Bulgarian Society
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Sofia branch

SCIENTIFIC MEETING

ABSTRACTS

Sofia, 25 November, 2016

BULGARIAN SOCIETY

OF PHYSIOLOGICAL SCIENCES

SOFIA BRANCH

SCIENTIFIC MEETING

ABSTRACTS

25 November, 2016, Sofia, Bulgaria

LOCAL ORGANIZING COMMITTEE

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Programme Overview

Oral presentations

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Concentration Dependence Of The Steady State Current Of Cyclic Processes On Example Of The Sodium-potassium Pump

14.15 – 14.30 Vladimir G. Dimitrov, Alexander G. Dimitrov, Todor I. Arabadzhiev, Nonna A. Dimitrova

Differences In Extracellular Potentials Produced By Straight And Curved Skeletal Muscle Fibers

14.30 – 14.45 Athanasia-Faidra T. Katsiou, Dimitrios D. Samaras, Christos D. Samaras, Vasileios P. Stamatopoulos, Nikolaos S. Statharakos, Christos D. Papageorgiou

Hormetic Effects Of Reactive Oxygen Species Generated By Regular Exercise

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Corticosteroid Interactions With Human Serum Albumin

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Effects Of Synthetic Neuropeptides (Neurotensins) On Drug-induced Neurodegenerative Disorders

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Role Of Kyotorphin In The Behavioral Changes Induced By An Experimental Model Of Alzheimer's Disease In Rats

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Treatment With Agomelatine After Status Epilepticus Suppress Epilepsy-associated Depression Without Ameliorating Spontaneous Motor Seizures In The Rat Model Of Temporal Lobe Epilepsy

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Study Role Of AT_1 Receptors On Spatial Memory Impairment In The Kainate Model Of Epilepsy

CONCENTRATION DEPENDENCE OF THE STEADY STATE CURRENT OF CYCLIC PROCESSES ON EXAMPLE OF THE SODIUM-POTASSIUM PUMP

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Many stationary currents in living cells are caused by cyclic processes. The standard way to describe them involves identification of the cycle states, estimation of the rate constants for transitions between the states and integration of the equations that reflect processes in the cycle. This approach results in complex expressions for the steady state current that require knowledge of all the rate constants. This is an obstacle for using such expressions in a general case. This enforces introducing simplifications in description of the current. The approach most often used in the existing models is considerable reduction in the number of states in the cycle. Such approximations inevitably lead to more complex expressions for the effective rate constants. In contrast to this approach we assumed existence of a sufficient number of states so that each transition to reflect an elementary process. Then a linear dependence of rate constants on involved concentrations is expected according to the mass action law. This allows expression for the steady state current to be transformed from a function of rate constants into a function of concentrations. Thus a general expression for the steady state current is obtained. The current would depend on the available free energy and on a set of properly defined affinities. As a result, to write the steady state current, one requires knowledge of neither the cycle states nor the rate constants. The general expression obtained for the steady state current, provides also a way to estimate the quality of the phenomenological models and gives a framework for their expansion over much wider parameter set.

DIFFERENCES IN EXTRACELLULAR POTENTIALS PRODUCED BY STRAIGHT AND CURVED SKELETAL MUSCLE FIBERS

Vladimir G. Dimitrov, Alexander G. Dimitrov, Todor I. Arabadzhiev, Nonna A. Dimitrova

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After 3 and 6 seconds of isometric maximal voluntary contraction and one second of resting the main phase of the M-response remained unchanged in amplitude and duration. But its terminal phase experienced shortening, increase in amplitude and earlier appearance (Rodrigues-Falces et al. 2016). It was not obvious to explain such findings with used models. Muscle potentials were modeled by the authors as sum of potentials produced by straight parallel muscle fibers. The actual fibers are not straight, even in muscles whose geometry is considered to be simple. There is cone like gathering of muscle fibers around the tendon aponeurosis in *m.biceps brachii*. Muscle fibers located closer to the muscle axis could end earlier. Fibers located further away from the muscle axis have longer curved portion. The aim of the present study is to clarify the effects of such changes on extracellular potentials detected with surface electrode. We used mathematical modeling and compare the extracellular potentials produced by straight and curved muscle fibers. We found generation of additional phase appearing when the action potential passed through the curved part of the muscle fiber. The additional phase has dipole character, like the terminal phases. Curving caused change in position and orientation of the muscle fiber cross sections along the fiber length. When curving increased the solid angle of observation of muscle fiber cross section from the position of the virtual electrode, the additional phase had the opposite sign comparing to the terminal phase. When curving decreased the solid angle of observation from the position of the virtual electrode, the additional phase had the same sign as the terminal phase. The effects of changes in the solid angle were similar to changes in the cell cross section. The fiber curvature should be taken into account when the changes in M-response are analyzed.

HORMETIC EFFECTS OF REACTIVE OXYGEN SPECIES GENERATED BY REGULAR EXERCISE

Athanasia-Faidra T. Katsiou, Dimitrios D. Samaras, Christos D. Samaras, Vasileios P. Stamatopoulos, Nikolaos S. Statharakos, Christos D. Papageorgiou*

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The reactive oxygen species (ROS) are one of the most common unstable molecules produces by the body during normal metabolism. They are also generated by various endogenous systems, exposure to different physiochemical conditions or pathological states. To be able to combat the adverse effects of free radicals, human body triggers the massive production of different antioxidants or accelerates their intake from foods. A balance between free radicals and antioxidants is necessary for proper physiological function. If free radicals overwhelm the body's ability to regulate them, a condition known as oxidative stress ensues.

Scientific studies have demonstrated that long intense exercise such as endurance training, may cause an overwhelming of body's antioxidant defenses, leading to excessive oxidative stress and harmful outcomes. On the other hand regular moderate exercise provides benefits upregulating defense against oxidative stress in good balance between the opposing dual roles. Thus, contrary to what is believed until now, oxidative stress is beneficial in small amounts. In fact it's essential, because prompts the body cells to become stronger over time by increasing antioxidant defense mechanisms. The beneficial consequences of regular exercise and harmful outcomes of exhaustive exercise due to amount of ROS production fit well with the concept of hormesis. However, mounting epidemiological data have proven that exercise decreases the incidence of oxidative stress-associated diseases and this is a result of exercise-induced adaptation. Unexpectedly, several prospective clinical intervention studies showed that cotreatment with antioxidants inhibits ROS signal transduction and prevents the adaptive response.

Key words: exercise, hormesis, reactive oxygen species

CORTICOSTEROID INTERACTIONS WITH HUMAN SERUM ALBUMIN

Anelia Zasheva, Silviya Abarova, Boris Tenchov

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Characterization of the thermodynamics of drug binding with blood plasma (BP) proteins is of essential importance for a better understanding of drug absorption, distribution and turnover in the circulation. Human serum albumin (HSA), the most prominent protein in plasma, plays a fundamental role in the transport of drugs.

Synthetic corticosteroids are a group of drugs used for treatment of various diseases, including lupus, inflammation, cerebral edema, etc. In the present study we investigated the interactions of betamethasone, methylprednisolone and dexamethasone with HSA.

To characterize the energetics of corticosteroid binding to HSA, we used Isothermal Titration Calorimetry (ITC) and Differential Scanning Calorimetry (DSC). DSC is a sensitive method for characterization of the stability and denaturational profiles of biological macromolecules in-solution. The ITC provides high sensitivity and flexibility in studies of protein-ligand binding, including the binding of drugs to their targets.

Aim: To characterize the mechanism of synthetic corticosteroid interactions with HSA.

The thermal effects of betamethasone, methylprednisolone and dexamethasone binding to HSA (Sigma Aldrich) was examined by ITC (Nano ITC, TA Instruments). ITC measurements were conducted in PBS (pH 7.4). The 1 ml sample cell was filled with 38 μM HSA. In the first experiment we loaded the 250 μl injection syringe with 0.5 mM solutions of dexamethasone, in the second – with methylprednisolone and, lastly, with betamethasone. The drug solutions were injected into the ITC cell in 10 μl increments in a total of 25 injections with 600 s intervals. The titration process was computer-controlled. The stirring speed was set at 250 rpm and the cell temperature was kept at 37 ^{o}C. The experimental results were processed using the calorimeter software.

Immediately after the ITC measurements the samples were degassed and loaded into the measuring cell of a Nano DSC (Nano DSC, TA Instruments), equipped with 300 μl measuring cells. PBS (pH 7.4) was used for the reference cell. Two subsequent scans were performed at a scanning rate of 1°C/min in the range from 20 °C to 110°C, under a pressure of 3 atm.

The ITC measurements demonstrated high binding affinity of the three glucocorticoids to HSA, as evidenced by the exothermic thermal effects. The thermograms of blood plasma recorded by DSC showed that betamethasone and methylprednisolone have higher binding affinity to HSA in comparison to dexamethasone. To see if there is binding between the glucocorticosteroids and other BP proteins, we also studied the interaction of methylprednisolone with the γ-globulin BP fraction. The results showed no interaction of the γ-globulins with methylprednisolone.

The present study demonstrates high binding affinity of synthetic glucocorticosteroids to HSA and helps to better understand the binding mechanism.

Key words: ITC, DSC, albumin, corticosteroids

INTERACTION BETWEEN GABAERGIC AND DOPAMINERGIC RETINAL SYSTEMS REVEALED BY ELECTRORETINOGRAM

Momchil Kostov, Elka Popova, Petia Kupenova

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Dopamine and GABA are among the major neurotransmitters in the retina, but the ways they interact with eachother are not fully understood. In this study we investigated the effect of the dopamine D_1 receptor blockade on the electroretinographic b-wave (ON response) and d-wave (OFF response) in intact frog eyecup preparations and in eyecups where the ionotropic GABA receptors were blocked. The D_1 receptors were blocked by using 75 μM SCH 23390, while the ionotropic GABA receptors were blocked by using 50 μM picrotoxin. The experiments were carried out on dark adapted eyecup preparations of frog (*Rana ridibunda*), continuously superfused with a Ringer solution and supplied with moistened O_2. Diffuse white light stimuli with a 5 s duration were presented repeatedly at interstimulus interval of 25 s. We found that the isolated D_1 receptor blockade with SCH 23390 caused moderate enhancement of the amplitude of the b-wave (to ~ 127%) and d-wave (to ~ 137%) without altering their latency. The isolated blockade of ionotropic GABA receptors with picrotoxin markedly enhanced the b- and d-wave amplitude (to ~ 300 % for the b-wave and to ~ 530 % for the d-wave) and significantly lengthened their latency. When SCH 23390 was applied on the background of the fully developed picrotoxin effect, it diminished the amplitude of the b- and d-waves in comparison to the corresponding values obtained during application of picrotoxin alone. Our results demonstrate that the enhancing effect of D_1 receptor blockade on the amplitude of the ERG b- and d-waves is not evident during ionotropic GABA receptor blockade, indicating an interaction between these neurotransmitter systems in frog retina. We suppose that the inhibitory effect of endogenous dopamine, mediated by D_1 receptors, on the ERG waves may be due to the dopamine-evoked GABA release.

EFFECTS OF SYNTHETIC NEUROPEPTIDES (NEUROTENSINS) ON DRUG-INDUCED NEURODEGENERATIVE DISORDERS

Stella Zaharinova[1], Silviya Abarova[1], Lyubka Tancheva[2], Svetlana Stoeva[2], Tamara Pajpanova[4], Rumiana Koynova[3], Boris Tenchov[1,3]

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Neurotensin (NT) is a neuropeptide and putative neurotransmitter expected to hold up the development of some neurodegenerative diseases (NDD). Differential scanning calorimetry (DSC) is a method for thermal analysis used to characterize the stability of the native conformations of biological macromolecules. Isothermal titration calorimetry (ITC) is used to characterize the interactions between proteins and their ligands, and to determine the thermodynamic parameters of these interactions. In this work, the thermodynamic properties of brain supernatants from rodents with drug-induced neurodenerative disorders (dementia and Parkinson's disease) were examined using DSC and ITC. Aim: To evaluate the impact of NT on animals with scopolamine-induced dementia and 6-hydroxydopamine-induced Parkinson's disease (PD) using DSC and ITC. Experimental models of scopolamine-induced dementia in male Albino mice (scopolamine 1 mg/kg, i.p., 11 days) and PD, induced in male Wistar rats via 6-OHDA i.c. injection, were verified by cognitive and biochemical tests. NT and its modifications NT2 and NT4 were applied i.p. for 11 days simultaneously with scopolamine. Animals were treated with NT4 i.p. for 5 days in dose 5 mg/kg before induction of PD. DSC and ITC measurements were performed on brain tissue supernatants isolated from healthy (controls) and treated with drugs animals.

The DSC measurements revealed significant differences between the brain proteome denaturational profiles of healthy animals and animals with drug-induced neurodegenerative disorders. They also demonstrate the recovery effect of NT4 on drug-induced NDD. The ITC measurements show high binding affinity of NT4 to human serum albumin (HSA) and suggest that HSA is a NT carrier in the blood stream.

Neurotensins have clearly expressed recovery effect on drug-induced neurodegenerative diseases, as demonstrated by the DSC and ITC. These methods provide information on the disease mechanisms at molecular level. They are appropriate for detection and characterization of compositional changes taking place in affected by NDD brain tissues and can be helpful in the future for further studies of these diseases.

Key words: DSC, ITC, Neurotensin, Parkinson's disease, Alzheimer's disease

ROLE OF KYOTORPHIN IN THE BEHAVIORAL CHANGES INDUCED BY AN EXPERIMENTAL MODEL OF ALZHEIMER'S DISEASE IN RATS

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Introduction: Alzheimer's disease (AD) is a widespread neurodegenerative disease associated with a progressive loss of memory, which occurs after prolonged pre-symptomatic phase. Intracerebroventricular (ICV) injection of streptozotocin (STZ) in rats is one of the established experimental models of AD. In the process of searching for new biomarkers for early diagnosis of the disease, it was found reduced levels of endogenous dipeptide kyotorphin (KTP) in the cerebrospinal fluid of people suffering from AD, which is accompanied by increased content of phosphorylated tau protein. Our hypothesis is that the KTP plays a role in the pathophysiology of the AD and related behavior.

Methods: To accomplish our goal we used an experimental model of AD (STZ, icv) in rats with stereotaxically implanted guide cannulas into lateral cerebral ventricle. The dipeptide KTP was injected ICV, 14 days (7 days before and 7 days after STZ). Open field test was used for study the overall motor activity, exploratory behavior, habituation and anxiety. ANOVA with factors AD and KTP was used to analyze the results.

Results: One month after the injection of STZ, rats showed an increase in locomotor activity and rears, as well longer activity in the aversive central area of the apparatus. This data are indicative that AD induced hyperactivity and increased exploratory behavior in novel environment accompanied with decreased level of anxiety. Treatment with KTP abolished the increased exploratory activity and diminished anxiety to the control levels without changing of AD-induced hyperactivity.

This data showed slight but significant protective effect of intracerebral KTP against AD-induced behavioral abnormalities.

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DIABETES MELLITUS TYPE 2 - INDUCED CHANGES IN METABOLISM, BEHAVIOR AND OXIDATIVE STRESS IN BOTH NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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The aim of this study was to compare the main consequences of diabetes mellitus type 2 (DM2) on the metabolic, behavioral and biochemical processes in two different rat strains: the normotensive Wistar and Spontaneously hypertensive rats.

Experimental method of DM2 was induced by combination of high fat diet and intraperitoneal injection of streptozotocin at a dose of 30 mg/kg, b.w. Daily intake of water and food, urine excretion and weight gain were studied in metabolic cages. Locomotor and exploratory activity and anxiety-like behavior were examined by "open field" and "elevated plus maze" tests. The level of oxidative stress in blood plasma, brain prefrontal cortex and hippocampus was evaluated by SOD Assay Kit-WST, determination of O^{-2}, H2O2, DPPH - free radical and reduced form.

The experimental model of type 2 DM is characterized by less pronounced, but significant changes in the urinary excretion, weight gain and intake of water and food as compared to DM type 1 in both rat strains. DM2 induced a decrease in locomotor activity and increased anxiety level in Wistar and SHRs. The observed changes were more pronounced in SHR strain. DM2 induced an elevation of the total SOD activity in brain cortex and hippocampus accompanied with decrease in superoxide radical level in Wistar rats.

These results suggest that DM2 induced metabolic and behavioral changes together with an oxidative imbalance in both rat strains but these consequences were heavier in SHR strain.

Acknowledgments: *The research was supported by Grant15/2015 of the Medical University of Sofia, Bulgaria.*

TREATMENT WITH AGOMELATINE AFTER STATUS EPILEPTICUS SUPPRESS EPILEPSY-ASSOCIATED DEPRESSION WITHOUT AMELIORATING SPONTANEOUS MOTOR SEIZURES IN THE RAT MODEL OF TEMPORAL LOBE EPILEPSY

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Our previous studies revealed that long-term melatonin treatment during epileptogenesis attenuated seizure susceptibility, abnormal behavioral alterations and exerted neuroprotection in the CA1 area of the hippocampus and piriform cortex. In the present study, we aimed to explore the efficacy of melatonin analogue agomelatine, a MT_1/MT_2 melatoninergic receptor agonist/ 5-HT_{2C} receptor antagonist, used as an antidepressant, on seizure frequency and behavioral abnormalities after kainate-induced status epilepticus (SE). Agomelatine treatment (40 mg/kg, i.p. for 10 weeks) started two hours after onset of SE in Wistar rats. Spontaneous motor seizures (SMS) were 24-h hours/day video-monitored for up to 10 weeks. Different behavioral tests for anxiety, depression and spatial memory were executed in rats during the period of agomelatine treatment. Agomelatine shortened the latency for the appearance of the first SMS and increased their frequency during the 2nd and the 3rd week after SE and start of treatment. The melatonin analogue was unable to change hyper-locomotion, impulsivity and damage in the spatial memory in epileptic rats. However, the drug attenuated depressive behavior tested in splash test, forced swimming test, sucrose preference test and novelty-suppressed feeding test in the KA-treated rats. Our findings suggest that although agomelatine treatment after SE is able to ameliorate comorbid depression it can exacerbate concomitant behavioral changes.

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STUDY ROLE OF AT1 RECEPTORS ON SPATIAL MEMORY IMPAIRMENT IN THE KAINATE MODEL OF EPILEPSY

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Cognitive impairment is a major concern in temporal lobe epilepsy (TLE). Studies have reported controversial data regarding the involvement of angiotensin II (Ang II) in hippocampus-dependent spatial memory. Accumulated data support the idea of an upregulation of renin-angiotensin system (RAS) in this disease. The renin-angiotensin system is classically involved in the regulation of arterial blood pressure and spontaneously hypertensive rats (SHRs) have hyperactivated RAS. The selective Angiotensin II receptor type 1 (AT_1 receptor) antagonist losartan is shown to decrease arterial blood pressure and to exert a neuroprotective and anticonvulsant effect. Here, we aimed to explore the role of AT_1 receptor on spatial memory impairment and its expression in the KA-induced model of TLE in SHRs and normotensive Wistar rats. The radial arm maze test was used with 18-days training session protocol. Angiotensin AT_1 receptor was quantified by immunocytochemistry. Wistar rats with epilepsy were unable to improve their working and reference memory performance during repeated daily performance of the task. Angiotensin AT_1 receptors were up-regulated in the hippocampus of both SHRs and Wistar rats during the chronic phase of epilepsy. The selective AT1 receptor antagonist losartan (10 mg/kg diluted in drinking water for 8 weeks) alleviated the epilepsy impaired reference performance in Wistrar rats but did not improve the violated working memory. Despite epileptic and losartan treated SHRs performed the task worse compared to their control groups, they showed a decrease of their working and reference memory errors. This study suggests that antihypertensive drugs targeting AT_1 receptor may be considered as an additional strategy for investigating and influencing spatial memory damage in epilepsy.

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