption.

The aim of the study was the estimation of homocysteine, folic acid and vitamin B12 concentrations in alcohol dependent male patients.

71 males with clinical diagnosis of alcohol dependence (ICD-10) have been examined. The investigated parameters have been determined in blood serum, the homocysteine by means of immunochemical method, vitamin B12 and folic acid by means of immunoenzymatic assay.

Serum homocysteine concentration was significantly higher and serum folic acid concentration was lower in alcohol dependent men than in control adolescents. Mean concentrations of folic acid and vitamin B12 were significantly lower in patients with hyperhomocysteinemia than in men with normal homocysteine concentration. The highest correlation were indeed noticed between folate deficiency and the intensity of hyperhomocysteinemia.

The development of hyperhomocysteinemia is associated with alcohol dependence that is also probable cause of folate and vitamin B12 deficiency.

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Biochemical markers of bones metabolism (ctx and osteocalcin) in alcohol dependent women

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The aim of this study was an assessment of bones’ metabolism in alcohol dependent women.

Method: Study group consists of 37 alcohol dependent women. Mean age of subjects was 38 yrs (std 8 yrs), mean age of onset was 33 yrs (std 7 yrs), mean duration of illness was 6 yrs (std 3 yrs).

Osteocalcin concentration was completed using LUMItest Osteocalcin by BRAHMS (0—35 ng/ml), β-crosslaps (β-ctx) concentration was completed using β-CrossLaps/serum by Roche; (0—0.32 ng/ml), testosterone concentration was completed using Testoster-one test (0.06—0.82 ng/ml).

Results: In study grup the concentration of osteocalcin was 23.17 ± 7.15 ng/ml, ctx 0.38 ± 0.16 ng/ml.

Subjects with higher concentration of ctx and osteocalcin had statistically significant lower level of testosterone than subjects with normal values of ctx and osteocalcin (ctx: 0.41 ± 0.20 vs. 0.57 ± 0.39 ng/ml, p=0.046; osteocalcin: 0.39 ± 0.18 ng/ml vs. 0.51 ± 0.37 ng/ml, p=0.039)

There was a positive correlation between concentration of ctx and osteocalcin (p=0.001).

There was no statistically significant correlations between age of subjects, duration of illness, age of onset and ctx concentration or osteocalcin.

Conclusions: 1. In study group there was a positive correlation between concentration ctx and osteocalcin.. It suggests between bone formation and bone resorption. 2. There was no correlations between age of subjects, duration of illness, age of onset and ctx concentration or osteocalcin.

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Dynamics of activity of the microsomal oxidation system during liver regeneration. Effect of heparin

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Clearance of endogenous toxins and xenobiotics is one of the main functions of the liver. Antipyrine was one of the first model metabolic drugs used and has remained a popular tool for metabolic studies to probe the xenobiotic hepatic metabolic capacity to dispose of xenobiotics. The clearance of antipyrine is cytochrome P450-dependent.

The present study was aimed at an examination of the liver metabolic capacity for drug clearance during its regeneration and to estimate the effect of heparin on the restoration of the drug-metabolizing function of the liver. The studies were carried out on 30 male Wistar rats weighing 200–250 g. The liver regeneration was initiated by 68% partial hepatectomy (PH). The animals
were divided into two experimental groups. Group I was administered 0.85% NaCl solution (1.3 ml/kg per day, subcutaneously). Group II received heparin (1 ml/kg = 130 U/kg per day, subcutaneously). Control group was sham-operated.

The blood content of antipyrine was studied on days 2, 4, 6, 8, 10, 12 and 14 after PH. Blood was collected at 60, 120 and 240 min from the tail. The pharmacokinetic parameters of antipyrine were calculated according to the single-cell model. It was found in Group I, that on 4 days after PH the drug-metabolizing liver function was considerably decreased on day 4 (MRT=8.52 h, kel=0.13 h⁻¹, t1/2=5.83 h). The liver drug-metabolizing function increased two times on day 6 in comparison with day 4 after PH, and was restored completely on day 14. Heparin administration did not effect the dynamics of restoration of the activity of liver microsomal oxidation system.

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**Effect of physical exercise on leptin concentration in human blood**

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Leptin, a product of the (ob) gene, is a 16 kDa peptide hormone expressed abundantly in the adipose tissue which controls obesity by reducing food intake and body weight via increasing energy expenditure. The results of some investigations correlating the effect of physical exercise on serum leptin level indicate negative correlation while others have not managed to find any correlation. The aim of the present study was to correlate the level of physical exercise with serum leptin level, parameters of lipid metabolism and level of energy expenditure. A group of cyclists comprising 16 women and 26 men was investigated. Serum level of leptin was found to be elevated in women as compared to men, both before and after physical exercise on a bicycle cycloergometer. In the whole group of sportsmen, there was found a positive correlation of serum leptin concentration and BMI or apolipoprotein B. On the other hand there was a negative correlation between serum leptin concentration and level of energy expenditure, maximum oxygen consumption as well as maximal lung ventilation, which suggests that serum leptin level seems to be lower in humans of better physical performance.

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**Amino acids concentrations in blood serum, liver and kidney of rats intoxicated with Pb and Cd and received certain bioflavonoids**

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Bioflavonoids (or flavonoids) are a large group of natural polyphenolic derivatives that are ubiquitous in all vascular plants. These compounds are important for plants, but also for humans and animals. Long interaction between plant bioflavonoids and human has stimulated much interest in the biochemical and physiological activities of these chemicals. Bioflavonoids possess significant antihepatoxic, gastrointestinal, anti-inflammatory and antitumor activities. In addition, their properties for metal chelation support the role as preventative antioxidants in terms of inhibiting transition metal-catalysed free radical formation. However, the extent of absorption of bioflavonoids is an important unsolved problem. Animal studies show that bioflavonoids present in foods are to be considered non-absorbable because they are bound to sugars as beta-glycosides (with the exception of catechins). Only free flavonoids, without a sugar molecule are thought to be able to pass through the gut wall.

The aim of our study was to determine the effect of quercetin and catechin administered together with lead or cadmium on the level free amino acids in serum blood, and some tissues of experimental rats.

The experiment was conducted on Wistar male rats, weighting 170–200 g. The animals were divided into six groups, each of 5 rats.

Groups I–III of animals obtained plumbum nitrate in amount of 500 mg/dm³ in reduction to unalloyed metal and additionally group II 200 mg/dm³ of quercetin, groups III 200 mg/dm³ of catechin. The IV – VI groups
obtained the same that groups I–III but they received instead of lead cadmium chloride in amount of 500 mg/dm³ in reduction to unalloyed metal.

The all groups of rats were on a normal diet (LSM dry food) and they got solutions ad libitum.

After 6 weeks the animals were anaesthetised with 0.5 ml of 5% ketamine. Liver, kidneys and blood serum were excised. Liver and kidney were homogenised and deproteinised with 6% sulphosalicylic acid in lithium-citrates buffer (pH 2.8) and centrifuged (12000 rpm for 20 min). Blood serum was only deproteinised with the same buffer. Free amino acids were determined using Amino Acids Analyser (AAA 400) by Ingos Praha.

Addition of the bioflavonoid to metal solution caused increase of amino acids level in kidney in comparison to group that obtained metal solution only. This increase was more significant in case of catechin than of quercetin. In blood serum and liver amino acids level fluctuations were observed in dependence on examined group but also kind of amino acid and its function in organism.