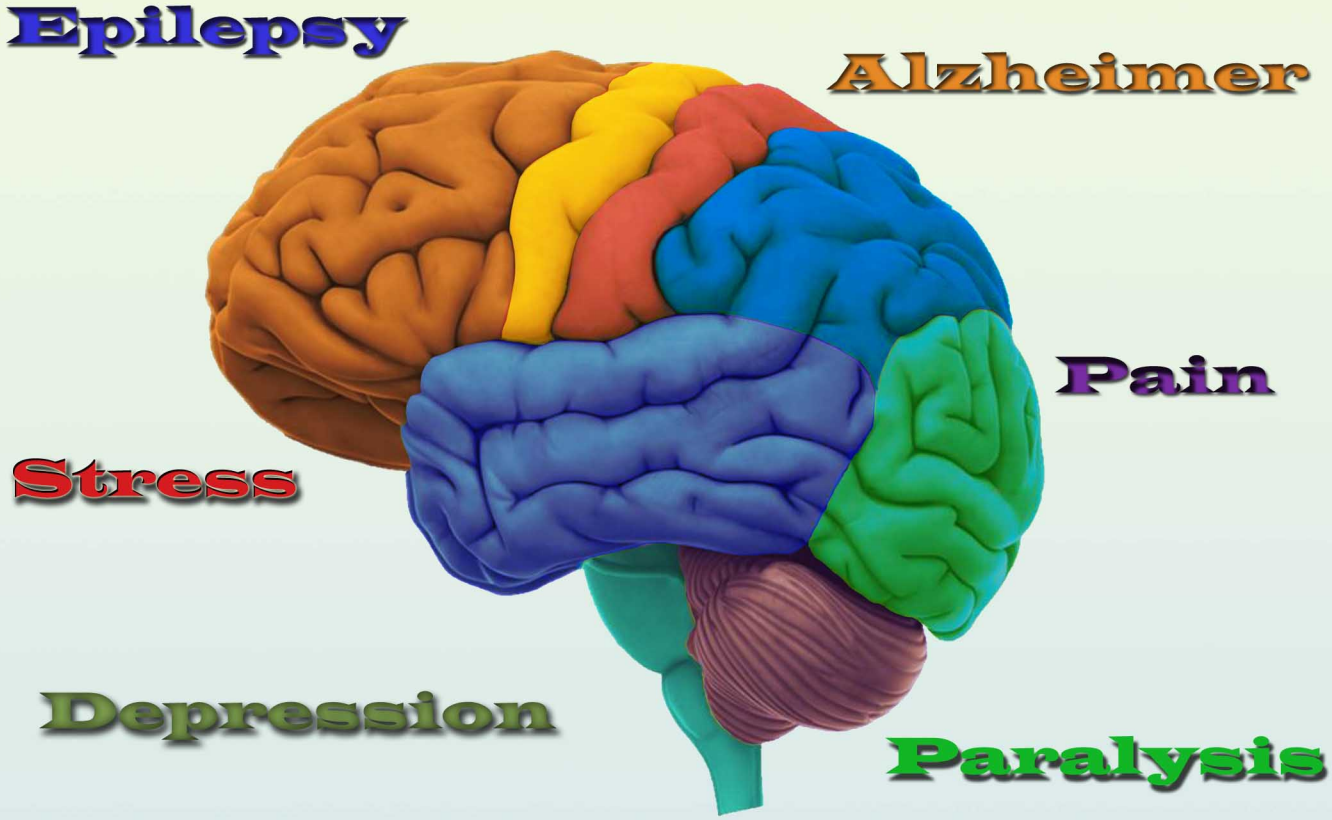
E-ISSN: 2149-7222 (Online)

Journal Cellular Neuroscience and Oxidative Stress



Abstract Book of 5th International Brain Research School

OPEN ACCESS and NO PUBLICATION FEE

http://dergipark.gov.tr/jcnos

Former name; Cell Membranes and Free Radical Research

Supp 1 Volume, 2020

Abstract Book of 5th International Brain Research School

16-22 November 2020, Isparta /TURKEY
http://2020.brs.org.tr

Journal of Cellular Neuroscience and Oxidative Stress

http://dergipark.gov.tr/jcnos

BSN Health Analyses, Innovation, Consultancy, Organization, Industry and Trade Limited Company

http://www.bsnsaglik.com.tr/

info@bsnsaglik.com.tr

Formerly known as:

Cell Membranes and Free Radical Research (2008 - 2014)

Supp 1 Volume, 2020

Supp 1 Volume, 2020
E-ISSN Number: 2149-7222 (Online)
Indexing: Google Scholar, Index Copernicus, Chemical Abstracts, Scopus (Elsevier),
EBSCOhost Research Database, Citation Index Database,

AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

5th International Brain Research School

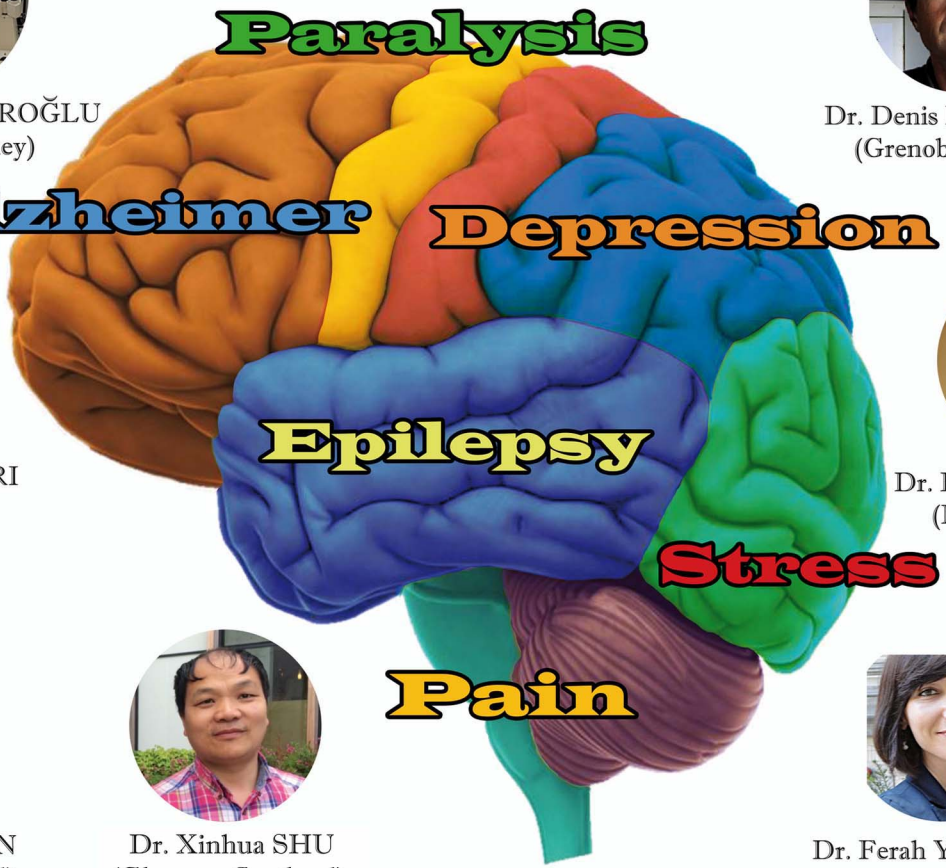
Web page; 2020.brs.org.tr

16 – 22 November 2020

Isparta / Turkey

Dr. Mustafa NAZIROĞLU
(Isparta, Turkey)

Dr. Denis ROUSSEAU
(Grenoble, France)



Dr. Marco CANEPARI
(Grenoble, France)

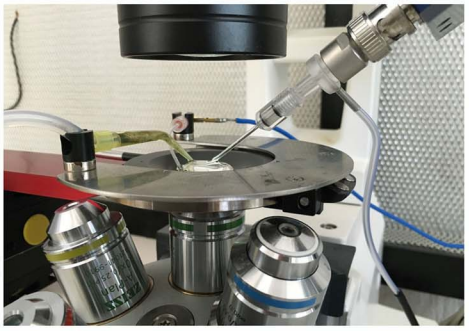
Dr. Plinio CASAROTTO
(Helsinki. Finland)

Dr. Simon HEBEISEN
(Biningen, Switzerland)

Dr. Xinhua SHU
(Glasgow, Scotland)

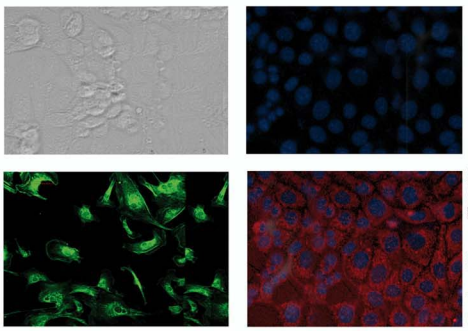
Dr. Ferah YILDIRIM
(Berlin, Germany)

Patch Clamp & Calcium Signaling



Cell Culture

Western Blot & Confocal Microscopy



Design: Yener Yazgan

Contact:
BSN SAĞLIK ANALİZ ARGE LTD ŞTİ
Göller Bölgesi Teknokenti, Çünür Mah.
102. Cadde No: 252/215, Isparta
Tel: 02462113641
e-mail; norobam@sdu.edu.tr

Isparta Turkey

INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

SÜLEYMAN DEMİREL ÜNİVERSİTESİ S.Demirel 1992

BSN SAĞLIK

5th International Brain Research School

_____ [Organization Committee] _____

Organization Chairman
Prof. Dr. Mustafa NAZIROĞLU
Department of Biophysics, School of Medicine
Suleyman Demirel University, Isparta, Turkey

Organization Vice Chairman
Assoc. Prof. Dr. Ömer ÇELİK
Department of Biophysics, School of Medicine
Suleyman Demirel University, Isparta, Turkey

Organization Secretariat
Ahmi ÖZ & Ramazan ÇINAR
Department of Biophysics, School of Medicine
Suleyman Demirel University, Isparta, Turkey

Accountant
Kenan YILDIZHAN & Yener YAZĞAN
Department of Biophysics, School of Medicine
Suleyman Demirel University, Isparta, Turkey

5th International Brain Research School

_____ [Scientific Committee] _____

5th International Brain Research School

_____ [Scientific Committee] _____

5th International Brain Research School

[CONTENTS]

Speakers

5th International Brain Research School

Oral Presentations

SPEAKERS

Speak No. 1

Calcium imaging and laser confocal microscopy analyses in the microglia

Mustafa NAZIROĞLU
Neuroscience Research Center, Suleyman Demirel University, Isparta, Turkey

Calcium ion (Ca^{2+}) acts as a second messenger. Several physiological and pathophysiological functions are modulated by the intracellular free Ca^{2+} concentration. For example, smooth muscle contraction and neurotransmitter release are induced by the Ca^{2+} influx. Apoptosis and cell death were also induced by the Ca^{2+} influx. Ca^{2+} passes the cell membranes via activation of several channels such as voltage gated calcium channels, ligand gated channels, and transient receptor potential (TRP) cation channels (Kumar et al. 2004).

Microglia are the resident immune cells of the central nervous system. The microglia monocyte-derived macrophages derived from the peripheral circulation act major and distinct actions in the several neuronal functions such as neuroinflammation and neuronal injury. The activations of microglia are modulated by several factors, including Ca^{2+} influxes (de Melo Reis et al. 2020).

There are several indicators for measurements of intracellular free Ca^{2+} concentration in the microglia. The main fluorescent indicators for measurements of intracellular free Ca^{2+} concentration in the microglia are Fluo-3 AM, Fluo-4 AM, and Fluo-8. Fluo-3 is used to image the spatial dynamics of Ca^{2+} signaling in the laser confocal microscope experiments. Fluo-4 AM has faster loads and brighter image, making it the preferred indicator for confocal microscopy imaging analyses. However, Fluo-3 AM and Fluo-4 AM are only moderately fluorescent in live cells upon esterase hydrolysis and they need high loading concentrations to maximize their cellular calcium responses. The problem was solved by development of Fluo-8 dyes. Recently, we measured Ca^{2+} dynamics via the activation of TRPM2 channels in the isolated mice microglia (Yıldızhan and Nazıroğlu 2020). In this presentation, I will summarize Ca^{2+} signaling and using the fluorescent dyes for Ca^{2+} imaging in the microglia.

In conclusion, intracellular free Ca^{2+} concentration in the microglia in the laser confocal microscope can be measured by using the indicators. In the measurement fluorescent dye, the Fluo-8 seems best fluorescent dye in the microglia.

Keywords; Calcium signaling, Laser confocal microscope analyses; Calcium fluorescent indicators; Microglia.

References

de Melo Reis RA, Freitas HR, de Mello FG. 2020. Cell calcium imaging as a reliable method to study neuron-glial circuits. Front Neurosci. 14:569361.

Kumar VS, Gopalakrishnan A, Nazıroğlu M, Rajanikant GK. 2014. Calcium ion--the key player in cerebral ischemia. Curr Med Chem. 21(18):2065-2075.

Yıldızhan K, Nazıroğlu M. 2020. Glutathione depletion and parkinsonian neurotoxin MPP^+-induced TRPM2 channel activation play central roles in oxidative cytotoxicity and inflammation in microglia. Mol Neurobiol. 57(8):3508-3525.

SPEAKERS

Speak No. 2

A mouse model for age-related macular degeneration

Xinhua Shu[1,2,3]

[1]Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, UK
[2]Department of Vision Science, Glasgow Caledonian University, Glasgow, UK
[3]School of Basic Medical Sciences, Shaoyang University, Shaoyang, P.R. China

Age-related macular degeneration (AMD) is the most common cause of blind registration in the Western world, accounting for 45% of all registrations in the UK. Early AMD is characterised by the presence of lipid-rich deposits in the macula without vision loss; late AMD is associated with atrophic changes (dry AMD) and/or pathological angiogenesis (wet AMD) in the macula. Cholesterol-rich extracellular lesions are the hallmark features of early- and intermediate-stage AMD. Apolipoproteins, cholesterol and cholesteryl ester deposits have been identified underneath the retinal pigment epithelium (RPE) cells of AMD patients. Genetic studies have also identified several cholesterol-related genes (APOE, CETP, ABCA1, and LIPC) as risk factors for AMD. Excess cholesterol is removed from peripheral cells by the reverse cholesterol transport (RCT) pathway, by which HDL return excess cellular cholesterol to the liver for excretion in bile. Cholesterol deposits in AMD suggest defective RCT may play an important role in the pathogenesis of AMD. In this talk, I will discuss our work in retinal cell lines and in a mouse model to elucidate the disease mechanisms and to develop new treatment for AMD. I will demonstrate how to dissect mouse retina and retinal pigment epithelial (RPE) cells.

SPEAKERS

Speak No. 3

Western-blot, PCR, and immunofluorescence analysis in mitochondrial biogenesis studies

Denis ROUSSEAU
Institut National Polytechnique PHELMA, Grenoble Alpes University, Grenoble Cedex, France

Mitochondria are providing an essential amount of energy to the cell, to achieve in homeostasis, metabolic increases, proliferation and differentiation processes. Also, mitochondrial deficiencies have severe or lethal impacts on cell viability. Among the 3000 proteins involved in mitochondrial activities, ATAD3 is a major one as essential for mitochondrial biogenesis, vital as early as embryonic implantation.

In order to see its impact at animal level, we have used ATAD3+/- mice to investigate its role in running training and in high calorie diet.

We found here that ATAD3 expression level avoids running capacity improvement and has a strong effect on weight increase, underlying its important role in mitochondrial mass regulations.

Prior to this presentation we will emphasize on the potential of Western-blot, PCR and Immunofluorescence analysis in biomedical researches.

Keywords: Mitochondria; ATAD3; Western-blot; PCR; immunofluorescence.

SPEAKERS

Speak No. 4

Drug-induced plasticity: How and where?

Plinio CASAROTTO
Neuroscience Center-HILIFE, University of Helsinki, Helsinki, Finland.

It is unclear how binding antidepressant drugs reopen juvenile-like plasticity and give rise to the clinical antidepressant effect. Recent studies have shown that several interventions reopen plasticity in adult mice and that brain-derived neurotrophic factor (BDNF) and its receptor TRKB play a crucial role in this process. In adults, chronic treatment with fluoxetine facilitates the extinction of fear-related memories and promotes the shift in ocular dominance. Interestingly, our lab has recently observed that the increased activation of TRKB induced by these drugs is dependent on cholesterol and that a highly conserved inverted cholesterol recognition amino acid consensus CARC is present in the transmembrane region (TMR) of TRKB. Using *in silico*, *in vitro* and *in vivo* approaches we found that both typical and fast-acting antidepressants bind to TRKB CARC. Cholesterol stabilized a specific configuration of TRKB dimers and facilitated its activation by BDNF. Mutation of the TRKB cholesterol interaction site impairs BDNF-mediated plasticity and cellular and behavioral responses to antidepressants *in vitro* and *in vivo*. Animals lacking TRKB in specific neuronal subpopulations are also resistant to the effects of antidepressants. We suggest that binding to and facilitation of TRKB activity is a common mechanism for antidepressant action, and propose a framework for how molecular effects of antidepressants are translated into clinical mood recovery.

Keywords: Drug; Plasticity; Antidepressant.

SPEAKERS

Speak No. 5

Principles of Ca^{2+} imaging using low-affinity indicators

Marco CANEPARI
University Grenoble Alpes, CNRS, LIPhy, Grenoble, France

In this lecture I will introduce the principles of Ca^{2+} imaging using low-affinity indicators and its applications to monitor Ca^{2+} dynamics under physiological conditions in native systems, i.e. neurons in *ex-vivo* or *in vivo* preparations. First, I will analyze in detail the issue of competition of the Ca^{2+} indicator with the endogenous Ca^{2+} buffers expressed by the cell and how Ca^{2+} imaging can be performed to monitor the free Ca^{2+} concentration without perturbing the physiological Ca^{2+} homeostasis. Second, how will show how the extremely fast kinetics of low-affinity indicators can be used to monitor fast Ca^{2+} currents to disclose the real kinetics of native voltage-gated Ca^{2+} channels, in particular by combining Ca^{2+} imaging with membrane potential imaging. I will finally extend these concepts to Na^{+} imaging to monitor in detail the origin of an action potential.

Keywords: Ca^{2+} imaging; Low-affinity indicators; Voltage gated calcium channels.

SPEAKERS

Speak No. 6

Transcriptional and epigenetic dysregulation in Huntington's disease

Ferah YILDIRIM
Charité - Universitätsmedizin Berlin, Department of Neuropsychiatry, Berlin, Germany

No Abstract

SPEAKERS

Speak No. 7

Voltage gated sodium channels and epilepsy

Simon HEBEISEN
B'SYS GmbH, The Ionchannel Company, Witterswil, Switzerland

Epilepsy is the fourth most common neurological disorder and affects people of all ages. Medication for epilepsy is often life-long and has a major impact on the quality of life - mostly being related to substantial adverse effects. Therefore, over 30% of people with epilepsy do not achieve sufficient seizure control whilst effective medication being available.

Ion channels are often primary targets of anticonvulsant drugs. They can either act as blockers for voltage gated sodium and calcium channels or as activators for potassium or chloride channels. Additionally, modulators of ligand gated ion channels (GABA or Glutamate receptors) are frequently used to treat epilepsy.

Employing a panel of functional electrophysiological assays using fluorescence based methods and patch-clamping on a broad range of voltage and ligand gated ion channels, we were able to successfully screen for drugs with a beneficial action profile. In successful leads we found drugs that selectively interacted with TTX sensitive, neuronal voltage gated sodium channels. Activation and fast inactivation were unchanged, while an increased affinity in the slow inactivated state was observed. This is a new mode of action for anticonvulsive drugs. In contrast, traditional anticonvulsant drugs often show their major effects on the fast inactivated state of voltage gated sodium channels. One of the tested drugs showed substantial shifts of the voltage dependence of the slow inactivation only for $Na_V1.2$ and 1.6. This favours this drug for treating patients with diseases with compromised $Na_V1.1$ function in interneurons, such as Alzheimer's disease. To improve anticonvulsants even further, compound libraries are screened to identify drugs with multiple ion channel effects like blockers of TTX sensitive Na_V channels and T type calcium channels.

Keywords: Epilepsy, voltage gated sodium channels, state dependent inactivation, patch-clamp technique

Oral Presentations

Oral Presentation 1

Interaction between hypoxia and mitochondrial oxidative stress in retina. Focus on TRPM2 channel

Dilek ÖZKAYA
Department of Ophthalmology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

In the presence of excessive reactive oxygen species (ROS) generation in the mitochondria, the ROS induce destructive action on macromolecules in retina. Hypoxia (HPX) via retinal vessel occlusions in retina results in excessive generation of ROS in mitochondria and has a main role in progression of several retinal degeneration such as glaucoma and diabetic retinopathy (Liu et al. 2020).

HPX-induced excessive Ca^{2+} influx via activation of cell membrane channels, including TRP channels, induces excessive mitochondrial ROS generation, cell death, and apoptosis in neuronal cells. A member of the TRP superfamily is TRP melastatin 2 (TRPM2) Ca^{2+} channel (Nazıroğlu et al. 2012). The Ca^{2+} permeable TRPM2 channel is activated in eye cells such as ARPE-19 retina and primary human corneal epithelial cells by ADP-ribose (ADPR) and ROS, although it is inhibited by chemicals such as 2-aminoethyl diphenylborinate, (2-APB) and N-(p-amylcinnamoyl) anthranilic acid (ACA) (Meléndez García et al. 2016, Zheng et al. 2018). It is well-known that an increase of TRPM2 activation causes the increase of Ca^{2+} uptake into mitochondria. In turn, this leads to excessive generation of ROS via increase of mitochondrial membrane depolarization, resulting in apoptosis, cell death, and down-regulation of survival signaling (Liu et al. 2020). HPX can also inhibit the mitochondrial inner membrane electron transport chain, reduce the mitochondrial membrane depolarization and cause an increase in mitochondrial membrane permeability, leading to the release of pro-apoptotic factors such as caspase -3 and -9 and ROS into the cytosol of eye cells (Liu et al. 2020). However, HPX-evoked cell death and retina degeneration in the retina cell lines, including ARPE-19 retina cells was diminished via inhibition of TRPM2 channels by ACA, 2-APB, and antioxidant treatments (Zheng et al. 2018). I will discuss protective actions of antioxidants via inhibition of TRPM2 channel on the HPX-induced ROS in the retina cells.

In summary, exposures to the hypoxia are accompanied by increased ROS, but decreased antioxidant levels, suggesting that excessive oxidative stress is a reason of HPX-induced retina injury. For clarifying the subject, future studies need on the HPX-induced oxidative stress in the retina cells of experimental animal and human

Key words: Antioxidants; Hypoxia; Retina; Mitochondria; Oxidative stress.

References

Liu L, Sha XY, Wu YN, Chen MT, Zhong JX. 2020. *Lycium barbarum* polysaccharides protects retinal ganglion cells against oxidative stress injury. Neural Regen Res. 15(8):1526-1531.

Meléndez García R, Arredondo Zamarripa D. et al. 2016. Prolactin protects retinal pigment epithelium by inhibiting sirtuin 2-dependent cell death. EBioMedicine. 7:35-49.

Zheng Q, Tan Q, Ren Y, Reinach PS, Li L, Ge C, Qu J, Chen W. 2018. Hyperosmotic stress-induced TRPM2 channel activation stimulates NLRP3 inflammasome activity in primary human corneal epithelial cells. Invest Ophthalmol Vis Sci. 59(8):3259-3268.

Oral Presentations

Oral Presentation 2

Are mobile phone and Wi-Fi frequencies induce oxidative stress in laryngotracheal mucosa?

Yusuf Çağdaş KUMBUL
Department of Otorhinolaryngology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.

Injuries of larynx and trachea through exposure of environmental factors such as smoking and environmental toxins induce injury of laryngotracheal mucosa (Hussain et al. 2015). Environmental factors-induced activation of inflammatory immune cells such as neutrophils and monocytes could increase the production of reactive oxygen species (ROS) to stimulate injury in the larynx and trachea. Hence, results of recent studies studying the oxidative related values in larynx cancer indicated the importance of the environmental exposures.

ROS such as superoxide and hydroxyl radicals are produced during the physiological functions such as phagocytosis and mitochondrial functions. If they will be scavenged by enzymatic and nonenzymatic antioxidant, they will not do hazardous action in the body cells. Lung is responsible from gas transport between alveoli and blood and its ROS levels were increased by the 900 MHz electromagnetic radiation exposure (Zong et al. 2015). Similar, the locations of larynx and trachea are present in the airway of respiration. Hence, both tissues are very sensitive to excessive production of ROS from oxygen. The 900 and 1800 MHz frequencies have been used in cell phone communication in several countries including Turkey, although 2450 MHz has been used as a Wi-Fi frequency in the countries (Alkis et al. 2019). Accumulating evidences indicate that the cell phone and Wi-Fi frequencies induce their hazardous effects in cells including laryngeal mucosa through excessive release of ROS. Results of a paper indicated that the antioxidant levels such as glutathione and glutathione peroxidase were diminished in the laryngeal mucosa of rats by the cell phone and Wi-Fi exposures, but ROS levels were increased in the mucosa by the exposures (Aynalı et al. 2013). In the oral presentation, I will review the results of recent papers on the electromagnetic radiation frequencies-induced ROS in the laryngeal mucosa. I will also discuss protective actions of antioxidants on the electromagnetic radiation frequencies-induced ROS in the laryngeal mucosa.

In summary, exposure to the cell phone and Wi-Fi frequencies are accompanied by increased ROS but decreased antioxidant levels, suggesting that oxidative stress is a reason of electromagnetic radiation-induced laryngotracheal pathophysiology. For clarifying the subject, future studies need on the Wi-Fi and mobile phone frequencies-induced oxidative stress in larynx samples of experimental animal and human.

Key words: Antioxidants; Laryngeal mucosa; Larynx cancer; Electromagnetic radiation; Oxidative stress.

References

Alkis ME, Bilgin HM, Akpolat V, Dasdag S, Yegin K, Yavas MC, Akdag MZ. 2019. Effect of 900-, 1800-, and 2100-MHz radiofrequency radiation on DNA and oxidative stress in brain. Electromagn Biol Med. 38(1):32-47.

Aynalı G, Nazıroğlu M, Çelik Ö, Doğan M, Yarıktaş M, Yasan H. 2013. Modulation of wireless (2.45 GHz)-induced oxidative toxicity in laryngotracheal mucosa of rat by melatonin. Eur Arch Otorhinolaryngol. 270:1695-700.

Hussain S, Manuel CT, Protsenko DE, Wong BJ. 2015. Electromechanical reshaping of ex vivo porcine trachea. The Laryngoscope, 125.7: 1628-1632.

Zong C, Ji Y, He Q, Zhu S, Qin F, Tong J, Cao Y. 2015. Adaptive response in mice exposed to 900 MHZ radiofrequency fields: bleomycin-induced DNA and oxidative damage/repair. Int J Radiat Biol. 91(3):270-276.

Oral Presentations

Oral Presentation 3

Diabetes induces neuropathic pain through activation of TRPV1 channel: A literature review

Ahmet ÖZŞİMŞEK
Department of Neuroscience, Faculty of Medicine, Alanya Alaaddin Keykubat University, Antalya, Turkey.

Type 1 diabetes is induced by destruction of pancreatic islet β cells. Neuropathic pain can arise from a wide variety injury to peripheral, including diabetes. The neuropathic pain is characterized by spontaneous pain, hyperalgesia and allodynia. It is well known that several types of pain, including neuropathic pain are induced by excessive Ca^{2+} influx. The excessive Ca^{2+} influx and overproduction of reactive oxygen species (ROS) have been linked to the neuronal death and neuropathic pain (Kahya et al. 2017).

TRPV1 is Ca^{2+} permeable cation channel. Cysteine groups have main roles for scavenging ROS and modifications of cysteine groups in the cell membrane are very important for the activation of TRPV1 channel (Ogawa et al. 2016). The TRPV1 channel is activated in dorsal root ganglion of diabetic experimental animals by the overproduction of ROS. Expression level of TRPV1 was high in the dorsal root ganglion of animals, resulting excessive Ca^{2+} influx. Hence, selective pharmacological blockade of TRPV1 via capsazepine showed that TRPV1 is crucially involved in diabetes-induced chemical pain sensation and heat hyperalgesia. This presentation describes the roles of TRPV1 in the peripheral pain pathophysiology in the diabetes mellitus. Inhibition of TRPV1 in diabetes channel could be important clinically.

In conclusion, results of experimental diabetes mellitus are involved in the Ca^{2+} entry-induced neuropathic pain via stimulation of TRPV1 channel. Inhibition of TRPV1 in the dorsal root ganglion of patients with diabetes mellitus may useful management and treatment of diabetic neuropathic pain.

Keywords: Diabetes mellitus; Peripheral pain; Oxidative stress; TRPV1 channel.

References

Brito R, Sheth S, Mukherjea D, Rybak LP, Ramkumar V. 2014. TRPV1: A Potential Drug Target for Treating Various Diseases. Cells 3(2):517-45.

Kahya MC, Nazıroğlu M, Övey İS. 2017. Modulation of diabetes-induced oxidative stress, apoptosis, and Ca2+ entry through TRPM2 and TRPV1 channels in dorsal root ganglion and hippocampus of diabetic rats by melatonin and selenium. Mol Neurobiol. 54:2345-2360.

Ogawa N, Kurokawa T, Fujiwara K, Polat OK, Badr H, Takahashi N, Mori Y. (2016) Functional and structural divergence in human TRPV1 channel subunits by oxidative cysteine modification. J Biol Chem. 291:4197-4210.

Oral Presentations

Oral Presentation 4

Resveratrol modulates MPP^+-induced TRPM2 channel activity in hippocampal neurons of mice

Kenan YILDIZHAN[1,2], Ramazan ÇINAR[3], Mustafa NAZIROĞLU[1,3,4]

[1]Department of Biophysics, Faculty of Medicine, Süleyman Demirel University, Isparta, Turkey
[2]Department of Biophysics, Faculty of Medicine, Van Yüzüncü Yıl University, Van, Turkey
[3]Department of Neuroscience, Health Science Institute, Suleyman Demirel University, Isparta, Turkey
[4]Drug Discovery Unit, BSN Health, Analysis and Innovation Ltd. Inc. Teknokent, Isparta, Turkey

Parkinson's disease (PD) is an age-related chronic neurodegenerative disease. Although it is known that hippocampal neurons are damaged in the PD (Hall et al. 2014), the molecular mechanism is still not fully clarified. The neurodegeneration in hippocampal neurons have been suggested to includes excessive production of reactive oxygen species (ROS). ROS, impairment of mitochondrial dysfunction and Ca^{2+} homeostasis, because it is incapable of regeneration (Yürüker et al. 2015). It is noteworthy in the literature that especially the change of calcium homeostasis triggers neuronal degeneration. Transient receptor potential melastatin 2 (TRPM2) is a unique calcium permeable nonselective cation channel and densest in numberless neuronal population. The TRPM2 channel is activated by ROS and ADP-ribose. Recently, protective role of resveratrol via inhibition of ROS-induced TRPM2 activation in substantia nigra of 1-methyl-4-phenylpyridinium ion (MPP^+)-induced PD model in mice was reported (Sun et al. 2018). Similar action of resveratrol may present in the hippocampus of mice with PD. The current study aimed to elucidate the effect of antioxidant resveratrol on TRPM2 mediated oxidative stress induced by MPP^+ exposure in the primary mouse hippocampal neurons that they were isolated from C57BL/6 neonatal (P0-P2) mice.

The neurons were divided into 4 groups as control, resveratrol (3,4',5-trihydroxystilben) (20 μM for 6 hour), MPP^+ (0.5 nM for 6 hour) and MPP^++resveratrol.

In the present results, we observed activation of TRPM2 in the hippocampal neurons by the MPP^+ treatment. TRPM2 channel expressions levels in the MPP^+ group were increased in hippocampal neurons by MPP^+ treatment. Also, intracellular free Ca^{2+} concentration and TRPM2 channels currents were high in the MPP^+ groups, although they were diminished by the resveratrol treatment. In addition, MPP^+-induced mitochondrial membrane depolarization, ROS, caspase 3, caspase 9 and apoptosis values were decreased by the resveratrol treatment.

In conclusion, this study reveals the protective effect of resveratrol against oxidative stress and apoptotic cell death induced by TRPM2 dysregulation in hippocampal neurons exposed to MPP^+.

Keywords: Apoptosis; Calcium signaling; Hippocampal neurons; Parkinson's disease; Oxidative stress; TRPM2.

Acknowledgement
The study was supported by BSN Health, Analysis and Innovation Ltd. Inc. Teknokent, Isparta, Turkey (Project No: 2019-06).

References

Hall H, Reyes S, Landeck N, Bye C, Leanza G, Double K, Thompson L, Halliday G, Kirik D. 2014. Hippocampal Lewy pathology and cholinergic dysfunction are associated with dementia in Parkinson's disease. Brain. 137(Pt 9):2493-508.

Sun Y, Sukumaran P, Selvaraj S, Cilz NI, Schaar A, Lei S, Singh BB. 2018. TRPM2 Promotes Neurotoxin MPP(+)/MPTP-Induced Cell Death. Mol Neurobiol. 55(1):409-420.

Yürüker V, Nazıroğlu M, Şenol N. 2015. Reduction in traumatic brain injury-induced oxidative stress, apoptosis, and calcium entry in rat hippocampus by melatonin: Possible involvement of TRPM2 channels. Metab Brain Dis. 30(1):223-31.

Oral Presentations

Oral Presentation 5

Involvement of TRPM2 channel in the experimental hypoxia: A critical review

Dilek DÜZGÜN ERGÜN
Department of Biophysics, Faculty of Medicine, Istanbul Aydin University, Istanbul, Turkey

Transient receptor potential (TRP) superfamily with seven subgroups is containing 28 members in mammalian. A subgroup of the TRP superfamily is TRP melastatin (TRPM). The TRPM subfamily is containing 8 members within the 4 subgroups. A member of the TRPM subgroups is TRPM2 channels. The TRPM2 has important functions in apoptosis and cell death (Clapham, 2003). The TRPM2 has ADP-ribose pyrophosphatase enzyme in the NUDT9-H domain. The channel needs to activation of the enzyme via oxidative stress and ADP-ribose. Excessive Ca^{2+} influx induces several pathophysiological pathways such as apoptosis and cell death. Hence, several oxidant factors including hypoxia induce apoptosis and cell death via activation of TRPM2 channel (Miller and Cheung, 2016). Oxidative stress and reactive oxygen species (ROS) are produced in the cells during the hypoxia. The excessive production of ROS is diminished by the treatment of antioxidants such as selenium and glutathione. Selenium is an essential trace element and it is a component of antioxidant enzyme glutathione peroxidase. Hence, selenium has strong antioxidant role on the hypoxia-induced ROS in several cells. A recent finding indicated modulator role of selenium on apoptosis and neuronal death TRPM2 channel in the hippocampus and dorsal root ganglion of rats (Balaban et al. 2017). Hence, similar action of selenium via inhibition of TRPM2 channel on the hypoxia-induced cell death and apoptosis might be present. In the oral presentation, I will summarize the literature review on the hypoxia-induced cell death and apoptosis via TRPM2 channel activation in several cells.

I concluded that hypoxia-induced apoptosis and oxidative stress productions were acted by Ca^{2+} entry via TRPM2 channel, although their productions were substantially reduced by antioxidants including selenium. However, the relationship between hypoxia and TRPM2 channel has not been clarified fully yet and it should be clarified by future studies.

Key words; TRPM2 channel; Hypoxia; Oxidative stress. Selenium.

References

Balaban H, Nazıroğlu M, Demirci K, Övey İS. 2017. The protective role of selenium on scopolamine-induced memory impairment, oxidative stress, and apoptosis in aged rats: The involvement of TRPM2 and TRPV1 channels. Mol Neurobiol. 54(4):2852-2868.

Clapham DE. 2003. TRP channels as cellular sensors. Nature. 426(6966):517-524.

Miller BA, Cheung JY. 2016. TRPM2 protects against tissue damage following oxidative stress and ischaemia-reperfusion. J Physiol. 594(15):4181-4191.

Oral Presentations

Oral Presentation 6

TRPM8 is activated in prostate cancer cells by oxidative stress

Sebahat ULUSAN
Student, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Prostate cancer is the most type of cancer and the second leading cause of cancer death among men of USA. It has been known for a long time that changes of intracellular calcium ion (Ca^{2+}) concentration and/or Ca^{2+} molecular pathways interfere with the second messenger signaling molecular pathways controlling apoptosis, proliferation, differentiation, secretion and migration. Prostate cancer cell proliferation is induced by the increase of the intracellular Ca^{2+} concentration. In addition, excessive Ca^{2+} influx induces overproduction of reactive oxygen species (ROS). Therefore, new anti-tumor treatment strategies with the aim of remodeling the Ca^{2+}-signaling and oxidative stress pathways have emerged in prostate cancer tumor cells (Bidaux et al. 2016).

Among Ca^{2+} channels, transient receptor potential melastatin 8 TRPM8 is thought to be a potential therapeutic target in the prostate cancer. Indeed, involvement of TRPM8 in the proliferation of prostate cancer was reported (Bidaux et al. 2016). TRPM8 is activated in cells by several stimuli such as cold, menthol and icilin (Chuang et al. 2004). A recent data indicated that TRPM8 is activated in prostate cancer cells by oxidative stress. In the young speaker presentation, I will summarize present reports on the oxidative stress and TRPM8 in the prostate cancer. My observations these suggests that inhibition of oxidative stress and TRPM8 could be an appropriate strategy to the treatment of prostate cancer.

Keywords: TRPM8 channel; Prostate cancer; Oxidative stress; Calcium signaling.

References

Baş E, Nazıroğlu M, Pecze L. 2019. ADP-Ribose and oxidative stress activate TRPM8 channel in prostate cancer and kidney cells. Sci Rep. 9(1):4100.

Bidaux G, Borowiec AS, Dubois C, Delcourt P, Schulz C, Vanden Abeele F, Lepage G, Desruelles E, Bokhobza A, Dewailly E, Slomianny C, Roudbaraki M, Héliot L, Bonnal JL, Mauroy B, Mariot P, Lemonnier L, Prevarskaya N. 2016. Targeting of short TRPM8 isoforms induces 4TM-TRPM8-dependent apoptosis in prostate cancer cells. Oncotarget 7(20):29063-29080.

Chuang HH, Neuhausser WM, Julius D. 2004. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. Neuron 43(6):859-869.

Oral Presentations

Oral Presentation 7

Effect of gallic acid on glutamate-induced oxidative cytotoxicity in hippocampal cell lines

Fatma Nihan CANKARA
Department of Pharmacology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey

Glutamate is the excitatory neurotransmitter of the central nervous system (CNS) and plays an important role in synaptic plasticity, learning, memory, and other cognitive functions. An increase in reactive oxygen species (ROS) that trigger neuronal cell death occurs as a result of excessive glutamate release. Therefore, protecting neurons against glutamate excitotoxicity may be an effective therapeutic approach for neurodegenerative diseases. In the current study, the effects of gallic acid (GA), which has high antioxidant capacity, on oxidative damage induced by glutamate in hippocampal cell lines were investigated.

GA (0.625, 1.25, 2.5, 5, 10, 20 μM) and L-glutamic acid (5 μM) were applied to the HT-22 cell line. After 12 hours of incubation, cell viability was evaluated by MTT test and glutathione, glutathione reductase, glutathione peroxidase, ROS, and lactate dehydrogenase levels were measured.

GA (2.5, 5, 10, and 20 μM) significantly attenuated decrease of cellular viability due to the glutamate administration. Additionally, GA (2.5, 5, 10 and 20 μM) decreased glutamate-induced oxidative stress and ROS generation. Furthermore, GA (2.5, 5, 10 and 20 μM) prevented oxidative stress related LDH release.

In conclusion, current results demonstrate that GA treatment decreased glutamate-induced cell death in the hippocampal cells which is considered as main mechanism of epilepsy. However, present data suggest antioxidant effect of GA has a main role in these effects.

Keywords: HT-22 cell line; Glutamate; Gallic Acid; Oxidative stress

Oral Presentations

Oral Presentation 8

Potential therapeutic use of dopamine receptor 2 agonist (cabergoline) in the treatment of endometriosis via inhibition of TRPV1 channel: A literature review

Elif İknur EKİCİ
Department of Obstetrics and Gynecology, Isparta City State Hospital, Isparta, Turkey

Endometriosis as a gynecological disease is characterized by the presence of endometrium like tissue-epithelium and stroma. It develops outside the uterine cavity and it causes pelvic pain and infertility in women. The etiology of pain in the endometriosis has not been fully clarified yet. However, excessive Ca^{2+} influx via activation of several cation channel has a main role in the etiology of endometriosis. Although several drug therapies present for the treatment of endometriosis, the drugs have their own limitations such as cost of treatment, side-effects and its short-term effect on the symptoms of endometriosis (Goenka et al. 2017).

Cabergoline is a dopamine receptor agonist and it has an inhibitor role on neoangiogenesis because of its explicit preventive activity in vascular endothelial growth factor receptor binding. Recently, protective role of cabergoline on pain of patients with stage IV endometriosis was reported. It is well known that excessive Ca^{2+} influx has a main role for induction of several pain types, including endometriosis-induced pain. Ca^{2+} pass the cell membrane via stimulation of cation channels. Cabergoline may induce the antipain action via inhibition of cation channel, including TRPV1 channel.

TRPV1 channel is a member of transient receptor potential superfamily and it is activated by several stimuli such as capsaicin, heat, acidic pH and oxidative stress. Its expression level was high in the dorsal root ganglion and trigeminal ganglia which were responsible for induction of neuropathic pain. Recent data indicated that there is a direct relationship between TRPV1 channel and endometriosis-associated pain in women. On the subject, Greaves et al. (2014) reported estrogen-dependent increase of TRPV1 expression in sensory neurons of women caused endometriosis-associated pain. It was reported that upregulation of TRPV1 in patients with endometriosis lesions and pain intensity has a potential role in pathophysiological mechanisms of endometriosis (Bohonyi et al. 2017).

Recently we observed protective action of cabergoline via inhibition of TRPV1 and TRPM2 on apoptozis and oxidative stress in neutrophils of patients with endometriosis (Ekici et al. 2020).

I have attempted to summarize the current interactions between cabergoline and TRPV1 channel for endometriosis.

In conclusion, TRPV1 activation is accompanied by increased endometrial pain, suggesting that TRPV1 is a cause of endometriosis-induced pain pathophysiology. For clarifying the subject, future studies need on TRPV1 channel in the endometrium and nerve of animal and human.

Key words: Cabergoline; Endometriosis; Pain; TRPV1.

References

Bohonyi N, Pohóczky K, Szalontai B, Perkecz A, Kovács K, Kajtár B, Orbán L, Varga T, Szegedi S, Bódis J, Helyes Z, Koppán M. 2017. Local upregulation of transient receptor potential ankyrin 1 and transient receptor potential vanilloid 1 ion channels in rectosigmoid deep infiltrating endometriosis. Mol Pain. 13:1744806917705564.

Ekici Eİ, Güney M, Nazıroğlu M. 2020. Protective effect of cabergoline on mitochondrial oxidative stress-induced apoptozis is mediated by modulations of TRPM2 in neutrophils of patiens with endometriosis. J Bioenerg Biomembr. 52(3):131-142.

Goenka L, George M, Sen M. 2017. A peek into the drug development scenario of endometriosis - A systematic review. Biomed Pharmacother. 90:575-585.

Greaves E, Grieve K, Horne AW, Saunders PT. 2014. Elevated peritoneal expression and estrogen regulation of nociceptive ion channels in endometriosis. J Clin Endocrinol Metab. 99(9):E1738-E1743.

Oral Presentations

Oral Presentation 9

Sirtuins: Key enzymes in age-related diseases

Saide MURATOĞLU
Department of Physiology, Faculty of Medicine, Yüksek İhtisas University, Ankara, Turkey

Aging is defined as the universal time-dependent process with loss of function for all living organisms. Sirtuins (SIRTs) are the Nicotinamide Adenine Diphosphate (NAD) linked histone/protein deacetylase family that can increase lifespan by maintaining genome stability, cell cycle, DNA repair, mitochondrial function, aging, inflammation, metabolism and oxidative stress regulation (Davalli et al. 2016). There are seven members in mammalian sirtuins. SIRTs according to their location; SIRT1 (also has important cytoplasmic functions), SIRT6 and SIRT7 in the nucleus; SIRT3, SIRT4 and SIRT5 are found in mitochondria. SIRT2, is the most abundant sirtuin in the cytoplasm and it can pass into the nucleus depending on the cell cycle (Haigis et al. 2010). SIRTs have emerged as potential targets that can be manipulated to counteract age-related diseases, metabolic, cardiovascular and neurodegenerative diseases, such as type 2 diabetes, Alzheimer and Parkinson (Donmez, 2012; Davalli et al. 2016). Activation of SIRT1 improves glucose homeostasis and insulin sensitivity; protects against high-fat diet-induced metabolic damage, inflammation and diabetes. SIRT2 increases with age. It is found brain, adipose tissue, liver, testis, skeletal muscle and kidney; and triggers the processes associated with stress-related premature aging of up-regulation (Haigis et al. 2010). SIRT3 has functions such as thermogenesis, metabolism, longevity, oxidative stress and cell apoptosis. The functions of SIRT4 are insulin secretion, TCA cycle, fatty acid oxidation, tumor and genome stability. SIRT5 is involved in processes such as urea cycle, ketone body synthesis, oxidative stress and cellular respiration. While SIRT6 plays a role in DNA repair, glucose homeostasis, genome stability, metabolism, inflammation, cancer, cardiovascular diseases; SIRT7 functions in rRDNA transcription, genome stability, oxidative stress (Finkel et al. 2009). This new family that will shed light on the mechanism of aging is exciting for the scientific world.

Keywords: Sirtuins, aging, oxidative stress, age-related diseases

References

Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D. 2016. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. Oxid Med Cell Longev. 3565127.

Donmez G. 2012. The neurobiology of sirtuins and their role in neurodegeneration. Trends Pharmacol Sci. 33(9):494–501.

Finkel T, Deng CX, Mostoslavsky R. 2009. Recent progress in the biology and physiology of sirtuins. Nature 460:587-591.

Haigis MC, Sinclair DA. 2010. Mammalian sirtuins: Biological insights and disease relevance. Annu Rev Pathol. 5:253–295.