Molecular Mechanisms of Regulation in the Nervous System

Tartu, Estonia

June 14-17, 2015
www.esn2015.ee
Acknowledgements

Organizer:

Supporter:

Exhibitor:
Dear Participants,

I am delighted to welcome you on behalf of the European Society for Neurochemistry (ESN) to the 21st meeting of our society in Tartu, Estonia. The ESN was founded in 1976 with the objectives to advance Neurochemistry for the public benefit and to promote the development of Neurochemistry in Europe. As it was always of special importance for our society to bring together European scientists from East and West, several ESN meetings have been held in East Europe. Following this old tradition of ESN, we are pleased to have now our first meeting in one of the Baltic States.

The program committee and the local organising committee have organised an outstanding meeting for our society. We have an excellent scientific program with prominent international plenary speakers, exciting symposia and minisymposia and a large number of excellent poster presentation. As new initiative, ESN has established now the ESN Young Members Symposia which will give our young members a platform to present their exciting scientific results as symposium talk at our international conference. Our meeting will offer heaps of opportunities for scientific exchange and interactions. The scientific content of our meeting will be nicely completed by several social events which will get us in close contact with Estonia and its history.

I am convinced that you will enjoy our meeting and that you will have a great time in Tartu.

Ralf Dringen

President of ESN
Dear Participants,

It is our great pleasure to welcome delegates of the European Society for Neurochemistry 21st meeting in Tartu, in the old university town on the bank of the river Emajõgi on June 14-17, 2015.

The University of Tartu was founded in 1632 by the Swedish king Gustavus Adolphus. For neurochemists Tartu is known as birthplace of experimental pharmacology, which was started here by Rudolf Buchheim and Oswald Schmiedeberg and embryology started by Karl Ernst von Baer in the middle of 19-th century. Today Tartu is a town of students, science and innovation, where modern scientific and corporate buildings stand side by side with historical ones. We hope that you find here a good atmosphere to discuss about your latest research developments and get inspiration for further studies. We are proud to host delegates from all around the world and we wish you interesting conference and enjoyable time in Tartu.

Welcome to Estonia and Welcome to Tartu!

Ago Rinken

*On behalf of the local organizing committee and the international programme committee*
International programme committee

Chair:
Professor Illana Gozes - The Lily and Avraham Gildor Chair for the Investigation of Growth Factors, Tel Aviv University, Israel

Members:
Prof Ralf Dringen – president of ESN, Centre for Biomolecular Interactions, University of Bremen, Germany
Prof. Philip Mark Beart - Florey Institute of Neuroscience & Mental Health, University of Melbourne, Australia
Dr. Natalia N Nalivaeva - School of Biochemical & Molecular Biology, University of Leeds, UK.
Prof. Ago Rinken – Institute of Chemistry, University of Tartu, Estonia
Prof. Juan Bolanos - Institute of Functional Biology and Genomics, University of Salamanca-CSIC, Spain
Prof. Mika Scheinin - Department of Pharmacology, University of Turku, Finland

Local organizing committee

Chair:
Prof. Ago Rinken – treasurer of ESN, Institute of Chemistry, University of Tartu; Board member of Estonian Society of Pharmacology, Estonia

Members:
Prof. Alessandro Prinetti - secretary of ESN, Department of Medical Biotechnology and Translational Medicine, University of Milano, Italy
Dr. Malle Kuum – president of ESP, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia
Dr. Anti Kalda – board member of ESP, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia
Dr. Annika Vaarmann – Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia
MSc Tiina Ööpik – Regulatory Manager for Generic Products, Sanofi-Aventis Estonia OÜ
Anni Allikalt – Institute of Chemistry, University of Tartu, Estonia
Sunday 14 June 2015

10.00–15.00  | **Sightseeing tour to Tartu including city tour in Tallinn**

**Venue: Dorpat Conference Centre**
Conference room: Peterson

16.00  | **ESN Council meeting**
Conference room: Struve I

18.00  | **Welcome**

18.15  | **Opening plenary lecture:** Hermona Soreq (ISRAEL)
MicroRNA-target interactions in neurodegenerative diseases (L1)
Chair: Ralf Dringen (GERMANY)

**Venue: Science Center AHAA**

19.30  | **Welcome reception,**
Science Center AHAA (Sadama 1, Tartu)

The abstracts are available online at http://www.springerplus.com/supplemens/4/S1
**Monday 15 June 2015**

**Venue: Dorpat Conference Centre**

Conference room: Struve I

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.00</td>
<td><strong>Special Lectures: Meet the Professors</strong></td>
<td>Chair: Alessandro Prinetti (ITALY), Ago Rinken (ESTONIA)</td>
</tr>
<tr>
<td></td>
<td>Alois Saria (AUSTRIA) Reacquisition of cocaine conditioned place preference and its inhibition by previous social interaction: Neurochemical and electrophysiological correlates in the nucleus accumbens corridor (L2)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ralf Dringen (GERMANY) Copper metabolism of astrocytes (L3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alexander Zharkovski (ESTONIA) Altered developmental neuroplasticity due to polysialyltransferase ST8SiaII deficiency in mice leads to schizophrenia-like phenotype (L4)</td>
<td></td>
</tr>
<tr>
<td>10.20</td>
<td>Morning coffee</td>
<td></td>
</tr>
</tbody>
</table>

Conference room: Struve I

<table>
<thead>
<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.45</td>
<td><strong>Symposium 1: Organized by GLISTEN (COST action CM1207) &quot;Signal transduction models in CNS – from calculations to in vivo assay&quot;</strong></td>
<td>Chair: Stephen Hill (UK), Graeme Milligan (UK)</td>
</tr>
<tr>
<td></td>
<td>Jens Carlsson (SWEDEN) Two steps forward, one step back: Successes and Failures in Structure-based Discovery of GPCR ligands (L5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carsten Hoffmann (GERMANY) Comparison of A1 and A2A receptor dynamics using FRET based receptor sensors (L6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Graeme Milligan (UK) Defining the organizational structure of dopamine and muscarinic acetylcholine receptors (L7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stephen Hill (UK) Investigation of GPCR allosterism and dimerization in single living cells using fluorescent ligands (L8)</td>
<td></td>
</tr>
<tr>
<td>12.45</td>
<td><strong>Lunch, posters and networking</strong></td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>13.30-14.30</td>
<td>Poster presentations. Odd poster numbers (1,3,5 etc)</td>
<td></td>
</tr>
<tr>
<td>15.00</td>
<td><strong>Parallel minisymposia: Three parallel symposia</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conference room: Peterson</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Minisymposium 1: Glioma Signalling: From Molecular Mechanisms to Therapy</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chair: Peter Brust (GERMANY)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eleonora Aronica (NETHERLANDS) Gliomas and epilepsy: Insights from neuropathological studies in humans (L9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maria P Abbracchio (ITALY) Purinergic transmission in brain tumors and its impact on drug development (L10)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bozena Kaminska (POLAND) Cannabinoid signalling in glioma cells (L11)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Patrick Roth (SWITZERLAND) The role of integrins in glioma biology and anti-glioma therapies (L12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conference room: Baer</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Minisymposium 2: Tau regulation/Alzheimer's disease and related dementia</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chair: Illana Gozes (ISRAEL), Amrit Mudher (UK)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Amrit Mudher (UK) Drosophila modelling axonal transport in the face of tau pathology (L13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Miguel Medina (SPAIN) Recent developments in tau-based therapeutics for Alzheimer’s disease and related dementia (L14)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Christiane Richter-Landsberg (GERMANY) The oligodendroglia cytoskeleton in health and disease (L15)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Carmen Laura Sayas (SPAIN) Tau regulates the localization and function of End Binding proteins in neuronal cells (L16)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conference room: Struve I</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Minisymposium 3: Biochemistry of GPCR system in CNS</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chair: Mika Scheinin (FINLAND), Jyrki Kukkonen (FINLAND)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jyrki Kukkonen (FINLAND) Multiple signaling pathways of orexin receptors (L17)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ülo Langel (SWEDEN/ESTONIA) Galanin receptor ligands (L18)</td>
<td></td>
</tr>
<tr>
<td>Time</td>
<td>Event</td>
<td></td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>17.00</td>
<td>Afternoon coffee</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Conference room: Struve I</td>
<td></td>
</tr>
<tr>
<td>17.30</td>
<td><strong>ESN business meeting</strong></td>
<td></td>
</tr>
<tr>
<td>19.30</td>
<td><strong>Sightseeing tour in Tartu with concert in St. John's Church</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&quot;There are two meeting points for the walking tour in Tartu – Dorpat hotel lobby and Tartu hotel lobby. Please be at the meeting point at 19.25&quot;</td>
<td></td>
</tr>
<tr>
<td>21.00</td>
<td><strong>C-Jam concert in St. John’s Church (Jaani 5, Tartu)</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>The special concert involves four men, four chairs, four cellos and music that charms the senses</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>If you are not attending the walking tour, you can come directly to the St. John’s Church</em></td>
<td></td>
</tr>
</tbody>
</table>
# Tuesday 16 June 2015

**Venue: Dorpat Conference Centre**

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.00</td>
<td><strong>ISN Lecture.</strong> Hannelore Ehrenreich (GERMANY)</td>
<td>Autism as a disease of the synapse: Search for mechanistic insight (L22) Chair: Illana Gozes (ISRAEL)</td>
</tr>
<tr>
<td>9.45</td>
<td>Morning coffee</td>
<td></td>
</tr>
<tr>
<td>10.15</td>
<td><strong>Young scientists’ minisymposia</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Conference room: Struve I**

*Young scientist symposium: Molecular Mechanism of disease*
Chair: Ralf Dringen (GERMANY)

- David Hicks (UK) Elucidating the mechanisms of TDP-43 aggregation in a cellular model of motor neuron disease (L23)
- Riccardo Maria Cristofani (ITALY) The role of dynein mediated transport in the clearance of misfolded proteins responsible for motoneuron diseases (L24)
- Silvia Lisa (GREECE) Effects of novel synthetic microneurotrophins in diabetic retinopathy (L25)
- Claudia Susana Fernandez Queiroga (PORTUGAL) Unravelling carbon monoxide protection in cerebral ischemia: from the organelle to the organism (L26)
- Anna Victorovna Churilova (RUSSIA) The effect of different modes of hypobaric hypoxia on the expression of transcription factor pCREB and pro-survival proteins BDNF and BCL-2 in rat neocortex and hippocampus (L27)

**Conference room: Baer**

*Young scientist symposium: Molecular mechanisms of disease, in vitro and in vivo*
Chair: Alessandro Prinetti (ITALY)
<table>
<thead>
<tr>
<th><strong>Anastassia Voronova (CANADA)</strong></th>
<th>Ankrd11 is a chromatin regulator involved in autism that is essential for neural development (L28)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Martina Gabrielli (ITALY)</strong></td>
<td>Active endocannabinoids are secreted on the surface of microglial microvesicles (L29)</td>
</tr>
<tr>
<td><strong>Katarzyna Nawrotek (POLAND)</strong></td>
<td>Peripheral nerve implants enriched with chemotactic factors for peripheral nervous tissue engineering (L30)</td>
</tr>
<tr>
<td><strong>Galyna Maleeva (UKRAINE)</strong></td>
<td>Ginkgolic acid specifically potentiates alpha 1 glycine receptors (L31)</td>
</tr>
<tr>
<td><strong>Charlotte Petters (GERMANY)</strong></td>
<td>Uptake, metabolism and toxicity of iron oxide nanoparticles in cultured microglia, astrocytes and neurons (L32)</td>
</tr>
</tbody>
</table>

| **12.30**                              | **Lunch, posters and networking**                                                                      |
| **13.30-14.30**                        | **Poster presentations. Even poster numbers (2, 4, 6 etc)**                                           |
| **14.30**                              | **Parallel minisymposia: Three parallel minisymposia**                                               |
| **Conference room: Struve I**          |                                                                                                        |
| **Minisymposium 4: Mitophagy: Insights into Neurodegeneration from Mitochondrial Dynamics** | Chair: Philip Beart (AUSTRALIA), Olga Corti (FRANCE)                                                  |
|                                        | Josef Kittler (UK) Regulation of mitochondrial trafficking and function by the mitochondrial GTPases Miro1 and Miro2 (L33) |
|                                        | Allen Kaasik (ESTONIA) Principles of mitochondrial fusion and fission cycle in neurons (L34)           |
|                                        | Philip Beart (AUSTRALIA) Bioenergetic failure, mitochondrial dysfunction and mitophagy (L35)          |
|                                        | Olga Corti (FRANCE) Mechanisms of mitochondrial quality control in autosomal recessive Parkinson's disease (L36) |
| **Conference room: Baer**              |                                                                                                        |
| **Minisymposium 5: Gene regulation and epigenetics** | Chair: Natalia Nalivaeva (UK)                                                                        |
|                                        | Illana Gozes (ISRAEL) Activity-dependent neuroprotective protein (ADNP): from autism to Alzheimer's disease (L37) |
Tõnis Timmusk (ESTONIA) Neuronal-activity regulated gene expression: emphasis on BDNF

Elena Rybnikova (RUSSIA) Epigenetic mechanisms of hypoxic preconditioning

Natalia Nalivaeva (UK) Role of amyloid precursor protein (APP) in regulation of neuronal genes

Conference room: Peterson

**Minisymposium 6: Nrf2, HO-1 and CO: signaling, neuroprotection and modulation of inflammation**
Chair: Helena L. A. Vieira (PORTUGAL), Roberto Motterlini (ITALY)

Roberto Motterlini (ITALY) Anti-inflammatory activities of carbon monoxide-releasing molecules (CO-RMs) in the brain

Helena L. A. Vieira (PORTUGAL) Carbon monoxide targeting mitochondria in astrocytes: modulation of cell metabolism, redox response and cell death

Antonio Cuadrado (SPAIN) Transcription Factor Nrf2: a novel target to modulate inflammatory and neuroprotective responses in Parkinson’s disease

Sylvain Doré (Canada) Neuroprotective effect of carbon monoxide and Nrf2 in cerebral ischemia

16.15  Afternoon coffee

Conference room: Struve I

16.30  **ESN Young Lectureship Award**
Chair: Anthony Turner (UK)

Nico Mitro (ITALY) The lipogenic regulator Sterol Regulatory Element Binding Factor-1c is required to maintain peripheral nerve structure and function

Anna Cariboni (ITALY) The molecular control of GnRH neuron development

Conference room: Struve I

**Satellite symposium**
Chair: Anti Kalda (ESTONIA)
<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>17:15</td>
<td><strong>Springer Online presentation</strong></td>
</tr>
<tr>
<td>17:35</td>
<td><strong>Bruker presentation:</strong> Multimodal preclinical Imaging. Applications in neuroscience</td>
</tr>
<tr>
<td></td>
<td><strong>Venue: University of Tartu Museum, Lossi 25, Tartu</strong></td>
</tr>
<tr>
<td>20:00</td>
<td><strong>Conference dinner</strong></td>
</tr>
<tr>
<td></td>
<td>Conference dinner will be held in the University of Tartu Museum that is located in the Toome hill. Please see the map on the back of the programme book or request assistance from the Conference information desk. Evening at the museum offers besides networking and dinner also good music and entertaining comedy show by Comedy Estonia.</td>
</tr>
<tr>
<td>Time</td>
<td>Session</td>
</tr>
<tr>
<td>----------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>9.00</td>
<td><strong>Parallel minisymposia</strong></td>
</tr>
<tr>
<td>Conroom</td>
<td>Struve I</td>
</tr>
</tbody>
</table>
| **Minisymposium 7:** WNT signaling regulates brain function and provides novel opportunities for therapy  
Chair: Gunnar Schulte (SWEDEN) |
|          | Manuela Simonetti (GERMANY) WNT signaling mechanisms in nociception and sensitization of afferent neurons (L48) |
|          | Ernest Arenas (SWEDEN) WNT signaling in midbrain dopaminergic neuron development and cell replacement therapies for Parkinson’s disease (L49) |
|          | Gunnar Schulte (SWEDEN) WNT signaling in microglia and the glioma microenvironment (L50) |
| Conroom  | Baer                                                                     |
| **Minisymposium 8:** From RNA- to proteo-toxicity in motoneuron diseases  
Chair: Angelo Poletti (ITALY), Maria Teresa Carri’ (ITALY) |
|          | Mauro Cozzolino (ITALY) Translational repression in the pathogenesis of FUS- and c9orf72-dependent ALS (L51) |
|          | Pietro Fratta (UK) Post-transcriptional modifications caused by TDP-43 mutations in mouse and man (L52) |
|          | Tilmann Achsel (BELGIUM) Sm-like proteins in the pathogenesis of Spinal Muscular Atrophy (L53) |
|          | Maria Teresa Carri’ (ITALY) Diverse roles of FUS in Amyotrophic Lateral Sclerosis (L54) |
|          | Angelo Poletti (ITALY) The protein quality control system in motoneuron diseases (L55) |
| Conroom  | Peterson                                                                |
| **Minisymposium 9:** Neuropeptides  
Chair: Ülo Langel (SWEDEN) |
<p>|          | Dora Reglodi (HUNGARY) Neurochemical changes in different brain regions induced by PACAP - relations to neuroprotection (L56) |</p>
<table>
<thead>
<tr>
<th>Barbara Kofler (Austria) Contribution of the galanin system to inflammation (L57)</th>
</tr>
</thead>
<tbody>
<tr>
<td>David Vaudry (FRANCE) Characterization of neuropeptides which control cerebellar granule cell survival, migration and differentiation (L58)</td>
</tr>
<tr>
<td>Jolanta Zawilska (Poland) A new face of orexins action - neuroprotection (L59)</td>
</tr>
</tbody>
</table>

Conference room: Struve I

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.15</td>
<td>Closing remarks</td>
</tr>
<tr>
<td>11.30</td>
<td>Farewell lunch</td>
</tr>
<tr>
<td>12.30-17.00</td>
<td>Sightseeing tour to Tallinn</td>
</tr>
<tr>
<td></td>
<td>Visit to the Põltsamaa Wine Cellar, Lunch at the Põhjaka Manor, English-speaking guide during the whole bus tour. Only for pre-registered participants.</td>
</tr>
<tr>
<td></td>
<td>Bus stops in Tallinn: Tallinn Airport and Tallinn City Center</td>
</tr>
</tbody>
</table>

Meeting point for the sightseeing tour (for registered participants) is Dorpat hotel, Soola 6
Poster presentations of ESN2015 Conference

Abstracts P1-P57 are available online at http://www.springerplus.com/supplements/4/S1
Abstracts P58-P61 are printed in the end of this programme book.

<table>
<thead>
<tr>
<th>Poster No</th>
<th>Poster presentation title</th>
<th>Poster authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>Characterization of ligand binding to dopamine receptors with fluorescence anisotropy based assay</td>
<td>Anni Allikalt, Ago Rinken</td>
</tr>
<tr>
<td>P2</td>
<td>The effect of P2Y12 receptor inhibition in chronic inflammatory pain</td>
<td>Katinka Bekő, Ádám Dénes, Gergely Horváth, Beáta Sperlágh</td>
</tr>
<tr>
<td>P3</td>
<td>Acute Stimulation of Glycolytic Flux in Cultured Primary Astrocytes by the Tyrosine Kinase Inhibitor Tyrphostin 23</td>
<td>Eva-Maria Blumrich, Reshma Kadam, Ralf Dringen</td>
</tr>
<tr>
<td>P4</td>
<td>Increase of the FGFR1 signaling in the FGFR1-5-HT1A heteroreceptor complex in midbrain raphe 5-HT neuron systems via allosteric receptor-receptor interaction</td>
<td>Dasiel O Borroto-Escuela, Manuel Narvaez, Antonio Jiménez-Beristain, Julia Oflijan, Luca Pinton, Michael Di Palma, Alexander O Tarakanov, Giuseppa Mudó, Luigi F Agnati, Natale Belluardo, Kjell Fuxe</td>
</tr>
<tr>
<td>P5</td>
<td>Copper oxide nanoparticles: Synthesis, toxic potential and modulation of astrocytic metabolism</td>
<td>Felix Bulcke, Ralf Dringen, Karsten Thiel</td>
</tr>
<tr>
<td>P6</td>
<td>Alteration of the protein quality control system in motor neuron and muscle expressing mutant proteins causing ALS and SBMA</td>
<td>Maria Elena Cicardi, Valeria Crippa, Riccardo Cristofani, Paola Rusmini, Angelo Poletti</td>
</tr>
<tr>
<td>P7</td>
<td>Neuroprotection by Nerve Growth Factor (NGF) involves modulation of neuronal autophagy</td>
<td>Anna Maria Colangelo, Michele Papa, Miluscia Berbenni, Marco Pizzocri, Vita M Ippolito, Valentina Riganti, Lilia Alberghina</td>
</tr>
<tr>
<td>P8</td>
<td>Brain extracellular levels of dimethylarginines (ADMA, SDMA) and cGMP and their modulation by exogenous S-adenosylmethionine in rats with acute liver failure</td>
<td>Anna Czarnecka, Jan Albrecht, Magdalena Zielińska</td>
</tr>
<tr>
<td>P9</td>
<td>Exogenous carbon monoxide improves neuronal differentiation: a near-death experience</td>
<td>Ana S Almeida, Melissa Vieira, Diogo Fortunato, Helena LA Vieira</td>
</tr>
<tr>
<td>P10</td>
<td>sRage plasma level in association with multiple sclerosis risk in Slovak population</td>
<td>Dusan Dobrota, Daniel Cierny, Jozef Michalik, Egon Kurca, Jan Lehotsky</td>
</tr>
<tr>
<td>P11</td>
<td>Characteristics of adenine nucleotides content in acute hypoxia by mathematical analysis methods</td>
<td>Elena Erlykina, Albina Moshkova</td>
</tr>
<tr>
<td>P12</td>
<td>Extracellular acidification leads to mitochondrial depolarization with following free radical formation in rat brain synaptosomes</td>
<td>Sergei Fedorovich, Tatyana Pekun, Sviatlana Hrynevich, Tatyana Waseem</td>
</tr>
<tr>
<td>P14</td>
<td>The effects of optical, electrical and chemical stimulation on serotonin release from median raphe and hippocampus of mice</td>
<td>Flora Goloncser, Romeo Ando, Dora Zelena, Jozsef Haller, Beata Sperlagh</td>
</tr>
<tr>
<td>P15</td>
<td>Identification of the antigen recognized by rHIgM22, a remyelination-promoting human monoclonal antibody</td>
<td>Sara Grassi, Simona Prioni, Yana Zorina, Livia Cabitta, Sandro Sonnino, Alessandro Prinetti</td>
</tr>
<tr>
<td>P16</td>
<td>Investigation of P2X7R Involvement in Maternal Poly(i:C) Exposure Evoked Autistic Features in Mice</td>
<td>Gergely Horváth, Lilla Otrokocsi, Ágnes Kittel, Mária Baranyi, Beáta Sperlágh</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P17</td>
<td>Effect of extracellular vesicles derived from distinct brain cells on Aβ toxicity and assembly: focus on Microglia derived vesicles</td>
<td>Pooja Joshi, Elena Turola, Ana Ruiz, Alessandra Bergami, Dacia Libera, Luisa Benussi, Clemens Falkner, Paola Giussani, Giuseppe Magnani, Giancarlo Comi, San Raffaele, Giuseppe Legname, Roberta Ghidoni, Roberto Furlan, Michela Matteoli, Claudia Verderio</td>
</tr>
<tr>
<td>P18</td>
<td>GnRH level regulation in the hypothalamus of female rats of different age</td>
<td>Andrew Korenevsky, Alexander Arutjunyan, Ulia Milyutina, Gleb Kerkeshko, Michael Stepanov, Irina Zaloznyaya</td>
</tr>
<tr>
<td>P19</td>
<td>Toxicity of amyloid beta 1-40 and 1-42 on SH-SY5Y cell line</td>
<td>Jekaterina Krishtal, Olga Bragina, Kristel Metsla, Peep Palumaa, Vello Tõugu</td>
</tr>
<tr>
<td>P20</td>
<td>Arsenic induced oxidative stress and mitochondrial dysfunction in rat brain</td>
<td>Vijay Kumar, Chandra Prakash</td>
</tr>
<tr>
<td>P21</td>
<td>Neuroprotection exerted by ischemic preconditioning in rat hippocampus involves extracellular signal receptor changes</td>
<td>Jan Lehotsky, Maria Kovalska, Barbara Tothova, Zuzana Tatarkova, Peter Kaplan</td>
</tr>
<tr>
<td>P22</td>
<td>Wolfram syndrome 1: from ER stress to impaired mitochondrial dynamics and neuronal development</td>
<td>Mailis Liiv, Michal Cagalinec, Zuzana Hodurova, Annika Vaarmann, Merle Mandel, Akbar Zeb, Malle Kuum, Miriam Ann Hickey, Dzhamilja Safiulina, Vinay Choubey, Eero Vasar, Vladimir Veksler, Allen Kaasik</td>
</tr>
<tr>
<td>P23</td>
<td>Novel peptides for kinetic studies of ligand binding to melanocortin-4 receptors using fluorescence anisotropy</td>
<td>Reet Link, Sergei Kopanchuk, Ago Rinken</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P24</td>
<td>Survival of retinal ganglion cells in transgenic mice with deficiencies in sialyltransferases or neural cell adhesion molecule (NCAM) or after the administration of neuraminidase</td>
<td>Natalia Lobanovskaya, Tamara Zharkovsky, Külli Jaako, Monika Jürgenson, Anu Aonurm-Helm, Alexander Zharkovsky</td>
</tr>
<tr>
<td>P25</td>
<td>Mitochondrial biogenesis is rate limiting-factor for axonal growth</td>
<td>Merle Mandel, Annika Vaarmann, Akbar Zeb, Przemyslaw Wareski, Joanna Liiv, Malle Kuum, Eva Antsov, Mailis Liiv, Vinay Choubey, Allen Kaasik</td>
</tr>
<tr>
<td>P26</td>
<td>N-terminal Proteolytic Processing of G Protein-coupled Receptor 37 (GPR37)</td>
<td>Orvokki Mattila, Ulla Petäjä-Repo</td>
</tr>
<tr>
<td>P27</td>
<td>The S100B-RAGE pathway is dysregulated in the ALS-linked neuroinflammatory process</td>
<td>Fabrizio Michetti, Claudia Donno, Nadia D’Ambrosi</td>
</tr>
<tr>
<td>P28</td>
<td>Ammonia reduces intracellular ADMA level in cultured astrocytes and endothelial cells: possible involvement of increased y+LAT2-mediated efflux.</td>
<td>Krzysztof Milewski, Jan Albrecht, Wojciech Hilgier, Magdalena Zielińska</td>
</tr>
<tr>
<td>P29</td>
<td>Synapse and dendrite deficits induced by mutations in the X-linked intellectual disability gene Il1rapl1</td>
<td>Caterina Montani, Mariana Ramos-Brossier, Mariana Ramos-Brossier, Carlo Sala</td>
</tr>
<tr>
<td>P30</td>
<td>Organic cation/carnitine transporter (OCTN2) interaction proteome in rat astrocytes: Role of phosphatase PP2A</td>
<td>Katarzyna Nalecz, Barbara Juraszek</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P32</td>
<td>Validation of astrocytic reference genes for qRT-PCR in CO treatment studies</td>
<td>Sara Oliveira, Helena Vieira, Carlos Duarte</td>
</tr>
<tr>
<td>P33</td>
<td>Learned helplessness paradigm in P2xr7 wild type and knock out mice and its effect on synapses in the dentate gyrus</td>
<td>Lilla Otrokocsi, Flóra Gölöncsér, Beáta Sperlágh, Agnes Kittel</td>
</tr>
<tr>
<td>P34</td>
<td>Trophic support following peripheral axotomy show different behaviour of reactive microglia and astroglia in the ventral horn</td>
<td>Michele Papa, Anna Maria Colangelo, Leonilde Savarese, Ciro De Luca, Giovanni Cirillo, Lilia Alberghina</td>
</tr>
<tr>
<td>P35</td>
<td>LRRK2 modulates neuronal vesicles cycle through protein interactions</td>
<td>Giovanni Piccoli, Francesca Pischedda, Daniela Cirnaru, Elisa Greggio, Franco Onofri, Isabella Russo, Antonella Marte, Elisa Belluzzi, Michele Morari, Christian Joannes Gloeckner, Carla Perego, Silvia Marsicano</td>
</tr>
<tr>
<td>P36</td>
<td>GABAA agonist muscimol ameliorates learning/memory deficits in streptozocin-induced Alzheimer's disease non-transgenic rat model</td>
<td>Vladimirs Pilipenko, Jolanta Pupure, Juris Rumaks, Ulrika Beitnere, Zane Dzirkale, Raimonds Skumbins, Vija Klusa</td>
</tr>
<tr>
<td>P37</td>
<td>Evidence for the existence of dopamine D2R and Sigma 1 allosteric receptor-receptor interaction in the rat brain: role in brain plasticity and cocaine action</td>
<td>Luca Pinton, Dasiel O Borroto-Escuela, Manuel Narváez, Julia Ofl ijan, Luigi F Agnati, Kjell Fuxe</td>
</tr>
<tr>
<td>P38</td>
<td>The role of Negr1 in cortical development via NCAM-FGFR2 signaling</td>
<td>Francesca Pischedda, Joanna Szczurkowska, Maria Daniela Cirnaru, Laura Cancedda, Giovanni Piccoli</td>
</tr>
<tr>
<td>P39</td>
<td>Cortical synapses in acute hepatic encephalopathy: morphology and expression of proteins involved in synaptic transmission</td>
<td>Mariusz Popek, Małgorzata Frontczak-Baniewicz, Barbara Zabłocka, Jan Albrecht, Magdalena Zielińska</td>
</tr>
<tr>
<td>P40</td>
<td>Proteasome stress triggers differential cellular responses of neural cells</td>
<td>Peter Racay, Ivana Pilchova, Katarina Klacanova, Dusan Dobrota</td>
</tr>
<tr>
<td>------</td>
<td>---------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>P41</td>
<td>Miro1 overexpression protects against α-synuclein-induced mitochondrial loss in neuronal culture</td>
<td>Dzhamilja Safiulina, Vinay Choubey, Miriam Ann Hickey, Allen Kaasik</td>
</tr>
<tr>
<td>P42</td>
<td>Glycohydrolases in the central nervous system: the role of GBA2 in the neuronal differentiation</td>
<td>Maura Samarani, Nicoletta Loberto, Valentina Murdica, Domitilla Schiumarini, Simona Prioni, Alessandro Prinetti, Sandro Sonnino, Rosaria Bassi, Massimo Aureli</td>
</tr>
<tr>
<td>P43</td>
<td>A new potential mechanism of action of tianeptine – the effect on microglial cell activation</td>
<td>Joanna Slusarczyk, Ewa Trojan, Anna Piotrowska, Katarzyna Glombik, Joanna Mika, Agnieszka Basta-Kaim</td>
</tr>
<tr>
<td>P44</td>
<td>The effects of STZ-induced diabetes on cognition and brain amyloid in 5XFAD mouse model of Alzheimer’s disease</td>
<td>Katrin Sonn, Alexander Zharkovsky</td>
</tr>
<tr>
<td>P45</td>
<td>The effect of hypoxic postconditioning after severe hypobaric hypoxia on the expression of Cu, Zn-SOD in hippocampus and neocortex of rats</td>
<td>Sergei Alexandrovich Stroev, Tatiana Sergeevna Glushchenko, Ekaterina Isifovna Tjul’kova, Mikhail Olegovich Samoilov</td>
</tr>
<tr>
<td>P46</td>
<td>The brain acylcarnitine profile depends on nutritional state</td>
<td>Baiba Svalbe, Marina Makrecka-Kuka, Eduards Sevostjanovs, Maija Dambrova, Edgars Liepinsh</td>
</tr>
<tr>
<td>P47</td>
<td>Janus-faced taurine: protection or toxicity?</td>
<td>Andrey Taranukhin, Pirjo Saransaari, Simo Oja</td>
</tr>
<tr>
<td>P48</td>
<td>Peptidomic characterization of peptide processing in the hippocampus of Wfs1 knockout mice.</td>
<td>Karin Tein, Sergo Kasvandik, Eero Vasar, Anton Terasmaa</td>
</tr>
<tr>
<td>Page</td>
<td>Title</td>
<td>Authors</td>
</tr>
<tr>
<td>------</td>
<td>----------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>P49</td>
<td>Increased prolyl endopeptidase induces alterations in the expression of neural cell adhesion molecule and its polysialylation in SH-SY5Y neuroblastoma cells in vitro</td>
<td>Keiti Uibokand, Külli Jaako, Aveli Noortoots, Anti Kalda, Alexander Zharkovsky</td>
</tr>
<tr>
<td>P50</td>
<td>Corticosterone induces DNA methyltransferases expression in rat cortical neurons</td>
<td>Mari Urb, Kaili Anier, Terje Matsalu, Anu Aonurm-Helm, Tõnis Timmusk</td>
</tr>
<tr>
<td>P51</td>
<td>Novel positive allosteric modulators of sigma-1 receptor</td>
<td>Edijs Vavers, Liga Zvejniece, Grigory Veinberg, Baiba Svalbe, Ilona Domracheva, Reinis Vilskersts, Maija Dambrova</td>
</tr>
<tr>
<td>P52</td>
<td>Allosteric modulation of peptide ligand binding to Neuropeptide Y receptor Y1 revealed by fluorescence-based assay.</td>
<td>Santa Veiksina, Sergei Kopanchuk, Ago Rinken</td>
</tr>
<tr>
<td>P53</td>
<td>Neuroprotection by MKK7 inhibition in excitotoxicity</td>
<td>Alessandro Vercelli, Tiziana Borsello, Alessandra Sclip, Silvia Biggi, Ivan Enrico Repetto, Sara Cimini, Fabio Falleroni, Simone Tomasi, Riccardo Monti, Noemi Tonna, Federica Morelli, Valentina Grande, Oriano Marin, Fabio Bianco, Daniele di Marino</td>
</tr>
<tr>
<td>P54</td>
<td>Hypoxic postconditioning is an effective method of protection from severe hypoxia induced lipid peroxidation and neuronal apoptosis in rats</td>
<td>Oleg Vetrovoy, Ekaterina Tyulkova, Elena Rybnikova</td>
</tr>
<tr>
<td>P55</td>
<td>Effect of brevican deficiency on neuroplasticity mediating molecules</td>
<td>Solveig Weigel, Juliane Meissner, Alexander Zharkovsky, Thomas Arendt, Steffen Rossner, Markus Morawski</td>
</tr>
<tr>
<td>P56</td>
<td>The protective and toxic role of neuromelanins in brain aging and Parkinson's disease</td>
<td>Luigi Zecca, Chiara Bellei, Pierluigi Mauri, Luigi Casella, Carolina Cebrián, David Sulzer, Fabio A Zucca</td>
</tr>
<tr>
<td>P57</td>
<td>Role of caspase-3 in development of neuronal plasticity and memory</td>
<td>Igor Zhuravin, Nadezhda Dubrovskaya, Dmitrii Vasilev, Darya Kozlova, Natalia Tumanova, Anthony Turner, Natalia Nalivaeva</td>
</tr>
<tr>
<td>P58</td>
<td>Evaluation Of The Effect Of Melatonin On Peripheral Neuropathy In STZ-Induced Diabetic Rats Using The EDA</td>
<td>Ali Yucel Kara, Hale Acer, Selda Tasan, Ferhat Pektas, Nazan Dolu</td>
</tr>
<tr>
<td>P59</td>
<td>Effect of individual sports on Hemispheric Differences: An auditory event-related potentials study</td>
<td>Esin Erdogan, Nazan Dolu, Ferhat Pektas</td>
</tr>
<tr>
<td>P60</td>
<td>Analysis of late phase auditory evoked potentials in unmedicated children with non-medicated attention-deficit hyperactivity disorder</td>
<td>Nazan Dolu, Aysegul Guven, Sevgi Ozmen, Esra Demirci, Miray Altinkaynak, Ferhat Pektas, Sacide Yıldız</td>
</tr>
<tr>
<td>P61</td>
<td>Nitric oxide-mediated neurovascular coupling mediated in Alzheimer disease and under metabolic acidosis</td>
<td>João Laranjinha, Cátia Lourenço, Nuno Ferreira, Rui Barbosa</td>
</tr>
<tr>
<td>P62</td>
<td>New antagonists for the orphan G-protein coupled receptor GPR18, a new player in microglial migration</td>
<td>Clara Schoeder, Viktor Rempel, Tadeusz Karcz, Katarzyna Kieľ-Kononowicz, Christa E. Müller</td>
</tr>
<tr>
<td>Delegate</td>
<td>Email</td>
<td>Abstract No</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Maria Abbracchio, Italy</td>
<td><a href="mailto:mariapia.abbracchio@unimi.it">mariapia.abbracchio@unimi.it</a></td>
<td>L10</td>
</tr>
<tr>
<td>Tilmann Achsel, Belgia</td>
<td><a href="mailto:Tilmann.Achsel@cme.vib-kuleuven.be">Tilmann.Achsel@cme.vib-kuleuven.be</a></td>
<td>L53</td>
</tr>
<tr>
<td>Anni Allikalt, Estonia</td>
<td><a href="mailto:anni.allikalt@ut.ee">anni.allikalt@ut.ee</a></td>
<td>L19</td>
</tr>
<tr>
<td>Lembit Allikmets, Estonia</td>
<td><a href="mailto:lembit.allikmets@ut.ee">lembit.allikmets@ut.ee</a></td>
<td></td>
</tr>
<tr>
<td>Ana Sofia Almeida, Portugal</td>
<td><a href="mailto:asalmeida85@gmail.com">asalmeida85@gmail.com</a></td>
<td>L26, L42, P9</td>
</tr>
<tr>
<td>Kaii Anier, Estonia</td>
<td><a href="mailto:kaili.anier@ut.ee">kaili.anier@ut.ee</a></td>
<td>P50</td>
</tr>
<tr>
<td>Ernest Arenas, Sweden</td>
<td><a href="mailto:ernest.arenas@ki.se">ernest.arenas@ki.se</a></td>
<td>L49</td>
</tr>
<tr>
<td>Eleonora Aronica, The Netherlands</td>
<td><a href="mailto:e.aronica@amc.uva.nl">e.aronica@amc.uva.nl</a></td>
<td>L9</td>
</tr>
<tr>
<td>Philip Beart, Australia</td>
<td><a href="mailto:philip.beart@florey.edu.au">philip.beart@florey.edu.au</a></td>
<td>L35</td>
</tr>
<tr>
<td>Ulrika Beitnere, Latvia</td>
<td><a href="mailto:ulrika.beitnere@lu.lv">ulrika.beitnere@lu.lv</a></td>
<td>P39</td>
</tr>
<tr>
<td>Katinka Bekő, Hungary</td>
<td><a href="mailto:beko.katinka@koki.mta.hu">beko.katinka@koki.mta.hu</a></td>
<td>P2</td>
</tr>
<tr>
<td>Eva-Maria Blumrich, Germany</td>
<td><a href="mailto:eblumrich@uni-bremen.de">eblumrich@uni-bremen.de</a></td>
<td>P3</td>
</tr>
<tr>
<td>Dasiel Oscar Borroto Escuela, Sweden</td>
<td><a href="mailto:dasiel.borroto.escuela@ki.se">dasiel.borroto.escuela@ki.se</a></td>
<td>P4, P31, P37</td>
</tr>
<tr>
<td>Jo Brown, The Netherlands</td>
<td><a href="mailto:jbrown@kenes.com">jbrown@kenes.com</a></td>
<td></td>
</tr>
<tr>
<td>Peter Brust, Germany</td>
<td><a href="mailto:p.brust@hzdr.de">p.brust@hzdr.de</a></td>
<td></td>
</tr>
<tr>
<td>Felix Bulcke, Germany</td>
<td><a href="mailto:bulcke@uni-bremen.de">bulcke@uni-bremen.de</a></td>
<td>L3, P5</td>
</tr>
<tr>
<td>Jens Carlsson, Sweden</td>
<td><a href="mailto:jens.carlsson.lab@gmail.com">jens.carlsson.lab@gmail.com</a></td>
<td>L5</td>
</tr>
<tr>
<td>Maria Teresa Carri, Italy</td>
<td><a href="mailto:carri@bio.uniorma2.it">carri@bio.uniorma2.it</a></td>
<td>L54</td>
</tr>
<tr>
<td>Anna Churilova, Russian Federation</td>
<td><a href="mailto:annch05@mail.ru">annch05@mail.ru</a></td>
<td>L27</td>
</tr>
<tr>
<td>Maria Elena Cicardi, Italy</td>
<td><a href="mailto:maria.cicardi@unimi.it">maria.cicardi@unimi.it</a></td>
<td>L55, P6</td>
</tr>
<tr>
<td>Olga Corti, France</td>
<td><a href="mailto:olga.corti@upmc.fr">olga.corti@upmc.fr</a></td>
<td>L36</td>
</tr>
<tr>
<td>Mauro Cozzolino, Italy</td>
<td><a href="mailto:mauro.cozzolino@ift.cnr.it">mauro.cozzolino@ift.cnr.it</a></td>
<td>L51</td>
</tr>
<tr>
<td>Riccardo Cristofani, Italy</td>
<td><a href="mailto:riccardo.cristofani@unimi.it">riccardo.cristofani@unimi.it</a></td>
<td>L24, L55, P6</td>
</tr>
<tr>
<td>Antonio Cuadrado, Spain</td>
<td><a href="mailto:antonio.cuadrado@uam.es">antonio.cuadrado@uam.es</a></td>
<td>L43</td>
</tr>
<tr>
<td>Maija Dambrova, Latvia</td>
<td><a href="mailto:md@biomed.lu.lv">md@biomed.lu.lv</a></td>
<td>P46, P51</td>
</tr>
<tr>
<td>Jurijs Dmitrijevs, Latvia</td>
<td><a href="mailto:jurijs@genmedica.eu">jurijs@genmedica.eu</a></td>
<td></td>
</tr>
<tr>
<td>Dusan Dobrota, Slovakia</td>
<td><a href="mailto:dobrota@jfmed.uniba.sk">dobrota@jfmed.uniba.sk</a></td>
<td>P10, P40</td>
</tr>
<tr>
<td>Nazan Dolu, Turkey</td>
<td><a href="mailto:dolu@erciyes.edu.tr">dolu@erciyes.edu.tr</a></td>
<td>P58, P59, P60</td>
</tr>
<tr>
<td>Sylvain Doré, Canada</td>
<td><a href="mailto:sdore@ufl.edu">sdore@ufl.edu</a></td>
<td>L44</td>
</tr>
<tr>
<td>Angelika Dr. Goertzen, Germany</td>
<td><a href="mailto:goertzen@t-online.de">goertzen@t-online.de</a></td>
<td></td>
</tr>
<tr>
<td>Name</td>
<td>Email</td>
<td>Affiliation</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>--------------------------------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ruediger W. Dr. Veh, Germany</td>
<td><a href="mailto:ruediger.veh@charite.de">ruediger.veh@charite.de</a></td>
<td></td>
</tr>
<tr>
<td>Ralf Dringen, Germany</td>
<td><a href="mailto:ralf.dringen@uni-bremen.de">ralf.dringen@uni-bremen.de</a></td>
<td>L3, L32, P3, P5</td>
</tr>
<tr>
<td>Hannelore Ehrenreich, Germany</td>
<td><a href="mailto:ehrenreich@em.mpg.de">ehrenreich@em.mpg.de</a></td>
<td>L22</td>
</tr>
<tr>
<td>Aleksandra Ellert-Miklaszewska, Poland</td>
<td><a href="mailto:aellert@nencki.gov.pl">aellert@nencki.gov.pl</a></td>
<td>L11</td>
</tr>
<tr>
<td>Elena Erlykina, Russian Federation</td>
<td><a href="mailto:e-erlyk@ya.ru">e-erlyk@ya.ru</a></td>
<td>P11</td>
</tr>
<tr>
<td>Sergei Fedorovich, Belarus</td>
<td><a href="mailto:fedorovich@ibp.org.by">fedorovich@ibp.org.by</a></td>
<td>P12</td>
</tr>
<tr>
<td>Benjamin Fouquet, Germany</td>
<td><a href="mailto:Benjamin.Fouquet@bruker.com">Benjamin.Fouquet@bruker.com</a></td>
<td></td>
</tr>
<tr>
<td>Pietro Fratta, United Kingdom</td>
<td><a href="mailto:p.fratta@ucl.ac.uk">p.fratta@ucl.ac.uk</a></td>
<td>L52</td>
</tr>
<tr>
<td>Merlin Friedemann, Estonia</td>
<td><a href="mailto:merfrie@gmail.com">merfrie@gmail.com</a></td>
<td>P13</td>
</tr>
<tr>
<td>Martina Gabrielli, Italy</td>
<td><a href="mailto:martina.gabrielli@unimi.it">martina.gabrielli@unimi.it</a></td>
<td>L29</td>
</tr>
<tr>
<td>Zafiroula Georgoussi, Greece</td>
<td><a href="mailto:iro@bio.demokritos.gr">iro@bio.demokritos.gr</a></td>
<td>L21</td>
</tr>
<tr>
<td>Flora Goloncser, Hungary</td>
<td><a href="mailto:goloncser.flora@koki.hu">goloncser.flora@koki.hu</a></td>
<td>P14, P33</td>
</tr>
<tr>
<td>Illana Gozes, Germany</td>
<td><a href="mailto:bulcke@uni-bremen.de">bulcke@uni-bremen.de</a></td>
<td>L3, P5</td>
</tr>
<tr>
<td>Sara Grassi, Italy</td>
<td><a href="mailto:sara.grassi@unimi.it">sara.grassi@unimi.it</a></td>
<td>P15</td>
</tr>
<tr>
<td>Laura Hausmann, Germany</td>
<td><a href="mailto:Lhausmann@ukaachen.de">Lhausmann@ukaachen.de</a></td>
<td></td>
</tr>
<tr>
<td>Miriam Hickey, Estonia</td>
<td><a href="mailto:miriam.ann.hickey@ut.ee">miriam.ann.hickey@ut.ee</a></td>
<td>P22, P41</td>
</tr>
<tr>
<td>David Hicks, United Kingdom</td>
<td><a href="mailto:d.hicks@bradford.ac.uk">d.hicks@bradford.ac.uk</a></td>
<td>L23</td>
</tr>
<tr>
<td>Stephen Hill, United Kingdom</td>
<td><a href="mailto:steve.hill@nottingham.ac.uk">steve.hill@nottingham.ac.uk</a></td>
<td>L8</td>
</tr>
<tr>
<td>Carsten Hoffmann, Germany</td>
<td><a href="mailto:c.hoffmann@toxi.uni-wuerzburg.de">c.hoffmann@toxi.uni-wuerzburg.de</a></td>
<td>L6</td>
</tr>
<tr>
<td>Gergely Horváth, Hungary</td>
<td><a href="mailto:horvath.gergely@koki.hu">horvath.gergely@koki.hu</a></td>
<td>P2, P16</td>
</tr>
<tr>
<td>Kadri Isakar, Estonia</td>
<td><a href="mailto:Kadri.Isakar@brucker.com">Kadri.Isakar@brucker.com</a></td>
<td></td>
</tr>
<tr>
<td>Andrei Izvolski, Estonia</td>
<td><a href="mailto:Andrei.Izvolski@brucker.com">Andrei.Izvolski@brucker.com</a></td>
<td></td>
</tr>
<tr>
<td>Külli Jaako, Estonia</td>
<td><a href="mailto:kulli.jaako@ut.ee">kulli.jaako@ut.ee</a></td>
<td>P24, P49</td>
</tr>
<tr>
<td>Pooja Joshi, Italy</td>
<td><a href="mailto:pooja.joshi87@gmail.com">pooja.joshi87@gmail.com</a></td>
<td>P17</td>
</tr>
<tr>
<td>Allen Kaasik, Estonia</td>
<td><a href="mailto:allen.kaasik@ut.ee">allen.kaasik@ut.ee</a></td>
<td>L34, P22, P25, P41</td>
</tr>
<tr>
<td>Anti Kalda, Estonia</td>
<td><a href="mailto:anti.kalda@ut.ee">anti.kalda@ut.ee</a></td>
<td>P49</td>
</tr>
<tr>
<td>Ali Ycel Kara</td>
<td><a href="mailto:eganis@hotmail.com">eganis@hotmail.com</a></td>
<td>P58</td>
</tr>
<tr>
<td>Josef Kittler, United Kingdom</td>
<td><a href="mailto:j.kittler@ucl.ac.uk">j.kittler@ucl.ac.uk</a></td>
<td>L33</td>
</tr>
<tr>
<td>Barbara Kofler, Austria</td>
<td><a href="mailto:b.kofler@salk.at">b.kofler@salk.at</a></td>
<td>L57</td>
</tr>
<tr>
<td>Sergei Kopanchuk, Estonia</td>
<td><a href="mailto:sergei.kopanchuk@ut.ee">sergei.kopanchuk@ut.ee</a></td>
<td>L19, P23, P52</td>
</tr>
<tr>
<td>Andrew Korenevsky, Russian Federation</td>
<td><a href="mailto:a.korenevsky@yandex.ru">a.korenevsky@yandex.ru</a></td>
<td>P18</td>
</tr>
<tr>
<td>Name</td>
<td>Email</td>
<td>Page(s)</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>Jekaterina Krishtal, Estonia</td>
<td><a href="mailto:katerina.kristall@gmail.com">katerina.kristall@gmail.com</a></td>
<td>P19</td>
</tr>
<tr>
<td>Jyrki Kukkonen, Finland</td>
<td><a href="mailto:jyrki.kukkonen@helsinki.fi">jyrki.kukkonen@helsinki.fi</a></td>
<td>L17</td>
</tr>
<tr>
<td>Vijay Kumar, India</td>
<td><a href="mailto:vkshiwach@gmail.com">vkshiwach@gmail.com</a></td>
<td>P20</td>
</tr>
<tr>
<td>Malle Kuum, Estonia</td>
<td><a href="mailto:malle.kuum@ut.ee">malle.kuum@ut.ee</a></td>
<td>P22, P25</td>
</tr>
<tr>
<td>Ülo Langel, Sweden</td>
<td><a href="mailto:ulo@neurochem.su.se">ulo@neurochem.su.se</a></td>
<td>L18</td>
</tr>
<tr>
<td>João Laranjinha, Portugal</td>
<td><a href="mailto:laranjin@ci.uc.pt">laranjin@ci.uc.pt</a></td>
<td>P61</td>
</tr>
<tr>
<td>Jan Lehotsky, Slovakia</td>
<td><a href="mailto:lehotsky@jfmed.uniba.sk">lehotsky@jfmed.uniba.sk</a></td>
<td>P10, P21</td>
</tr>
<tr>
<td>Lucia Lichvarova, Estonia</td>
<td><a href="mailto:lucia.lichvarova@ut.ee">lucia.lichvarova@ut.ee</a></td>
<td></td>
</tr>
<tr>
<td>Mailis Liiv, Estonia</td>
<td><a href="mailto:siliam1@ut.ee">siliam1@ut.ee</a></td>
<td>P22, P25</td>
</tr>
<tr>
<td>Reet Link, Estonia</td>
<td><a href="mailto:reetlink@gmail.com">reetlink@gmail.com</a></td>
<td>L19, P23</td>
</tr>
<tr>
<td>Silvia Lisa, Greece</td>
<td><a href="mailto:silvialisa@gmail.com">silvialisa@gmail.com</a></td>
<td>L25</td>
</tr>
<tr>
<td>Natalia Lobanovskaya, Estonia</td>
<td><a href="mailto:natalia.lobanovskaya@gmail.com">natalia.lobanovskaya@gmail.com</a></td>
<td></td>
</tr>
<tr>
<td>Galyna Maleeva, France</td>
<td><a href="mailto:galina_maleeva@ukr.net">galina_maleeva@ukr.net</a></td>
<td>L31</td>
</tr>
<tr>
<td>Merle Mandel, Estonia</td>
<td><a href="mailto:merle.mandel@ut.ee">merle.mandel@ut.ee</a></td>
<td>P22, P25</td>
</tr>
<tr>
<td>Miguel Medina, Spain</td>
<td><a href="mailto:mmedina@ciberned.es">mmedina@ciberned.es</a></td>
<td>L14</td>
</tr>
<tr>
<td>Kristel Metsla, Estonia</td>
<td><a href="mailto:kristelmetsla@gmail.com">kristelmetsla@gmail.com</a></td>
<td>P19</td>
</tr>
<tr>
<td>Fabrizio Michetti, Italy</td>
<td><a href="mailto:fabrizio.michetti@rm.unicatt.it">fabrizio.michetti@rm.unicatt.it</a></td>
<td>P27</td>
</tr>
<tr>
<td>Krzysztof Milewski, Poland</td>
<td><a href="mailto:kmilewski@imdik.pan.pl">kmilewski@imdik.pan.pl</a></td>
<td>P28</td>
</tr>
<tr>
<td>Graeme Milligan, United Kingdom</td>
<td><a href="mailto:Graeme.Milligan@glasgow.ac.uk">Graeme.Milligan@glasgow.ac.uk</a></td>
<td>L7</td>
</tr>
<tr>
<td>Nico Mitro, Italy</td>
<td><a href="mailto:nico.mitro@unimi.it">nico.mitro@unimi.it</a></td>
<td>L45</td>
</tr>
<tr>
<td>Caterina Montani, Italy</td>
<td><a href="mailto:c.montani@in.cnr.it">c.montani@in.cnr.it</a></td>
<td>P29</td>
</tr>
<tr>
<td>Roberto Motterlini, France</td>
<td><a href="mailto:roberto.motterlini@inserm.fr">roberto.motterlini@inserm.fr</a></td>
<td>L41</td>
</tr>
<tr>
<td>Amritpal Mudher, United Kingdom</td>
<td><a href="mailto:a.mudher@soton.ac.uk">a.mudher@soton.ac.uk</a></td>
<td>L13</td>
</tr>
<tr>
<td>Katarzyna Nalecz, Poland</td>
<td><a href="mailto:k.nalecz@nencki.gov.pl">k.nalecz@nencki.gov.pl</a></td>
<td>P30</td>
</tr>
<tr>
<td>Natalia Nalivaeva, United Kingdom</td>
<td><a href="mailto:n.n.nalivaeva@leeds.ac.uk">n.n.nalivaeva@leeds.ac.uk</a></td>
<td>L40, P57</td>
</tr>
<tr>
<td>Katarzyna Nawrotek, Poland</td>
<td><a href="mailto:katarzyna.nawrotek@p.lodz.pl">katarzyna.nawrotek@p.lodz.pl</a></td>
<td>L30</td>
</tr>
<tr>
<td>Aveli Noortoots, Estonia</td>
<td><a href="mailto:aveli.noortoots@ut.ee">aveli.noortoots@ut.ee</a></td>
<td>P49</td>
</tr>
<tr>
<td>Julia Oflijan, Estonia</td>
<td><a href="mailto:juliaofli@gmail.com">juliaofli@gmail.com</a></td>
<td>P4, P31, P37</td>
</tr>
<tr>
<td>Sara Oliveira, Portugal</td>
<td><a href="mailto:sara.oliveira@cnc.uc.pt">sara.oliveira@cnc.uc.pt</a></td>
<td>P32</td>
</tr>
<tr>
<td>Lilla Otrokocsi, Hungary</td>
<td><a href="mailto:otrokocsi.lilla@koki.mta.hu">otrokocsi.lilla@koki.mta.hu</a></td>
<td>P16, P33</td>
</tr>
<tr>
<td>Michele Papa, Italy</td>
<td><a href="mailto:michelepapasun@gmail.com">michelepapasun@gmail.com</a></td>
<td>P7, P34</td>
</tr>
<tr>
<td>Ferhat Pektas</td>
<td><a href="mailto:ferhat_p@hotmail.com">ferhat_p@hotmail.com</a></td>
<td>P58, P59, P60</td>
</tr>
<tr>
<td>Charlotte Petters, Germany</td>
<td><a href="mailto:petters@uni-bremen.de">petters@uni-bremen.de</a></td>
<td>L32</td>
</tr>
<tr>
<td>Name</td>
<td>Email Address</td>
<td>Page Numbers</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------------------------------</td>
<td>--------------</td>
</tr>
<tr>
<td>Giovanni Piccoli, Italy</td>
<td><a href="mailto:giovanni.piccoli@hsr.it">giovanni.piccoli@hsr.it</a></td>
<td>P35, P38</td>
</tr>
<tr>
<td>Vladimirs Pilipenko, Latvia</td>
<td><a href="mailto:vladimirs.pilipenko@hotmail.com">vladimirs.pilipenko@hotmail.com</a></td>
<td>P36</td>
</tr>
<tr>
<td>Luca Pinton, Sweden</td>
<td><a href="mailto:lucapinton@hotmail.it">lucapinton@hotmail.it</a></td>
<td>P4, P31, P37</td>
</tr>
<tr>
<td>Francesca Pischedda, Italy</td>
<td><a href="mailto:francesca.pischedda@unimi.it">francesca.pischedda@unimi.it</a></td>
<td>P35, P38</td>
</tr>
<tr>
<td>Angelo Poletti, Italy</td>
<td><a href="mailto:angelo.poletti@unimi.it">angelo.poletti@unimi.it</a></td>
<td>L24, L55, P6</td>
</tr>
<tr>
<td>Mariusz Popek, Poland</td>
<td><a href="mailto:mpopek@imdik.pan.pl">mpopek@imdik.pan.pl</a></td>
<td>P39</td>
</tr>
<tr>
<td>Alessandro Prinetti, Italy</td>
<td><a href="mailto:alessandro.prinetti@unimi.it">alessandro.prinetti@unimi.it</a></td>
<td>P15, P42</td>
</tr>
<tr>
<td>Marite Punapart, Estonia</td>
<td><a href="mailto:marite.punapart@gmail.com">marite.punapart@gmail.com</a></td>
<td></td>
</tr>
<tr>
<td>Claudia Queiroga, Portugal</td>
<td><a href="mailto:claudia.queiroga@fcm.unl.pt">claudia.queiroga@fcm.unl.pt</a></td>
<td>L26, L42</td>
</tr>
<tr>
<td>Peter Racay, Slovakia</td>
<td><a href="mailto:racay@jfmed.uniba.sk">racay@jfmed.uniba.sk</a></td>
<td>P40</td>
</tr>
<tr>
<td>Dora Reglodi, Hungary</td>
<td><a href="mailto:dora.reglodi@aok.pte.hu">dora.reglodi@aok.pte.hu</a></td>
<td>L56</td>
</tr>
<tr>
<td>Christiane Richter-Landsberg, Germany</td>
<td><a href="mailto:christiane.richter.landsberg@uni-oldenburg.de">christiane.richter.landsberg@uni-oldenburg.de</a></td>
<td>L15</td>
</tr>
<tr>
<td>Ago Rinken, Estonia</td>
<td><a href="mailto:ago.rinken@ut.ee">ago.rinken@ut.ee</a></td>
<td>L19, P1, P23, P52</td>
</tr>
<tr>
<td>Patrick Roth, Czech Republic</td>
<td><a href="mailto:patrick.roth@usz.ch">patrick.roth@usz.ch</a></td>
<td>L12</td>
</tr>
<tr>
<td>Elena Rybnikova, Russian Federation</td>
<td><a href="mailto:rybnikova1@rambler.ru">rybnikova1@rambler.ru</a></td>
<td>L39, P54</td>
</tr>
<tr>
<td>Džamilja Safiulina, Estonia</td>
<td><a href="mailto:dzamilja.safiulina@ut.ee">dzamilja.safiulina@ut.ee</a></td>
<td></td>
</tr>
<tr>
<td>Maura Samarani, Italy</td>
<td><a href="mailto:maura.samarani@unimi.it">maura.samarani@unimi.it</a></td>
<td>P42</td>
</tr>
<tr>
<td>Alois Saria, Austria</td>
<td><a href="mailto:alois.saria@i-med.ac.at">alois.saria@i-med.ac.at</a></td>
<td>L2</td>
</tr>
<tr>
<td>C. Laura Sayas, Spain</td>
<td><a href="mailto:laura.sayas@ull.es">laura.sayas@ull.es</a></td>
<td>L16</td>
</tr>
<tr>
<td>Mika Scheinin, Finland</td>
<td><a href="mailto:mschein@utu.fi">mschein@utu.fi</a></td>
<td>L20</td>
</tr>
<tr>
<td>Clara Tabea Schoeder, Germany</td>
<td><a href="mailto:clara.schoeder@uni-bonn.de">clara.schoeder@uni-bonn.de</a></td>
<td>P62</td>
</tr>
<tr>
<td>Gunnar Schulte, Sweden</td>
<td><a href="mailto:gunnar.schulte@ki.se">gunnar.schulte@ki.se</a></td>
<td>L50</td>
</tr>
<tr>
<td>Manuela Simonetti, Germany</td>
<td><a href="mailto:manuela.simonetti@pharma.uni-heidelberg.de">manuela.simonetti@pharma.uni-heidelberg.de</a></td>
<td>L48</td>
</tr>
<tr>
<td>Joanna Slusarczyk, Poland</td>
<td><a href="mailto:slusarczyk_j@op.pl">slusarczyk_j@op.pl</a></td>
<td>P43</td>
</tr>
<tr>
<td>Katrin Sonn, Estonia</td>
<td><a href="mailto:katrin.sonn@gmail.com">katrin.sonn@gmail.com</a></td>
<td>P44</td>
</tr>
<tr>
<td>Hermona Soreq, Israel</td>
<td><a href="mailto:hermona.soreq@mail.huji.ac.il">hermona.soreq@mail.huji.ac.il</a></td>
<td>L1</td>
</tr>
<tr>
<td>Sergei Stroev, Russian Federation</td>
<td><a href="mailto:s_stroev@hotmail.com">s_stroev@hotmail.com</a></td>
<td>P45</td>
</tr>
<tr>
<td>Baiba Svalbe, Latvia</td>
<td><a href="mailto:svalbe@biomed.lu.lv">svalbe@biomed.lu.lv</a></td>
<td>P46, P51</td>
</tr>
<tr>
<td>Andrey Taranukhin, Italy</td>
<td><a href="mailto:andrey.taranukhin@uta.fi">andrey.taranukhin@uta.fi</a></td>
<td>P47</td>
</tr>
<tr>
<td>Anton Terasmaa, Estonia</td>
<td><a href="mailto:anton.terasmaa@ut.ee">anton.terasmaa@ut.ee</a></td>
<td>P48</td>
</tr>
<tr>
<td>Tõnis Timmusk, Estonia</td>
<td><a href="mailto:tonis.timmusk@ttu.ee">tonis.timmusk@ttu.ee</a></td>
<td>L38, P50</td>
</tr>
<tr>
<td>Name</td>
<td>Email</td>
<td>Page</td>
</tr>
<tr>
<td>-----------------------</td>
<td>--------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>Anthony Turner</td>
<td><a href="mailto:a.j.turner@leeds.ac.uk">a.j.turner@leeds.ac.uk</a></td>
<td>P57</td>
</tr>
<tr>
<td>Keiti Uibokand</td>
<td><a href="mailto:keiti.uibokand@ut.ee">keiti.uibokand@ut.ee</a></td>
<td>P49</td>
</tr>
<tr>
<td>Mari Urb</td>
<td><a href="mailto:mari.urb@ut.ee">mari.urb@ut.ee</a></td>
<td>P50</td>
</tr>
<tr>
<td>Annika Vaarmann</td>
<td><a href="mailto:annika.vaarmann@ut.ee">annika.vaarmann@ut.ee</a></td>
<td>P22, P25</td>
</tr>
<tr>
<td>David Vaudry</td>
<td><a href="mailto:david.vaudry@univ-rouen.fr">david.vaudry@univ-rouen.fr</a></td>
<td>L58</td>
</tr>
<tr>
<td>Edijs Vavers</td>
<td><a href="mailto:edijis@biomed.lu.lv">edijis@biomed.lu.lv</a></td>
<td>P51</td>
</tr>
<tr>
<td>Santa Veikšina</td>
<td><a href="mailto:santa.veiksa@ut.ee">santa.veiksa@ut.ee</a></td>
<td>L19, P52</td>
</tr>
<tr>
<td>Alessandro Vercelli</td>
<td><a href="mailto:alessandro.vercelli@unito.it">alessandro.vercelli@unito.it</a></td>
<td>L26, P53</td>
</tr>
<tr>
<td>Oleg Vetrovoy</td>
<td><a href="mailto:vov210292@yandex.ru">vov210292@yandex.ru</a></td>
<td>P54</td>
</tr>
<tr>
<td>Helena Vieira</td>
<td><a href="mailto:helena.vieira@fc.m.unl.pt">helena.vieira@fc.m.unl.pt</a></td>
<td>L26, L42, P9 P32</td>
</tr>
<tr>
<td>Anastassia Voronova</td>
<td><a href="mailto:anastassia.voronova@sickkids.ca">anastassia.voronova@sickkids.ca</a></td>
<td>L28</td>
</tr>
<tr>
<td>Solveig Weigel</td>
<td><a href="mailto:WeigelS@medizin.uni-leipzig.de">WeigelS@medizin.uni-leipzig.de</a></td>
<td>P55</td>
</tr>
<tr>
<td>Sacide Yildiz</td>
<td><a href="mailto:sacide.yldz@gmail.com">sacide.yldz@gmail.com</a></td>
<td>P60</td>
</tr>
<tr>
<td>Katarina Yrttimaa</td>
<td><a href="mailto:katariina.yrttimaa@basaali.fi">katariina.yrttimaa@basaali.fi</a></td>
<td></td>
</tr>
<tr>
<td>Jolanta Zawilska</td>
<td><a href="mailto:jolanta.zawilska@umed.lodz.pl">jolanta.zawilska@umed.lodz.pl</a></td>
<td>L59</td>
</tr>
<tr>
<td>Akbar Zeb</td>
<td><a href="mailto:akbar@ut.ee">akbar@ut.ee</a></td>
<td>P22, P25</td>
</tr>
<tr>
<td>Luigi Zecca</td>
<td><a href="mailto:luigi.zecca@itb.cnr.it">luigi.zecca@itb.cnr.it</a></td>
<td>P56</td>
</tr>
<tr>
<td>Alexander Zharkovsky</td>
<td><a href="mailto:aleksander.zarkovski@ut.ee">aleksander.zarkovski@ut.ee</a></td>
<td>L4, P24, P44, P49, P55</td>
</tr>
<tr>
<td>Igor Zhuravin</td>
<td><a href="mailto:i-zhur@mail.ru">i-zhur@mail.ru</a></td>
<td>P57</td>
</tr>
<tr>
<td>Liga Zvejniece</td>
<td><a href="mailto:liga@biomed.lu.lv">liga@biomed.lu.lv</a></td>
<td>P51</td>
</tr>
<tr>
<td>Tiina Ööpik</td>
<td><a href="mailto:tiina.oopik@sanofi.com">tiina.oopik@sanofi.com</a></td>
<td></td>
</tr>
</tbody>
</table>
Late breaking abstracts
Peripheral neuropathy is one of common complications in patients with diabetes mellitus. Diabetic neuropathy contains the findings of somatic and/or autonomic parts of the peripheral nervous system. Electrodermal activity (EDA) measures the activity of the eccrine sweat glands which are innervated by the sympathetic nerves and has been used in the evaluation of sympathetic skin response. In this study, the findings of peripheral neuropathy in streptozotocin (STZ) induced diabetic rats and the effects of melatonin on these findings were investigated by the EDA. 3-month-old Sprague-Dawley male rats were used. Rats were divided 4 groups as the control (0.9% NaCl, n=10), diabetes (STZ) (40 mg/kg STZ, single dose, n=6), melatonin (10 mg/kg, 30 days, n=10) and melatonin (started 3 days before STZ administration and continued for 30 days) + STZ (single dose, n=10). EDA recordings were taken and blood glucose levels were measured in all groups on the 15th and 30th days. Skin conductance levels (SCL, μmho) were calculated. Statistical analysis was performed with Kruskal-Wallis and Mann Whitney tests. SCL in the STZ-treated group was significantly lower than the control group on the 15th (p=0.005) and 30th days (p=0.001). SCL in the melatonin group was significantly lower than the control group on the 15th (p=0.001) and 30th days (p=0.001). SCL in the melatonin + STZ group was significantly higher than the STZ-treated group on the 15th (p=0.04) and 30th days (p=0.002). There was no difference in SCL between the control and melatonin + STZ groups on the 15th (p=0.48) and 30th days (p>0.05). Diabetes decreases the SCLs by causing peripheral neuropathy. Melatonin decreased the SCLs in healthy rats and increased the SCLs in experimental diabetic rats. These findings suggest that melatonin can inhibit sympathetic nervous system and has therapeutic effect on diabetic neuropathy.

Keywords: melatonin, electrodermal activity, diabetic neuropathy
Sport is known to improve cognitive function. Evoked potentials (EPs) are the changes in the spontaneous electrical activity of the brain following a sound, light etc. In this study, we investigated effect of individual sports on the cognitive function (especially attention) via auditory evoked potentials. We also evaluated hemispheric difference compared with sedentary group. This study was realized with 52 participants, between 18-25 old. Group 1 (n=10 ski), Group 2 (n=10 taekwondo, judo), Group 3 (n=10 tennis), Group 4 (n=10 swim) and Group 5 (n=12 sedentary). After the ethic approval, EPs were recorded with MP150 system and EEG amplificatory consist with this system. EPs were recorded from the haired skinned of parietal P3, P4 and temporal T3, T4 brain areas by means of Ag/AgCl electrodes which placed according to the 10-20 system. Recordings were taken with 160 oddball auditory stimuli (128 standards (Std), 32 targets (T)). The latency and amplitude of P300 waves were analyzed. Latency of P300 was statistically different in Group 5 when comparison of T3-T4 regions for Std (p<0.03) and Std+T(p<0.05) stimulus. Amplitude of P300 was different in Group 2 when comparison of T3-T4 regions for T (p<0.00), Std (p<0.00), Std+T (p<0.00) stimulus. There were differences in Group 3’s P300 wave amplitude when comparison of P3-P4 regions for Std (p<0.00) and Std+T (p<0.05) stimulus. There were differences in Group 5’s P300 wave amplitude when comparison of P3-P4 regions for T (p<0.03) and Std+T (p<0.05) stimulus. Between Group 1 with Group 4 was not found significant difference. Conclusion: According to these findings, there were difference between right and left hemisphere for ski, tennis and taekwondo, judo sports. We didn’t found this difference for ski and swim sports. So, we can say ski and swim sports effects all brain and hemispheres.

This study was supported Erciyes University Scientific Research Grant TYL-2014-5494.

Keywords: Individual sports , Hemispheric Differences, auditory event-related potentials
Analysis of late phase auditory evoked potentials in unmedicated children with non-medicated attention-deficit hyperactivity disorder

Nazan Dolu, Aysegul Guven, Sevgi Ozmen, Esra Demirci, Miray Altinkaynak, Ferhat Pektas, Sacide Yildiz
Erciyes University, Medical Faculty, Physiology Department Kayseri, Turkey

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common neuropsychiatric disorders of the childhood characterized by attention deficit, hyperactivity and impulse uncontrollability. Auditory evoked potentials (AEP) is a non-invasive, quantitative method used in the studies which cognitive functions are evaluated, and it can be used as an assist method for diagnosis in the evaluation of attention in ADHD patients. However, it hasn’t yet become a routine and there are very limited study non-medicated children with ADHD. In this study, we explored cognitive functions in non-medicated children with ADHD. Ten children with ADHD and 10 controls between the ages of 7-12 were participated. To obtain UP signal of the control group and the children with ADHD, MP 150 system and 4 EEG 100C amplifiers were used. Stimulation potentials were recorded from the regions of Frontal (Fz), Central (Cz), Parietal (Pz) and Occipital (Oz) AEP recordings were taken with oddball auditory stimuli. Auditory stimuli consist of 160 sounds consisting of rare coming (target, 32 stimulus) and frequently iterating with a beep tone (nontarget – 128 standard stimulus) tone explosion. P300 wave latency and amplitudes were analyzed for attention evaluation. The latency of P300 was statistically longer than control’s at Fz (p<0.009), Cz (p<0.004), Pz (p<0.000) and Oz (p<0.000) regions for target stimulus. The amplitude of P300 was also statistically lower for target stimulus at Fz (p<0.003) and Cz (p<0.03) regions in ADHD than control group. There were no statistically differences in other brain region. Cognitive impairment was found in ADHD. AEP can be an ideal method for investigating of cognitive functions in ADHD. This method can be used as routine of ADHD and other dementia diseases.

This study was supported by TÜBİTAK for Scientific Research Grant 114S470. Keywords. Attention-deficit-hyperactivity disorder, auditory evoked potentials, cognitive functions
Nitric oxide-mediated neurovascular coupling mediated in Alzheimer disease and under metabolic acidosis

João Laranjinha, Cátia Lourenço, Nuno Ferreira, Rui Barbosa
University of Coimbra, Portugal

Upon glutamatergic stimulation in vivo, neuronal-derived nitric oxide (\(\bullet\)NO) diffuses towards neighboring vessels, bridging neuronal activation with a localized increased blood flow in hippocampus (Lourenço et al. 2014). Brain function dependents on such a constant and well-regulated amount of blood flow, which provides substrates with temporal and regional precision, according to energy demands imposed by neural activity – the neurovascular coupling (NVC). On basis of quantitative and in vivo second-by-second recordings of \(\bullet\)NO dynamics, oxygen tension and local blood flow with stereotaxically inserted selective microelecrodes and laser Doppler flow probe in a mouse model of Alzheimer’s disease (AD), the triple transgenic mice (3xTg-AD), we found an impairment in the NVC in older 3xTg-AD mice, preceding obvious cognitive dysfunction. Evaluation of vascular responses suggested that the observed impairment in NVC in the AD animals is primarily due to cerebrovascular dysfunction rather than a dysfunctional signaling from neurons to blood vessels. Also, the changes observed in middle-aged AD mice recapitulate those observed in old control mice, pointing to the notion of AD as accelerated aging. A supporting mechanism particularly operative under metabolic acidosis, conditions in which enzymatic \(\bullet\)NO synthesis is compromised, involves the redox interaction of nitrite and ascorbate in the extracellular space in hippocampus, contributing to increases in blood flow. This work ascertains the significance of neuronal- and nitrite-derived NO as a direct mediator of the NVC for health and disease issues and forwards fundamental knowledge that might be conducive to the development of therapeutic strategies in brain aging and dysfunction, such as AD. Lourenço et al. 2014 Free Radic. Biol. Med. 73, 421-429.

This work was supported by FCT grants (Portugal) PTDC/BBB-BQB/321/2012.

Keywords: Nitric oxide, neurovascular coupling, Alzheimer’s disease
New antagonists for the orphan G-protein coupled receptor GPR18, a new player in microglial migration

Clara Schoeder¹, Viktor Rempel¹, Tadeusz Karcz², Katarzyna Kiec-Kononowicz², Christa E. Müller¹

¹Pharma-Zentrum Bonn, Pharmazeutisches Institut, Pharmazeutisches Chemie I, Germany
²Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University, Medical College, Poland

GPR18 is an orphan G protein-coupled receptor (GPCR) which interacts with some cannabinoid receptor ligands, for example Δ9-tetrahydrocannabinol (THC). Nevertheless, GPR18 is phylogenetically unrelated to the classical cannabinoid receptors CB1 and CB2. GPR18 was found to stimulate microglial migration in BV-2 cells, a murine immortalized microglial cell line.¹ N-Ara-chidonoylglycine was proposed as its endogenous agonist, but several groups, including ours, could not confirm that.² To further elucidate the (patho)physiological roles of GPR18 and to investigate its potential as a drug target, potent and selective ligands are required. In a screening campaign using our proprietary compound library (www.mueller-group.pharma.uni-bonn.de/bibliothek) bicyclic imidazole-4-one derivatives synthesized by the group of K. Kiec-Kononowicz³ (Cracow, Poland) were identified as a novel class of GPR18 antagonists. We subsequently investigated their structure-activity relationships at GPR18, and evaluated their selectivity versus the CB1, CB2 and the related orphan receptors GPR35 and GPR55.⁴ (Z)-2-(3-(4-Chlorobenzyloxy)benzylidene)-6,7-dihydro-2H-imidazo[2,1-b][1,3]thiazin-3(5H)-one was developed as a potent, selective GPR18 antagonist with an IC50 of 0.279 μM, >36-fold selectivity vs. CB1, GPR55 and GPR35 and 14-fold selectivity vs. CB2. It represents the most potent and selective GPR18 antagonist known to date.⁴

References:

Keywords: GPR18, microglia, GPR55
Conference venue

1. Dorpat Conference Centre (Turu 2, Tartu)

Social events

2. Science Center AHHA (Welcome reception, 14 June)
   (Sadama 1, Tartu)

3. University of Tartu Museum (Conference dinner, 16 June)
   (Lossi 25, Tartu)

4. St John’s Church (Concert, 15 June)
   (Jaani 5, Tartu)

5. Meeting point for the bus excursion to Tallinn, Dorpat hotel (17 June)
   (Soola 6, Tartu)
MicroRNAs (miRNAs) are short, 22–25 nucleotide long transcripts that may suppress entire signaling pathways by interacting with the 3'-untranslated region (3'-UTR) of coding mRNA targets, interrupting translation and inducing degradation of these targets. The long 3'-UTRs of brain transcripts compared to other tissues predict important roles for brain miRNAs. Supporting this notion, we found that brain miRNAs co-evolved with their target transcripts, that non-coding pseudogenes with miRNA recognition elements compete with brain coding mRNAs on their miRNA interactions, and that Single Nucleotide Polymorphisms (SNPs) on such pseudogenes are enriched in mental diseases including autism and schizophrenia, but not Alzheimer’s disease (AD). Focusing on evolutionarily conserved and primate-specific miRNA controllers of cholinergic signaling (‘CholinomiRs’), we find modified CholinomiR levels in the brain and/or nucleated blood cells of patients with AD and Parkinson’s disease, with treatment-related differences in their levels and prominent impact on the cognitive and anti-inflammatory consequences of cholinergic signals. Examples include the acetylcholinesterase (ACHE)-targeted evolutionarily conserved miR-132, whose levels decline drastically in the AD brain. Moreover, we found that interruption of ACHE miRNAs’ interaction with the primate-specific CholinomiR-608 in carriers of a SNP in the ACHE’s miR-608 binding site induces domino-like effects that reduce the levels of many other miR-608 targets. Young, healthy carriers of this SNP express 40% higher ACHE activity than others, potentially affecting the responsiveness to AD’s anti-ACHE therapeutics, and show elevated traits anxiety, inflammation and hypertension. Non-coding regions affecting miRNA-target interactions in neurodegenerative brains thus merit special attention.

Keywords Alzheimer’s disease, microRNAs, pseudogenes
Copper metabolism of astrocytes
Ralf Dringen, Ivo Scheiber, Felix Bulcke
Centre for Biomolecular Interactions Bremen, Faculty 2 (Biology/Chemistry)
University of Bremen, Bremen, Germany
SpringerPlus 2015, 4(Suppl 1):L3

Copper is an essential trace element which is involved in many important cellular functions [1]. However, excess of copper can impair cellular functions by copper-induced oxidative stress. In brain, astrocytes are considered to play a prominent role in antioxidative defence as well as in the copper homeostasis [1,2]. To investigate uptake, toxicity, storage and export of copper in astrocytes, we used primary rat astrocyte cultures as model system. Cultured astrocytes efficiently take up copper ions predominantly by the copper transporter Ctr1 and the divalent metal transporter DMT1. In addition, copper oxide nanoparticles are rapidly accumulated by astrocytes, most likely by endocytic processes. Astrocytes tolerate moderate increases in intracellular copper contents very well. However, if the specific cellular copper content exceeds after exposure to copper or copper oxide nanoparticles a threshold level of around 10 nmol copper/mg protein, accelerated production of reactive oxygen species and compromised cellular viability were observed even without exposure to sub-toxic concentrations of copper ions or copper oxide nanoparticles, astrocytes increase their copper storage capacity by upregulating the cellular contents of glutathione and metallothioneins. In addition, cultured astrocytes have the capacity to export copper ions which is likely to involve the copper-transporting ATPase 7A. The ability of astrocytes to efficiently accumulate, store and export copper ions suggests that astrocytes play a key role in brain copper homeostasis and that an impairment of astrocytic functions may be involved in diseases which are connected with disturbances in brain copper metabolism.

Keywords Astrocytes, metals, oxidative stress

References

Altered developmental neuroplasticity due to polysialyltransferase ST8SiaII deficiency in mice leads to schizophrenia-like phenotype
Alexander Zharkovsky
University of Tartu, Estonia
SpringerPlus 2015, 4(Suppl 1):L4

Post-translational modification of the neural cell adhesion molecule (NCAM) by polysialic acid (PSA) is crucial for nervous system development and brain plasticity. PSA attachment is catalyzed by the two polysialyltransferases, ST8Sial and ST8SiaIV. ST8Sial dominates during embryonic and early postnatal development while ST8SiaIV is the prevailing enzyme of the juvenile and adult brain. A growing body of evidence links aberrant levels of NCAM and PSA to neuropsychiatric disorders, including schizophrenia. To investigate whether polysialyltransferase deficiency might cause a schizophrenia-like phenotype, ST8Sial+/− mice, ST8SiaIV+/− mice and their wild-type littermates were assessed neuroanatomically and subjected to tests of cognition and sensory functions. ST8Sial+/− but not ST8SiaIV+/− mice displayed enlarged lateral ventricles and a size reduction of the thalamus accompanied by a smaller internal capsule and a highly disorganized pattern of thalamocortical and corticothalamic fibers. Loss of ST8Sial was associated with reduced phosphorylation of fibroblast growth factor receptor 1 in the frontal cortex of newborn mice and retarded neuronal differentiation of newly generated cells in the dentate gyrus of adults. ST8Sial+/− and ST8SiaIV+/− mice were both impaired in short- and long-term recognition memory, but only ST8Sial+/− mice displayed impaired working memory and a deficit in prepulse inhibition, which could be attenuated by clozapine treatment. Furthermore, ST8Sial+/− mice exhibited anhedonic behavior and increased sensitivity to amphetamine-induced hyperlocomotion. These data reveal that reduced polysialylation in ST8Sial+/− mice leads to pathological brain development and schizophrenia-like behavior. We therefore propose that ST8Sial deficiency has the potential to cause a neurodevelopmental predisposition to schizophrenia.

Acknowledgements The study was supported by the EU 6FPWP grant LSHM-CT-2005-512012.

Keywords Neural cell adhesion molecule, sialytransferase, neuroplasticity

Two steps forward, one step back: successes and failures in structure-based discovery of GPCR ligands
Jens Carlsson
Stockholm University, Sweden
SpringerPlus 2015, 4(Suppl 1):L5

G protein–coupled receptors (GPCRs) are intensely studied as drug targets and for their role in signaling. High-resolution crystal structures of GPCRs capturing different receptor conformations are now available, which have provided insights into the mechanism of activation and ligand selectivity for this important class of drug targets. I will first present a series of structure-based screens for novel ligands of the A2A adenosine receptor (A2AAR), which is a drug target for Parkinson’s disease (antagonists) and ischemia (agonists). As crystal structures for both inactive- and active-like receptor conformations of the A2AAR have now been determined, molecular docking screens for novel ligands can be performed. Virtual screens against different conformations of the A2AAR were carried out to investigate if structure-based methods can be used to identify agonists and antagonists. Our results shed light on the importance of access to crystal structures and the role of the chemical library in screens for ligands with specific pharmacological properties. For most GPCRs, no experimental coordinates are available and structure-based screens are forced to rely on homology models. However, it is still unclear if models of GPCRs are sufficiently accurate to be used in ligand discovery. The determination of crystal structures for dopamine and serotonin receptors, and the challenges to the community to predict these in the GPCR Dock competitions, have enabled us to carry out comparisons of ligand discovery from models versus crystal structures. Our results from these challenges reveal opportunities and limitations of the use of homology models in ligand discovery and design of selective lead candidates.

Acknowledgements Symposium organized by COST action CM1207 – GLUSTEN.

Keywords G protein–coupled receptors, chemical biology, virtual screening

Comparison of A1 and A2A receptor dynamics using FRET based receptor sensors
Carsten Hoffmann, Anette Stumpf
University of Wuerzburg, Germany

Recently published crystal structures of the adenosine A2A receptor in the active and inactive state have revealed static endpoints of the conformational changes associated with the activation process. To investigate the activation dynamics of different adenosine receptor subtypes we used fluorescence resonance energy transfer (FRET) measurements of a modified A1 and A2A receptor construct (A1R, A2AR). Those optical probes were designed by fusion of the cyan fluorescent protein (CFP) to the C-terminus of the receptor and insertion of the fluorescein arsenical hairpin binder (FlAsH) motif into the 3rd intracellular loop. Based on the ligand binding pocket revealed from the crystal structure 10 optical probes including individual mutations were created for each receptor. To compare A1R and A2AR dynamics, we established HEK293 cell lines stably expressing these optical probes and investigated the signal amplitude and the receptor activation kinetics in living cells. We identified 3 different effects of these mutations. One class causes problems in membrane localization of the A1R but not the A2AR. The 2nd group is involved in binding of the ribose moiety and has stronger effects in the A1R compared to the A2AR.
A2AR. The 3rd class consists of the mutants that are involved in binding of the adenine moiety and have similar effects for adenosine and theophylline binding for the A2AR. Thus, our study provides evidence that amino acids serve different functions within the A1R and A2AR ligand binding pocket. In summary the different signal amplitudes and different activation kinetics are indicative for a different activation behavior of the A1R and A2AR and the data from the receptor mutants support these findings and gives new insight into the A1R-structure.

Acknowledgements Symposium organized by COST action CM1207 – GLUSTEN.

Keywords Receptor dynamics, adenosine receptor, FRET

L7
Defining the organizational structure of dopamine and muscarinic acetylcholine receptors
Graeme Milligan1, María José Varela Liste1, Gianluigi Caltabiano Caltabiano2, Elisa Alvarez-Curto1, Sara Marsango1
1University of Glasgow, UK; 2Universitat Autònoma de Barcelona, Spain
SpringerPlus 2015, 4(Suppl 1):L7

G protein-coupled receptors, including the M3 muscarinic acetylcholine receptor and the dopamine D3 receptor, can form homo-oligomers. However, the basis of these interactions and the overall organizational structure of such oligomers are poorly understood. Combinations of site-directed mutagenesis and homogenous time-resolved FRET studies that assessed interactions between receptor protomers at the surface of transfected cells indicated important contributions of regions of transmembrane domains I, IV, V, VI and VII, as well as intracellular helix VIII, to the overall organization. Molecular modelling studies based on both these results and an X-ray structure of the inactive state of the M3 receptor bound by the antagonist/inverse agonist tiotropium were then employed. The results could be accommodated fully by models in which a proportion of the cell surface M3 receptor population is a tetramer with rhombic, but not linear, orientation. This is consistent with previous studies based on spectrally-resolved, multi-photon FRET. Modelling studies suggest, furthermore, an important role for molecules of cholesterol at the dimer + dimer interface of the tetramer, consistent with the presence of cholesterol at key locations in many G protein-coupled receptor crystal structures. Mutants that displayed disrupted quaternary organization were often poorly expressed and showed immature N-glycosylation. Sustained treatment of cells expressing such mutants with the muscarinic receptor inverse agonist atropine increased cellular levels and restored both cell surface delivery and quaternary organization to many of the mutants. These observations suggest that organization as a tetramer may occur before plasma membrane delivery and may be a key step in cellular quality control assessment.

Acknowledgements Symposium organized by COST action CM1207 – GLUSTEN.

Keywords Dopamine, acetylcholine receptor structure

L8
Investigation of GPCR allosterism and dimerization in single living cells using fluorescent ligands
Stehen Hill
University of Nottingham, UK
SpringerPlus 2015, 4(Suppl 1):L8

Previous work, using fluorescent adenosine receptor agonists and antagonists, has provided novel insights into the allosteric regulation of adenosine A3 (A3AR) and A1 (A1AR) receptors by adenosic ligands and receptor dimerization in single living cells [1,2]. We have also used a fluorescent analogue of CGP12177 to investigate ligand binding to the human β1-adrenoceptor. This work has demonstrated that there is negative cooperativity between the two different ligand-binding conformations of the β1-adrenoceptor activated by catecholamines and CGP12177 respectively [3]. Finally, we have used fluorescence correlation spectroscopy (FCS) to investigate ligand binding to A1AR and A3AR in small 0.2 μm² microdomains of single living cells [4]. FCS studies with a fluorescent A3-agonist have enabled high affinity labeling of the active conformation (R*) of the receptor [4]. We have also used a fluorescent adenosine A3-antagonist (CA200645) to study the binding characteristics of antagonist-occupied receptor conformations (R) in membrane microdomains of single cells [5]. Investigation of the dissociation kinetics of CA200645 provided further support for allosteric regulation of this receptor by homodimerization [5].

Acknowledgements Symposium organized by COST action CM1207 – GLUSTEN.

Keywords Ligand binding, fluorescence, cooperativity

References

L9
Gliomas and epilepsy: insights from neuropathological studies in humans
Eleonora Aronica
Department of (Neu)Pathology, Academic Medical Center and
Swammerdam Institute for Life Sciences, Center for Neuroscience University of Amsterdam, The Netherlands, SEIN – Stichting Epilepsie Instellingen Nederland, Heemstede, The Netherlands
SpringerPlus 2015, 4(Suppl 1):L8

Brain tumors represent a recognized cause of epilepsy in both children and adults. In principle, any tumor (extra-axial, intra-axial, benign or malignant, common or uncommon) can cause seizures. However, patients with supratentorial low-grade glial tumors are more likely to develop epilepsy. Several clinical studies emphasize that pharmacologically intractable epilepsy critically affects the daily life of patients with brain tumors, even if the tumor is under control. Recently, the term of long-term epilepsy associated tumor (LEAT) has been introduced. LEATs are low grade, slowly growing, cortically-based tumors which predominantly occur in young patients with long histories (often 2 years or more) of drug-resistant epilepsy. Glioneuronal tumors (GNT), including gangliogliomas (GG) and dysembryoplastic neuroepithelial tumors (DNTs), represent the most common tumor within the spectrum of LEAT. The advent of the neurosurgical treatment of epilepsy-associated brain lesions confirmed the strong epileptogenicity of these tumor entities. Understanding the mechanisms that underlie epileptogenesis in LEATs is essential to identify new treatment targets and to develop an effective therapy. Mechanisms such as alterations of the balance between excitation and inhibition and alterations in neuron-glia interactions might be involved. Astroglial cells express functional receptors for a variety of neurotransmitters and may critically modulate synaptic transmission. In addition, an increasing number of observations indicate that pro-epileptogenic inflammatory pathways are activated in GNT and may contribute to the onset and progression of seizures. The recent advances and likely candidate mechanisms and molecules involved in tumor-associated epileptogenesis will be discussed.

Acknowledgements EA is supported by the National Epilepsy Fund – “Power of the Small”, the Hersenstichting Nederland (NEF 013-1) and KIKI (Stichting Kinderen Kankervrij).

Keywords Glioma, glioneuronal tumor, epilepsy

L10
Purinergic transmission in brain tumors and its impact on drug development
Maria Abbracchio, Stefania Cerutti, Davide Lecca
Dept Pharmacol Sci, University of Milan, Italy

Extracellular nucleoside and nucleotides acting via adenosine/ P1 and nucleotide P2 (P2X/P2Y) purinoceptors are fundamental signaling molecules controlling the survival and proliferation of astrocytes and oligodendrocytes (Cerutti and Abbracchio, Adv Exp Med Biol. 2013;986:13). The malignant transformation of these cells to
progressively more aggressive tumors (respectively, astrocytomas and anaplastic glioblastoma, and glioblastoma multiforme, containing proliferating cells resembling Oligodendrocytes Precursor Cells, OPCs) confers growth advantage and chemoresistance. Characterization of the specific P1 and P2 receptors on these tumors may unveil new strategies to reduce cancer growth and/or promote differentiation to non-cancerous glial phenotypes. The adenosine A3 receptor (A3AR) has emerged as a potential target. Under hypoxia, a condition typical of gliomas’ core, A3AR mediates chemoresistance via the PI3K/Akt pathway (leading to inactivation of the pro-apoptotic Bad protein) and by upregulating matrix metalloproteinase-9, that degrades extracellular matrix and promotes migration of glioma cells towards healthy brain regions (Ceruti and Abbracchio, and ref therein). Thus, inhibition of A3AR with selective antagonists could represent an appealing therapeutic approach. More recently, the P2X7 receptor has been recently found to be over-expressed in grade IV human gliomas (Monif et al., J Inflammation, 2014;11:25) and its blockade with the synthetic antagonist Brilliant Blue G decreased tumour cell number. Finally, treatment of human glioblastoma multiforme cells with UDP, UDP-glucose or LDTD4, that act as agonists at the new P2Y-like GPR17 receptor, reduced the formation of glioma spheres, suggesting that GPR17 stimulation on highly proliferating tumor OPCs may drive their differentiation to the oligodendroglial fate, negatively affecting both tumor proliferation and self-renewal (Dougherty et al., Cancer Res. 2012;72:4856).

Keywords Cell differentiation, purinoreceptors, new targets in glioma

L11
Cannabinoid signalling in glioma cells
Bozena Kaminska, Aleksandra Ellert-Miklaszewska
Nencki Institute of Experimental Biology, Warsaw, Poland
SpringerPlus 2015, 4(Suppl 1):L11

Cannabinoids, originally derived from Cannabis sativa, as well as their endogenous and synthetic counterparts, were shown to induce apoptosis of glioma cells in vitro and tumour regression in vivo via their specific receptors, cannabinoid receptors CB1 and/or CB2. CB2 are aberrantly expressed in human gliomas and glioma cell lines. Most of the analysed gliomas expressed significant levels of CB2 receptor and the extent of CB2 expression in the tumour specimens was related to tumour malignancy. A synthetic cannabinoid, WIN 55,212-2, down-regulated the Akt and Erk signalling pathways in C6 glioma cells that resulted in reduction of phosphorylated Bad levels, mitochondrial depolarization and activation of caspase cascade leading to apoptosis. We examined whether synthetic cannabinoids with different receptor specificity: WIN55,212-2 (a non-selective CB1/CB2 agonist) and JWH133 (a CB2-selective agonist) affect survival of four human glioma cell lines and three primary human glioma cell lines. WIN-55,212-2 decreased cell viability in all examined cell lines and induced cell death. Susceptibility of the cells to JWH133 treatment correlated with the CB2 expression. Cannabinoids triggered a decrease of mitochondrial membrane potential, cleavage of caspase-9 and effector caspases. Induction of cell death by cannabinoid treatment led to the generation of a pro-apoptotic sphingolipid ceramide and disruption of signalling pathways crucial for regulation of proliferation and survival. Increased ceramide levels induced ER-stress and autophagy in drug-treated glioblastoma cells. We conclude that cannabinoids are efficient inhibitors of human glioma cells growth, once the cells express specific type of cannabinoid receptor.

Acknowledgements Studies supported by a grant 2012/04/A/ NZ3/00630 from the National Science Center, Poland

Keywords Cannabinoids, signal transduction, cell death

L12
The role of integrins in glioma biology and anti-glioma therapies
Patrick Roth
University of Zurich, Switzerland
SpringerPlus 2015, 4(Suppl 1):L12

Integrins are a group of molecules expressed by various cells including glioma cells and endothelial cells within the tumor. There are 18 known alpha and beta integrin subunits which form a heterodimer. Integrins regulate different cellular processes such as proliferation, adhesion, motility and survival as shown in numerous preclinical models. Furthermore, integrins control the activity of the transforming growth factor (TGF)-beta pathway and are involved in the process of angiogenesis which is indispensable for continued tumor growth. Because of the high expression levels of some integrins on glioma cells and their numerous functions, inhibition of integrin signaling has been considered a promising strategy for the treatment of glioma patients. Besides blocking antibodies which are currently under clinical investigation in other cancer entities, the integrin inhibitor cilengitide has been tested within several trials in glioblastoma patients over the last years. Cilengitide is a cyclic RDG peptide which targets integrins alphavbeta3 and alphavbeta5. Based on the results of smaller, initial trials suggesting an activity of cilengitide against glioblastoma, 2 larger trials were subsequently performed. However, both trials, which combined temozolomide-based chemoradiation with cilengitide failed to demonstrate an improved outcome with the addition of cilengitide. Ongoing translational analyses suggest that integrin levels in the tumor tissue are neither prognostic nor predict response to cilengitide. While the clinical development of cilengitide has been stopped, integrin inhibition with more effective agents may still be a promising approach in clinical neuroendology.

Keywords Glioma, integrin, TGF-beta

L13
Drosophila modelling axonal transport in the face of tau pathology
Amrit Mudher
University of Southampton, UK

Alzheimer’s disease (AD) is a devastating neurodegenerative disease causing irreversible cognitive decline in the elderly. There is no disease-modifying therapy for this condition and the mechanisms underpinning neuronal dysfunction and neurodegeneration are unclear. Compromised cytoskeletal integrity within neurons is reported in AD. This is believed to result from loss-of-function of the microtubule-associated protein tau, which becomes hyper-phosphorylated and deposits into neurofilibrillar tangles in AD. We have developed a Drosophila model of tauopathy in which abnormal human tau mediates neuronal dysfunction characterised by microtubule destabilisation, axonal transport disruption, synaptic defects and behavioural impairments. Here we show that a microtubule-stabilising drug, NAPVSIPQ (NAP), prevents as well as reverses these phenotypes even after they have become established. Moreover, it does not alter abnormal tau levels indicating that it by-passes toxic tau altogether. Thus, microtubule stabilisation is a disease-modifying therapeutic strategy protecting against tau-mediated neuronal dysfunction, which holds great promise for tauopathies like AD.

Keywords Microtubule stabilisation, tau, NAP

L14
Recent developments in tau-based therapeutics for Alzheimer’s disease and related dementias
Miguel Medina
CIBERNED and CIEN Foundation, Madrid, Spain
SpringerPlus 2015, 4(Suppl 1):L14

Current therapies for Alzheimer’s disease (AD) and related disorders have demonstrated very modest, symptomatic efficacy, leaving an unmet medical need for new, more effective therapies. While drug development efforts in the last two decades have primarily focused on the amyloid cascade hypothesis, with disappointing results so far, tau-based strategies have received little attention until recently despite that the presence of extensive tau pathology is central to the disease. The discovery at the turn of the century of mutations within the tau gene that cause fronto-temporal dementia demonstrated that tau dysfunction was per se sufficient to cause neuronal loss and clinical dementia. Development of tau pathology is associated with progressive neuronal loss and cognitive decline and is the common
underlying cause of a group of neurodegenerative disorders collectively known as “taupathies”. Tauopathies are clinically, morphologically and biochemically heterogeneous neurodegenerative diseases characterized by the deposition of abnormal tau protein in the brain. The neuropathological phenotypes are distinguished based on the involvement of different anatomical areas, cell types and presence of distinct isoforms of tau in the pathological deposits. Thus, the spectrum of tauopathy entities expands beyond the traditionally discussed disease forms. Emerging evidence strongly suggests that accumulation of abnormal tau is mediated through spreading of seeds of the protein from cell to cell. This prion-like mechanism would support the concept that in AD brains, tau pathology initiates in a very small part of the brain many years before becoming symptomatic, spreading slowly and progressively to the whole brain following an anatomically defined pattern. Emerging therapeutic strategies aimed at treating the underlying causes of the tau pathology will be discussed, including some novel therapeutic approaches on the verge of providing new treatment paradigms in upcoming years.

Keywords Tau, Alzheimer, dementia

L15
The oligodendroglia cytoskeleton in health and disease
Christiane Richter-Landsberg
University of Oldenburg, Molecular Neurobiology, Germany
SpringerPlus 2015, 4(Suppl 1):L15

Oligodendrocytes, the myelin forming cells of the CNS, enwrap neuronal axons and form multimellular myelin sheets. They are derived from oligodendrocyte precursor cells which migrate from the subventricular zone into the different regions of the brain. Differentiation from the early progenitor to the mature multiprocessed oligodendrocyte is characterized by different morphological stages. To support cell morphology and establish and maintain the myelin membrane, an intact, spatially organized cytoskeleton with dynamic properties is essential. In particular microtubules and their associated proteins play an important role. A variety of microtubule binding proteins, including tau, are present in oligodendrocytes. Oligodendrocytes in culture express all six isoforms of tau which are developmentally regulated. Tau proteins are present in immature and mature oligodendrocytes and specifically prominent in the branching points of the cellular processes. Downregulation of tau impairs cell differentiation and the process of early myelination. In neurodegenerative diseases collectively termed tauopathies, fibrillary tau accumulations occur not only in neurons but also in glia. Tau positive coiled bodies originating in oligodendrocytes are characteristic for the brains of patients with frontotemporal dementias, such as corticobasal degeneration and progressive supranuclear palsy. These aggregates are further characterized by the presence of heat shock proteins and ubiquitin, indicating that stress situations are causally related to the pathogenesis. In this respect, proteasomal dysfunctions have been discussed to be involved in neurodegenerative disorders and the aging process. Data on the consequences of proteolytic stress in oligodendrocytes and the protein aggregation process will be presented. Our data demonstrate that an intact cytoskeleton is essential for cellular defense mechanisms.

Keywords Oligodendrocytes, microtubules, neurodegenerative diseases

L16
Tau regulates the localization and function of End Binding proteins in neuronal cells
Carmen Laura Sayas1,2,3, Elena Tortosa, Flavia Bottali, Sacnictie Ramirez-Rios, Isabelle Arnal, Jesús Ávila
1Centre for Biomedical Research of the Canary Islands (CIBICAN), Spain; 2Institute for Biomedical Technologies (ITB), Italy; 3University of La Laguna (ULL), Spain
SpringerPlus 2015, 4(Suppl 1):L16

Tau is a classical microtubule-associated protein known to regulate microtubule stability in neurons. In our study, we have addressed the putative cross-talk between tau and End binding proteins 1 and 3 (EB1/3), the core microtubule plus-end tracking proteins (+TIPs), in differentiating neuronal cells. We show that tau and EB proteins interact directly and that the cellular distribution and mobility of EB proteins depends on tau localization and expression levels. Moreover, our data reveal that tau is essential for the proper localization of EB1 at the medial-distal region of the axon shaft in developing neurons. In summary, we provide evidence for a new function of tau protein as a direct regulator of EB1/3 proteins. This further indicates that the interplay between classical MAPs and core +TIPs may be important for the fine-tuned regulation of microtubule dynamics and stability during neuronal differentiation.

Keywords Tau, end binding proteins, microtubule dynamics

L17
Multiple signaling pathways of orexin receptors
Jyrki Kukkonen
University of Helsinki, Finland
SpringerPlus 2015, 4(Suppl 1):L17

Orexin/hypocretin peptides are central for the regulation of the sleep/arousal states, and have, in addition, a role in the regulation of e.g. metabolism, addiction, stress response and pain gating. Orexin responses are mediated by G-protein-coupled OX1 and OX2 orexin receptors. Orexin signaling (in different cell types) is very versatile, ranging from excitation to induction of cell death. Orexin receptor coupling is promiscuous, engaging members of at least three different families of G-proteins, namely Gi, Gs and Gq, and some non-G-protein mediators as well. Preferred G-protein-coupling of the receptors appears different in different tissues, but the mechanism determining this is unknown. The primary signal transducers of orexin receptors very effectively activate phospholipase cascades, including PLA2, PLC and PLD, and also PLC-diacylglycerol lipase-mediated endocannabinoid generation. These cascades may play a significant role in the regulation of K+ and non-selective cation channels, which are the effectors for the orexin-mediated neuronal excitation. In some cell types, orexin receptor stimulation induces programmed cell death. Some different mechanisms for his have been proposed, but the picture is still incomplete. Orexin receptors have been a target for multiple drug discovery projects. Main focus has been on the antagonists, with insomnia as the indication. For agonist drugs there are some ongoing smaller academic and semi-academic projects. Use of orexin receptor agonists is suggested to be beneficial in narcolepsy (as peptide replacement therapy) and putatively in other sleep/wakefulness disturbances, metabolic disorders and cancer.

Keywords Orexin, hypocretin, phospholipase

L18
Galanin receptor ligands
Ülo Langel
Stockholm University, Sweden
SpringerPlus 2015, 4(Suppl 1):L18

The effect of galanin is mediated through three GPCR subtypes, GalR1-3. The limited number of specific ligands to the galanin receptor subtypes has hindered the understanding of the individual effects of each receptor subtype. This review summarizes the current data of the importance of the galanin receptor subtypes and receptor subtype specific agonists and antagonists and their involvement in different biological and pathological functions.

Keywords Galanin, neuropeptide, ligand

L19
Application of fluorescence methods for characterization of ligand binding to G protein coupled receptors
Ago Rinken1, Anni Allikalt2, Sergei Kopanchuk3, Reet Link1, Santa Veikšina1
1 Institute of Chemistry, University of Tartu, Estonia; 2Institute of Chemistry, University of Tartu, Estonia, Competence Centre on Health Technologies, Tartu, Estonia
SpringerPlus 2015, 4(Suppl 1):L19

G protein coupled receptors (GPCR) comprise a large family of transmembrane proteins involved in the regulation of signal transduction
through the cell membrane in response to various extracellular stimuli. GPCRs have become important targets of many drugs for treatments of very different diseases. During the last decade several fluorescence-based methods have been implemented for the characterization of signal transduction via GPCRs, starting from ligand binding and including several steps leading up to a response on the level of gene regulation. We have proposed the fluorescence anisotropy (FA) and fluorescence intensity (FI) assay to investigate fluorescent ligand binding properties to different GPCRs (Veikisana et al., 2010). The implementations of budded baculoviruses that display G protein-coupled receptors on their surfaces have significantly increased sensitivity and applicability of these assays (Veikisana et al., 2014). The developed novel assay systems opened new possibilities for real-time monitoring of ligand binding to their receptors for understanding their particular kinetic properties. These assays are also compatible for homogenous HTS suitable for ligand screening. There has been implemented assay systems for receptors of peptides like melanocortin (MC4R) and neuropeptide Y (NPY1R) as well as for receptors of monoamines like dopamine (D1DAR) and serotonin (5-HT1AR).

Acknowledgements This work has been financed by Estonian Ministry of Education and Science (IUT 20-17) and by the European Regional Development Fund (TK114, 30020).

Keywords GPCR, Fluorescence anisotropy, budded baculoviruses

L20 Imaging of a2C-adrenoceptors in the living brain: a method to monitor noradrenaline release?
Mika Scheinin1, Jussi Lehto1, Jarkko Johansson1, Pauliina Luoto2, Eveliina Arponen1, Lauri Vuorilehto1, Harry Scheinin1, Juha Rouyu1
1University of Turku, Finland; 2Turku PET Centre, TYKS, Finland; 6Onion Corporation, Finland
SpringerPlus 2015, 4(Suppl 1):L20

Objectives: The PET tracer [11C]ORM-13070 was recently validated for receptor occupancy analysis of brain a2C-adrenoceptors, and PET experiments in monkeys and humans indicated that tracer uptake into the caudate and putamen was reduced by interventions that increased synaptic noradrenaline concentrations in the brain [1-3]. This study aimed to confirm the sensitivity of [11C]ORM-13070 binding to increased levels of synaptic noradrenaline.

Methods: PET imaging of the brain was performed with a 3D High Resolution Research Tomograph. Eight subjects underwent a control [11C]ORM-13070 PET scan and two PET scans after two different noradrenaline challenges, i.e. a sub-anesthetic infusion of ketamine and oral intake of atomoxetine combined with cold stimulation. Tracer uptake in the caudate nucleus and putamen was described with AUC values in scan time windows of 10-20 min and 5-30 min post injection, and quantified with the ratio method. Voxel-based analysis was performed with average B/F images. Both challenges caused small but statistically significant (10–20%, p < 0.05) reductions in tracer uptake in both target regions. Voxel-based analysis revealed significant clusters in the dorsal putamen with both challenges. Ketamine was associated with significant elevations in circulating noradrenaline and adrenaline levels, while the atomoxetine + cold treatment was not. Strong experimental support was gained for the feasibility of [11C]ORM-13070 PET imaging of brain noradrenergic neurotransmission.

Keywords Adrenoceptor, noradrenaline, positron emission tomography

References

L21 The other side of opioid receptor signaling: regulation by protein–protein interaction
Zafiroula Georgoussi
Institute of Biosciences and Applications, Laboratory of Cellular Signaling and Molecular Pharmacology, National Centre for Scientific Research "Demokritos", Greece
SpringerPlus 2015, 4(Suppl 1):L21

Opioid receptors (OR) are widely known as mediators of the analgesic effects of opioids and also contribute to the development of tolerance and dependence. Moreover, opioids are implicated in cell proliferation and survival. How opioids modulate these downstream signaling pathways is a research receiving a lot of attention. Our group aims to define the signaling pathways through which opioid receptors participate in these physiological processes. Emphasis is given to unconventional interacting partners of the μ and δ-opioid receptors such as the Regulators of G protein signalling (RGS) proteins and STAT5B (Georgoussi et al. 2012). Evidence will be presented with which RGS proteins opioid receptors interact, how RGS members confer selectivity to receptors to choose a specific subset of G proteins, how activation of opioid receptors result in recruitment of RGS proteins to the plasma membrane and exert a differential modulatory effect in ERK1,2 phosphorylation, agonist-driven adenylyl cyclase inhibition and internalization of the opioid receptors (Papakonstantinou et al., 2015). Moreover evidence will be presented on how STAT5B associates with the δ-opioid receptor and forms selective pairs with selective Go, Gβγ subunits and RGS proteins, and how activation of the δ-opioid receptor with selective agonists promotes a multi-component signaling complex involving the STAT5B transcription factor and other signaling intermediates to mediate neuronal survival and neurite outgrowth (Georganta et al., 2013). Understanding the mechanism that control OR signaling is important to address problems related to phenomena such as pain perception, tolerance and dependence that occur upon chronic opiate administration and define whether disruption of such interactions may contribute to the development of novel therapeutic strategies.

Acknowledgements Supported by the EU “Normolife”-LSHC-CT2006-037733 and the GSRT, Excellence II - 3722, “NO-ALGOS”. Z.G participates in the EU COST Action CM1207 (GListen).

Keywords Opioid receptors, RGS proteins, STAT5

L22 Autism as a disease of the synapse: search for mechanistic insight
Hannelore Ehrenreich
Clinical Neuroscience, Max Planck Institute of Experimental Medicine, Göttingen, Germany
SpringerPlus 2015, 4(Suppl 1):L22

Autism spectrum disorders (ASD) are heterogeneous, heritable neurodevelopmental conditions, affecting ~0.5% of the population across cultures, with a ~4:1 male/female ratio. ASD are characterized by social interaction and communication deficits, restricted interests, repetitive behaviors, and reduced cognitive flexibility. Causes likely converge at the synapse, as shown by mutations of synaptic genes or mutations causing quantitative alterations in synaptic protein expression. Neuroligin4 (NLGN4X) mutations are among the most frequent causes of heritable ASD. But monogenic forms altogether account for 1200 schizophrenic subjects and validated it in Asperger autists. We hypothesized that a coincidence of unfortunate normal expression. Neuroligin4 (NLGN4X) mutations are among the most frequent causes of heritable ASD. But monogenic forms altogether account for 1200 schizophrenic subjects and validated it in Asperger autists. We hypothesized that a coincidence of unfortunate normal variation in synaptic or synapse-regulating genes rather than mutations underlies most autistic phenotypes. We identified ‘prenatal’ variants in synaptic genes, which in aggregate are associated with high autism severity. A transcranial magnetic stimulation study on respective individuals revealed enhanced glutamatergic and GABAergic activity. IPS-derived cortical neurons from these subjects are now functionally characterized.

Keywords Autism, synapse, ambra1
SpringerPlus 2015, Volume 4 Suppl 1
http://www.springerplus.com/supplements/4/S1

L23
Elucidating the mechanisms of TDP-43 aggregation in a cellular model of motor neuron disease
David Hicks, Marcus Rattray
University of Bradford, UK

Motor neuron disease (MND) is a neurodegenerative disease of mid-life, with average survival of 3–5 years after diagnosis. The only approved treatment, riluzole, is only moderately effective and so there is a great need for novel therapeutics. TAR DNA binding protein 43 (TDP-43) has held a particular centrality in MND research since its discovery as the principal protein component of the characteristic protein aggregates found in the disease. Subsequent work has investigated its role as an RNA-binding protein and regulator of approximately 3000 RNA transcripts. In the majority of disease cases, both familial and sporadic, normally nuclear TDP-43 is sequestered in the cytoplasm in protein aggregates. Using differentiated motor neuron-like cells (NSC-34) and primary motor neurons, we have treated cells with the disease-relevant ER stressor tunicamycin. Overnight treatment with a low concentration of tunicamycin resulted in the formation of aggregates immunoreactive for endogenous TDP-43, as visualised by immunocytochemistry and Western blotting of the RIPA-insoluble fraction. We have found that these aggregates do not co-stain for TIAR, a well-known marker of stress granules. However, using the oxidative stressor sodium arsenite, we are able to induce formation of stress granules, though they do not co-stain for TDP-43. The tunicamycin treatment also led to a moderate decrease in cell viability, as shown by MTT and Trypan Blue Exclusion assays. In this study, we have recapitulated a known pathological process (ER stress) of MND in vitro, which resulted in the formation of TDP-43 aggregates. The signalling pathways driving TDP-43 aggregation are unknown, hence work detailing these mechanisms is likely to present wide scope for therapeutic intervention.

Keywords TDP-43, MND, aggregation

L24
The role of dynein mediated transport in the clearance of misfolded proteins responsible for motoneuron diseases
Riccardo Cristofori, Eilis Giorgetti, Alessandro Boncoraglio, Marco Meroni, Mariarita Galbiati, Paola Rusmini, Elio Messi, Angelo Poletti
Università degli Studi di Milano, Italy
SpringerPlus 2015, 4(Suppl 1):L24

Spinal and bulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are motoneuron diseases. A mutation in the androgen receptor (ARpolyQ) gene is responsible for SBMA. Mutations in the SOQ1, in the TDP-43, in the FUS-TLS or in the C9ORF72 genes are responsible for familiar form of ALS. The mutated coded proteins misfold and aggregates. Efficient protein quality control (PQC) is required for the maintenance of physiological and soluble protein pool in affected motoneuron. The balance between autophagy and ubiquitin-proteasome system (UPS) plays a vital role in the clearance of misfolded and aggregated proteins. Degradation of SBMA and ALS misfolded proteins. Dynein binds the chaperone BAG3 and transports the mutant proteins at microtubule degradation of SBMA and ALS misfolded proteins. Dynein binds the co-chaperone BAG3 and transports the mutant proteins at microtubule organization center where misfolded proteins interact, aggregate and can be degraded by autophagy. However, here misfolded proteins may blocks autophagy flux. In NSC34 cells, dynein is sequestered into ARpolyQ aggregates suggesting the role of dynein into aggregates formation process. Unexpectedly, the silencing of dynein heavy chain resulted in a drastic reduction of ARpolyQ retained in filter retardation assay (FRA). Moreover, dynein silencing drastically altered autophagic markers localization (LC3 and p62) by immunofluorescence. Notably, treatment with a dynein inhibitor (EHNA) drastically reduced the retention of ARpolyQ, mutSOQ1 and mutTDP43 aggregates in FRA, even when autophagy was inhibited with 3-MA. Conversely UPS blockage with MG132 counteracted the reduction induced by altered dynein transports. RTq-PCR on NSC34 cells treated with EHNA showed an increased BAG1:BAG3 ratio that can targeting the misfolded proteins to UPS. Moreover, in NSC34 cells, EHNA increased the degradation of proteasome reporter GFPu, while BAG1 overexpression reduced the level of aggregates retained in FTA. These data suggest that, when autophagy is overload, by misfolded proteins, dynein inhibition restores the physiological and soluble protein pool via UPS.

Keywords MNDs, PQC, dynein

L25
Effects of novel synthetic microneurotrophins in diabetic retinopathy
Silvia Lisa, Ruth Iban-Arias, Despina Kokona, Ioannis Charalampopoulo, Achilles Gravanis, Kyriaky Thermos
University of Creta, Greece

Diabetic retinopathy (DR) is a neovascular, inflammatory and neurodegenerative disease. The neovascular component is treatable but no therapeutic agents are available for the other two components. Early changes in the diabetic retina include neuronal death of amacrine and retinal ganglion cells (RGC) (Barber et al., J Clin Invest, 1998), and elevated levels of inflammatory mediators (Yoshimura et al., Plos One, 2009). Nerve Growth factor (NGF) receptors, namely TrkA and p75NTR, are expressed in RGC. The TrkA receptor activates prosurvival, while p75NTR activates inflammatory and apoptotic pathways (Mysona et al., Expert Rev Ophthalmol, 2014). Dehydroepiandrosterone (DHEA) binds to both receptors, mimicking NGF. It affords anti-apoptotic, neuroprotective and anti-inflammatory effects in the retina (Kokona et al., Neuro-pharmacol, 2012, Straub et al., J Clin Endocrinol, 1998). The therapeutic use of DHEA is restricted due to its metabolic products. The main objective of this study was to investigate and compare the neuroprotective and anti-inflammatory effects of DHEA and its spiro-epoxy derivatives BNN27 and BNN20 (Cologepoulou et al., J Med Chem, 2009) (not metabolized to estrogens and androgens), in the rat streptozotocin model of DR. BNN27, via TrkA activation, protected in a dose-dependent manner (2, 10, 50 mg/kg, ip) BNOS (brain nitric oxide synthetase) and TH (tyrosine hydroxylase) expressing amacrine cells and ganglion axons (NFL immunoreactivity) similar to DHEA’s actions, while BNN20 was less effective. BNN27 activated the TrkA prosurvival signaling pathway ERK1/2 kinase. It reduced the activation of SAPK/JNK kinase and the expression of p75NTR. BNN27 also increased the expression of anti-inflammatory cytokines (IL10). These results suggest that NGF TrkA receptor is involved in the neuroprotective and anti-inflammatory effects of BNN27 and is a valuable target via which BNN27 could afford efficacious therapeutics for the treatment of DR.

Acknowledgements Funded by GGET. ARISTEIAI.

Keywords Diabetic retinopathy, Microneurotrophins, neuroprotection

L26
Unravelling carbon monoxide protection in cerebral ischemia: from the organelle to the organism
Claudia Queiroga1, Ana Almeida1, Simone Tomasi2, AlessandroVercelli2, Paula Alves3, Helena Vieira1
1Chronic Diseases Research Center, Universidade Nova de Lisboa, Portugal; 2Neuroscience Institute Cavaleiros Oottolengo, Italy; 3Instituto de Biologia Experimental e Tecnológica/Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Portugal
SpringerPlus 2015, 4(Suppl 1):L26

Perinatal complications are a serious clinical problem, in particular hypoxic-ischemic (HI) episodes, caused by birth asphyxia or uterine and fetal blood flow interruption. HI corresponds to 23% of neonatal deaths, being one of the top 20 leading causes of disease burden. Preconditioning (PC) is a stimulation below the injury threshold that activates endogenous protective mechanisms to prevent damage. Low doses of carbon monoxide (CO) play a beneficial role through PC induction. Herein, CO cytoprotection was explored in distinct brain models. The used experimental models range from monolayers of astrocytes, co-cultures of neurons and astrocytes, to the whole organism from the organelle to the organism.
communication towards neuronal survival. Purinergic molecules are the main mediators for this non-cell autonomous effect. Our results seem to indicate that the main pathway involved includes ATP release from astrocytes, its metabolism and A2A receptor binding to initiate protective mechanisms within the neurons. Rat pups were exposed to CO before hypoxia-ischemia induction (Vannucci model). 24 h after HI the brains were collected for cell death and tissue protection assessment (histological and immunohistochemical analysis). It was found limited apoptosis in hippocampus following cerebral ischemia: lower cytochrome c release and caspase-3 activation yielding an increased Bcl-2 expression. Altogether, one can conclude that there is not just a unique pathway for the CO-induced endogenous protection, brain tolerance is the result of a complex cellular change in response to injury. Indeed, CO regulates cell death pathways and modulates cellular metabolism. **Keywords** Carbon monoxide, preconditioning, ischemia

**L27**

The effect of different modes of hypobaric hypoxia on the expression of transcription factor pCREB and pro-survival proteins BDNF and BCL-2 in rat neocortex and hippocampus

Anna Churilova, Michail Samoilov
Pavlov Institute of Physiology of Russian Academy of Sciences, Russian Federation

Severe hypobaric hypoxia (180 mm Hg) is a harmful stimulus that induces structural and functional injuries of susceptible brain neurons in the neocortex and hippocampus. In contrast, moderate hypobaric hypoxia (360 mm Hg) activates endogenous cellular defensive mechanisms. The rate of neuroprotection afforded by the mild hypoxia depends on the quantity of hypoxic sessions. In particular, preconditioning (pre-exposure) by three trials of mild hypoxia protects from deleterious effects of subsequent severe hypoxia whereas preconditioning with one mild hypoxic trial does not. The molecular mechanisms induced by mild hypoxic preconditioning are unclear. Pro-survival proteins, such as neurotrophic factor BDNF and anti-apoptotic factor Bcl-2 are supposed to be involved in this process. In the present study, the effects of three-trial and one-trial hypoxic preconditioning on the expression of pro-survival proteins BDNF and Bcl-2 as well as their upstream activator pCREB, have been studied in the neocortex and hippocampus of rats. As revealed by quantitative immunocytochemistry, the severe hypobaric hypoxia didn’t affect or down-regulated the neuronal levels of pCREB, BDNF and Bcl-2 at 3–24 h after the exposure. The one-trial preconditioning did not change this effect of severe hypoxia. In contrast, preconditioning by three trials of mild hypoxia (360 Torr, 2 h, 24 h intervals, 3 times) significantly enhanced the pCREB, BDNF and Bcl-2 neuronal expression in response to severe hypoxic challenge. Three-trial mild hypoxia alone also up-regulated the expression of molecular factors examined in the neocortex and hippocampus at 24 h whereas one trial of the mild hypoxia did not. The results of the present study indicate that development of the neuronal hypoxic tolerance induced by the three-trial, in contrast to one-trial, mild hypoxic preconditioning is apparently largely associated with the activation of CREB, as well as BDNF and Bcl-2 overexpression.

**Keywords** Hypoxic preconditioning, brain hypoxic tolerance, pCREB

**L28**

Ankrd11 is a chromatin regulator involved in autism that is essential for neural development

Anastassia Voronova, Denis Gallagher, Mark Zander, Gonzalo Cancino, Alexa Bramall, MP Krause, C Abad, M Tekin, PM Neilen, DF Callen, SW Scherer, GM Keller, DR Kaplan, K Walz and FD Miller
Hospital for Sick Children, Canada

SpringerPlus 2015, 4(Suppl 1) L28

Ankrd11 is a potential transcriptional regulator that is implicated in cognitive dysfunction and ASD, but has no known function in the brain. We show that Ankrd11 is expressed in the embryonic cortex, and that when it was knocked-down in murine cortical precursors this caused decreased proliferation, reduced neurogenesis, and aberrant neuronal positioning in developing cortex. Knockdown of Ankrd11 in human forebrain neural precursors phenocopied Ankrd11 knockdown in murine neural precursors. Decreased proliferation of embryonic and adult neural precursors and neuronal mispositioning were also observed in Yoda mice carrying a point mutation in the histone deacetylase (HDAC)-binding domain of Ankrd11. Yoda mice also displayed ASD-like behavioural abnormalities. Consistent with a role for Ankrd11 in histone acetylation, Ankrd11 was associated with chromatin and co-localized with HDAC3. Also, expression and histone acetylation of Ankrd11 target genes were altered in Yoda neural precursors. Finally, the Ankrd11 knockdown-mediated decrease in precursor proliferation was rescued by inhibiting histone acetyltransferase activity or expressing HDAC3. Thus, Ankrd11 is a crucial epigenetic regulator of neural development that regulates histone acetylation and gene expression, thereby providing a likely explanation for its association with cognitive dysfunction and ASD.

**Keywords** Chromatin, stem cells, brain development

**L29**

Active endocannabinoids are secreted on the surface of microglial microvesicles

Martina Gabrielli1, Natalia Battista1, Loredana Riganti1, Ilaria Prada1, Flavia Antonucci1, Laura Cantone, Marta Lombardi, Michela Matteoli, Mauro Maccarrone, Claudia Verderio

1Department of Medical Biotechnology and Translational Medicine, University of Milano, Milano, Italy; 2Faculty of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy, European Center for Brain Research/IRCCS Santa Lucia Foundation, Rome, Italy; 3CNR-IN Neuroscience Institute, Department of Medicine, Milan, Italy

SpringerPlus 2015, 4(Suppl 1) L29

Endocannabinoids (eCBs) are bioactive lipids which primarily influence synaptic communication in the nervous system. They are synthesized by neurons but also by microglia, especially under inflammation. To exert their function, eCBs travel across the intercellular space. However, how eCBs move extracellularly remains obscure. Our recent evidence indicates that reactive microglia release extracellular vesicles (EVs), which may represent an ideal vehicle for the transport of hydrophobic eCBs. Hence, in this study we investigated whether microglial EVs carry eCBs and may influence neurotransmission. First we analyzed the eCB content of EVs and found a clear enrichment of anandamide (AEA) in EVs relative to parental microglia. This analysis revealed higher AEA levels in EVs shed from the plasma membrane (microvesicles), compared to those which originate from the endocytic compartment (exosomes). To bioassay the activity of vesicular AEA, we used patch clamp analysis of miniature inhibitory post-synaptic currents (mIPSC) on hippocampal neurons in vitro. Exposure of neurons to microvesicles (MVs) induced a significant decrease in mIPSC frequency, mimicking the action of CB1R agonists. The involvement of vesicular AEA in this phenomenon was inferred from the ability of the CB1R antagonist SR141716A to block the reduction of mIPSC frequency evoked by MVs. Western blot analysis showed that MVs induces an increase in ERK phosphorylation, which was completely inhibited by SR141716A. This indicate that CB1R activation by AEA-storing MVs translates into downstream signaling. Finally, consistent with a surface localization of AEA, MVs membranes maintained their capability to decrease mIPSC frequency. Overall, this study shows that microglial MVs carry AEA on their surface to stimulate CB1R on target GABAergic neurons thus playing a crucial role in the modulation of inhibitory transmission.

**Keywords** Endocannabinoids, extracellular vesicles, microglia-neuron signaling

**Reference**

Peripheral nervous tissue engineering
Katarzyna Nawrottek, Michał Tyłman, Jacek Balcerzak, Kamil Kamiński
Lodz University of Technology, Poland
SpringerPlus 2015, 4(Suppl 1):L30
Peripheral nerve injuries are an important part of everyday medical practice since there are reported over 600,000 cases in Europe and in the United States annually [1]. Many of such nerve injuries cause gaps between nerve stumps. Without intervention, they can lead to the formation of a stumpy neuroma, what can result in functional impairment of the nerve fiber. The current approaches to regeneration of damaged peripheral nerves include: autografting, allografting, and, last but not least, the implantation of polymeric tubes and conduits between nerve stumps. Nerve autografting is considered as the “gold standard” technique for the repair of peripheral nerve discontinuities, but it has a number of limitations, such as the requirement for the second surgical procedure to harvest the graft tissue, the donor site morbidity, additional injuries and scarring as well as the increased recovery time. Allografts (e.g., cadaver nerve grafts) and xenografts (e.g. animal nerve grafts) can be an alternative to autografts, but their main drawbacks are in the high possibility of an unfavorable immune response. The most promising materials for peripheral nerve conduits preparation are natural biopolymers. They are obtained from natural sources, exhibit similar properties to the tissues they are replacing and reveal good cell adhesion. Furthermore, they tend to accelerate regeneration processes due to specific chemical interactions within the human body, e.g. with extracellular matrix (ECM) molecules. The purpose of our study is to create a conduit with properties that will mimic the ones of the extracellular matrix of the peripheral nervous system. Our focus is put on natural polymers, especially chitosan. In addition, we use chemotactic factors which exhibit properties beneficial in regeneration of the peripheral nervous tissue.

Keywords Biomaterials, hydrogel, nerve regeneration

Reference

Ginkgolic acid specifically potentiates alpha 1 glycine receptors
Galyna Malieva1, Svetlana Buldakovà, Piotr Bregestovski2
1Bogomoletz Institute of Physiology, Ukraine, 2Institute of Neuroscience des Systèmes, Aix-Marseille University, France
SpringerPlus 2015, 4(Suppl 1):L31
Ginkgo biloba extract is a neuroactive agent that is widely used for correction of age-associated impairment of memory, attention deficit and other cognitive functions. It has been shown that ginkgolides and bilobalides, Ginkgo biloba extract components, are potent blockers of glycine receptors [1,2]. However, the effect of ginkgolic acid, the other Ginkgo biloba extract constituent, on ligand-gated ion channels was not investigated. In the present study, using patch-clamp technique and transient transfection of different subunits in CHO cells we have shown that glycine receptors (GlyRs) are modulated by ginkgolic acid in a subunit-specific manner. After pre-application of ginkgolic acid (0.5–2 min), glycine-induced currents mediated by α1 GlyRs were strongly potentiated, while currents mediated by α2 GlyRs exhibited weak inhibition. There was no significant effect of ginkgolic acid on amplitudes of currents mediated by α3 GlyR or on GABAARs composed of glycine and abolishment of the inhibitory effect, typical for wild type α2 GlyR. Our results suggest that (i) ginkgolic acid selectively enhances the function of α1 GlyRs and attenuates the function of α2 GlyRs, (ii) mutation of α2 subunit converts effect of ginkgolic acid from inhibition to potentiation.

Keywords Glycine receptor, ginkgolic acid, patch-clamp

References

Regulation of mitochondrial trafficking, function and quality control by the mitochondrial GTPases Miro1 and Miro2
Josef Kittler
University College London, UK
SpringerPlus 2015, 4(Suppl 1):L33
Regulated trafficking of mitochondria in neurons is essential for providing ATP at the correct spatial location to power neural function and computation, and for providing calcium buffering at sites of calcium entry or release. Indeed the regulation of mitochondrial distribution, morphology and function are proposed to play an important role in neuronal development and survival but the regulatory mechanisms remain unclear. Miro family proteins (Miro1 and Miro2 in mammals) contain a transmembrane domain locating them to the mitochondrial inner membrane and reveal good cell adhesion. Furthermore, they tend to accelerate regeneration processes due to specific chemical interactions within the human body, e.g. with extracellular matrix (ECM) molecules. The purpose of our study is to create a conduit with properties that will mimic the ones of the extracellular matrix of the peripheral nervous system. Our focus is put on natural polymers, especially chitosan. In addition, we use chemotactic factors which exhibit properties beneficial in regeneration of the peripheral nervous tissue.

Keywords Biomaterials, hydrogel, nerve regeneration

Reference
in regulating neuronal development, function and pathology will also be explored.

Keywords Mitochondria, mitophagy, parkinson's

L34 Principles of mitochondrial fusion and fission cycle in neurons
Allen Kaasik
University of Tartu, Estonia
SpringerPlus 2015, 4(Suppl 1):L34

Mitochondrial fusion-fission dynamics play a crucial role in many important cell processes. These dynamics control mitochondrial morphology, which in turn influences several important mitochondrial properties including mitochondrial bioenergetics and quality control, and they appear to be affected in several neurodegenerative diseases. The molecular machineries behind mitochondrial fusion and fission events are relatively well known. The regulation of fusion and fission events beyond the molecular machinery involved is less clear, fusion and fission are not random occurrences but form a cycle whereby fission typically follows fusion. Mitochondrial fission machinery may somehow sense mitochondrial length and become active when the mitochondrion is oversized and cease when mitochondria are smaller. In contrast, mitochondrial fusion events depend heavily on mitochondrial trafficking. Fusion only takes place when two mitochondria meet and motile mitochondria will be more likely to encounter one another. In cultured cortical neurons, for example, only one in every 14th contact between mitochondria results in fusion. The purpose of this presentation is to provide insight into the complex crosstalk between different processes involved in mitochondrial fusion-fission dynamics and to discuss the potential physiological purpose of mitochondrial fusion and fission.

Keywords Mitochondrial dynamics, neurons, mitophagy

L35 Bioenergetic failure, mitochondrial dysfunction and mitophagy
Philip Beart
University of Melbourne, Australia

Mitophagy specifically describes autophagy of damaged or dysfunctional mitochondria and occurs in programmed cell death when mitochondria fragment and remodel their cristae. Cellular bioenergetics is entwined with mitochondrial dynamics, and mitochondrial insults, including depolarization and inhibition of electron transport chain, trigger mitochondrial fragmentation. Here we investigated mitophagy in neurons during manipulation of mitochondrial bioenergetics. Dysfunction of mitochondria was induced by pharmacological inhibition of respiratory chain complexes I-V (rotenone, 3-nitropropionic acid, antimycin A, KCN & oligomycin, respectively) in primary cultures of cerebellar granule cells. The extent of bioenergetic failure was determined by measuring [ATP], depolarization of mitochondrial membrane potential and decrease in oxygen consumption rate in the Seahorse XF-24. All stressors produced mitochondrial dysfunction as shown by concentration- and time-dependent decline in [ATP] over 4–24 h. Complexes I, III or IV showed rapid loss of mitochondrial membrane potential and decreases in oxygen consumption rate over 4–24 h. Autophagolysosomal flux was increased as shown by increased LC3-II and labelling of acidic vacuoles with monodansylcarmine. Immunocytochemistry for PINK1 showed translocation of PINK1 from cytoplasm to mitochondria after injury, indicating likely involvement of mitophagy during bioenergetic dysfunction. Transfection of fluorescent pH-biosensor Rosella (Rosado CJ, et al. Autophagy. 2008;4:205) targeting mitochondria indicated the pH of the mitochondrial location dropped with the inhibition of complex I and II, implying acidification of mitochondria, presumably in acidic vesicles undergoing mitophagy. Dieback of neuronal arbor observed here paralleled that seen with a GFP-plasmid. Together these data indicate that bioenergetic dysfunction produces mitophagy in primary neurons and is likely to be involved in neuronal dynamics.

Keywords Mitophagy, PINK1, autophagolysosome

L36 Mechanisms of mitochondrial quality control in autosomal recessive Parkinson's disease
Olga Corti
Institut du Cerveau et de la Moelle épinière, Paris, France

The genes encoding the E3 ubiquitin protein ligase Parkin (PARK2) and the mitochondrial serine/threonine kinase PINK1 (PARK6) are mutated in clinically similar, autosomal recessive early onset Parkinson's disease (PD) forms. Over the past ten years, a number of studies in different model systems have demonstrated that PINK1 and Parkin regulate jointly several processes relevant to maintenance of mitochondrial quality, including mitochondrial trafficking and dynamics, mitophagy and biogenesis. By using a combination of approaches of cell biology, confocal imaging and biochemistry in different cell models, we recently showed that loss of protein import efficiency triggers recruitment of Parkin by PINK1 in proximity of the translocase of outer mitochondrial membrane (TOM). We provided evidence that the degradation of specific TOM subunits plays a key role in initiating the autophagic degradation of damaged mitochondria. We also showed that PINK1 and Parkin interact with the TOM machinery on polarized mitochondria. Our results suggests that this interaction modulates the import of the multifunctional mitochondrion-protective matrix enzyme 17beta-hydroxysteroid dehydrogenase 10, which is depleted in Parkin-deficient mice and Parkinson's disease patients. Electron and confocal microscopy, and calcium imaging approaches used to characterize the enoplasmic reticulum (ER)-mitochondria interface, a compartment previously linked to neurodegenerative processes, revealed enhanced juxtaposition between these organelles, associated with increased ER-to-mitochondria calcium transfer in cells from Parkin-deficient mice and patients with PARK2 mutations. Our current work aims at investigating the relevance of mitochondrial quality control mechanisms regulated by PINK1 and Parkin in different cell types of the central nervous system, to evaluate the contribution of each of them to the physiopathology of autosomal recessive Parkinson's disease.

Keywords Parkinson's disease, mitophagy, ER/mitochondria interface

L37 Activity-dependent neuroprotective protein (ADNP): from autism to Alzheimer's disease
Illana Gozes
The Lily and Avraham Gildor Chair for the Investigation of Growth Factors and the Elton Laboratory for Molecular Neuroendocrinology, Department of Human Genetics and Biochemistry, Sackler Faculty of Medicine, Sagol School of Neuroscience and Adams Super Center for Brain Studies, Tel Aviv University, Tel Aviv University, Tel Aviv 69978, Israel
SpringerPlus 2015, 4(Suppl 1):L37

We have previously discovered activity-dependent neuroprotective protein (ADNP) as a major regulatory gene, a component of the SWI/SNF chromatin remodeling complex, essential for brain formation. Others found ADNP as a most frequent autism spectrum disorder (ASD)-associated gene. Furthermore, ADNP is the only protein significantly decreasing in the serum of Alzheimer’s disease (AD) patients. Our most recent results revealed sex-related learning/memory differences in mice, reflecting hippocampal expression changes in ADNP and ADNP-controlled AD/ASD risk genes 1. Hippocampal ADNP transcript content was doubled in male vs. female mice, with females showing equal expression to ADNP+/− males and no significant genotype-associated reduction. Increased male ADNP expression was replicated in human postmortem hippocampal samples. The hippocampal transcript for ApoE (the major risk gene for AD) was doubled in female mice compared with males, and further doubled in the ADNP+/− females, contrasting a decrease in young ADNP+/− males. ADNP regulates >400 genes during embryonic development, with ApoE being a major target. Other AD related proteins regulated by ADNP include tau (with pathological tau constituting the neurofibrillary tangles and with AD being the major tauopathy). Furthermore, ADNP associates with microtubule end binding proteins, controlling dendritic spine density, which is compromised in AD and ASD. Previously, overexpression
of the eukaryotic translation initiation factor 4E (eIF4E) led to ASD-like phenotype in mice and we have shown that hippocampal eIF4E expression was specifically increased in young ADNP+-/- male mice. Understanding ADNP expression, positioned as a master regulator of key ASD and AO risk genes, introduces a novel concept of hippocampal gene-regulated sexual dimorphism toward gender-based biology and therapeutics.

Acknowledgements

Funded by AMIN, ISF, and the Israeli Ministry of Science.

Keywords

ADNP, Autism, Alzheimer's disease

Reference


L38

Neuronal-activity regulated gene expression: emphasis on BDNF

Tõnis Tõnnusk

Tallinn University of Technology, Estonia

SpringerPlus 2015, 4(Suppl 1):L38

Neurotrophin brain-derived neurotrophic factor (BDNF) is a growth factor that has important roles in the development and functioning of nervous system by promoting the survival, differentiation and synaptic plasticity of specific neuronal populations. Modifiability of neuronal connectivity by formation of new synapses, and alteration of the strength and stability of existing synapses, is regarded as the main cellular basis for memory and long-term behavioral adaptations. The gene encoding BDNF is considered to be one of the master genes of synaptic plasticity. BDNF has also received particular interest for its deregulation in nervous system disorders. Decreases of BDNF and its receptor TrkB levels and activity are accompanied by and are believed to lead to several pathologies, particularly nervous system diseases like neurodegenerative, psychiatric and cognitive diseases. Therefore knowledge about the regulatory mechanisms of BDNF gene is important both for understanding of nervous system function and for finding new drug targets. Results of our studies on the molecular mechanisms of neuronal activity-regulated expression of BDNF gene in the nervous system will be presented and discussed.

Acknowledgements

Supported by by Estonian Research Council (institutional research funding IUT19-18), National R&D program „Biotechnology“ (grant AR12030), Norwegian Financial Mechanism (grant EMP128) and Estonian Academy of Sciences.

Keywords

BDNF, synaptic plasticity, gene regulation

L39

Epigenetic mechanisms of hypoxic preconditioning

Elena Rybnikova, Mikhail Samoilov

Pavlov Institute of Physiology, Russian Academy of Sciences, Russian Federation


Hypoxia preconditioning is a pre-exposure to the repetitive mild hypoxia which results in development of brain hypoxic/ischemic tolerance and cross-tolerance to injurious factors of another nature. The endogenous defense processes mobilized by hypoxia preconditioning and resulting in formation of brain tolerance are based on evolutionary acquired gene-determined mechanisms of neuroprotection and adaptation. Key event is an activation of pro-adaptive transcriptional factors HIF-1, CREB, NF-kB, c-Fos, NGFI-A and down-stream expression of their target genes in vulnerable brain neurons. An important role can thus be suggested for the epigenetic regulation of gene expression, in particular, acetylation of core nucleosome histones leading to changes in chromatin structure which ensure access of the transcriptional factors activated by the preconditioning to the promoters of target genes. It has been shown that hypoxic preconditioning considerably increases the acetylation status of all histones and, particularly, H3 in the neurons of rat hippocampus and neocortex, whereas injurious severe hypoxia causes global repression of histone acetylation. Diverse effects of the severe hypoxia and mild hypoxic preconditioning on the methylation of DNA and histones have also been observed. The complex of the epigenetic modifications induced by the hypoxic preconditioning is attributed to the relaxed DNA which becomes available for activation of gene expression by pro-adaptive transcriptional factors up-regulated by the preconditioning.

Acknowledgements

The work was supported by Russian Foundation for Basic Research (grant No. 14-04-00516).

Keywords

Hypoxic preconditioning, brain hypoxic/ischemic tolerance, epigenetic mechanisms

L40

Role of amyloid precursor protein (APP) in regulation of neuronal genes

Natalia N Nalivaeva1,2

1I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry RAS, St. Petersburg, 19423, Russian Federation; 2School of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, UK

SpringerPlus 2015, 4(Suppl 1):L40

Understanding the mechanisms regulating gene expression in the course of development and adaptation of the organism to the permanently changing environment and in the case of pathology is fundamentally important. Special attention in recent years has been paid to the processes of epigenetic regulation of neuronal genes involving chromatin modifications at the level of DNA methylation and histone acetylation. In these processes an important role belongs to the histone deacetylases (HDAC) which control gene silencing. Recently it was shown that the amyloid precursor protein (APP) and its intracellular domain (AICD) participate in regulation of expression of a number of neuronal genes, including those involved in amyloid metabolism and clearance. The list of APP-regulated genes includes the major amyloid-degrading enzyme neprilysin (NEP), a transport protein transthyretin (TTR), aquaporin and others. Our studies strongly indicate that AICD regulation of NEP and TTR is APP isoform-dependent and cell-type specific. In this process AICD competes for gene regulation with HDACs and treatment of cells or animals with HDAC inhibitors such as valproic acid results in up-regulation of NEP and TTR mRNA and protein levels and increased NEP activity leading to a reduction in total cellular amyloid (AB) peptide levels. Regulation of other proteins, e.g. acetylcholinesterase, does not involve AICD but requires full length APP molecules. APP overexpression in neuronal cells was also shown to affect the levels of HDAC gene products which might explain its role in gene regulation. Further studies of the APP interactome are important for better understanding of its role in brain development and functioning and for designing the drugs protecting the brain against neurodegeneration, in particular Alzheimer's disease.

Acknowledgements

Supported by RFBR (13-04-00388), Program of RAS "Fundamental Sciences for Medicine": ARUK.

Keywords

Amyloid precursor protein, neprilysin, histone decetylases

L41

Anti-inflammatory activities of carbon monoxide-releasing molecules (CO-RMs) in the brain

Roberto Motterlini, Roberta Foresti

INSERM-France

SpringerPlus 2015, 4(Suppl 1):L41

The transcription factor Nrf2 and its downstream target heme oxygenase-1 (HO-1) are ubiquitous protective systems against oxidative stress, inflammation and tissue injury. The products of HO-1 enzymatic activity, biliverdin and carbon monoxide (CO), possess interesting anti-oxidant and anti-inflammatory properties suggesting that exploitation of this pathway as a target for drug discovery may offer therapeutic avenues in a variety of disorders [1]. In this context we have developed carbon monoxide-releasing molecules (CO-RMs), a class of compounds that deliver CO in a controlled fashion and exert a variety of pharmacological effects [2]. Data will be presented showing that CO-RMs modulate neuroinflammation and neuroprotection in vitro and in vivo. In BV2 microglia cells challenged with thrombin and interferon gamma, CO-RMs reduced the production of inflammatory mediators both in normoxic and hypoxic conditions. Similarly, in a rat model of collagenase-induced intracerebral hemorrhage, CO-RMs...
modulated microglia activation and the production of TNF-α while partially protecting against brain damage [3]. The mechanism(s) by which CO liberated from CO-RMs exert protection remains elusive. However, our laboratory has recently produced findings in BV2 microglia pointing to mitochondria as a plausible target for the anti-inflammatory action of CO. Studies are also currently ongoing on the synthesis and characterization of novel hybrid molecules that potently activate Nrf2 and simultaneously release CO in order to maximize the anti-inflammatory effects of the HO-1 pathway [4].

Acknowledgements

Supported by grants from ANR (MITO-CO) and the University of Paris-Est Creteil (CHORM) and a European Fellowship (Marie Curie IF).

Keywords

HO-1, carbon monoxide, microglia

References


L43

Transcription Factor Nrf2: a novel target to modulate inflammatory and neuroprotective responses in Parkinson's disease

Antonio Cuadrado

Centro de Investigación en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Instituto de Investigación Sanitaria La Paz (IDIAPAZ), Departamento de Bioquímica e Instituto de Investigaciones Biomédicas “Alberto Sols” (CIBERNED), Instituto de Investigación Sanitaria La Paz (IdiPAZ) and European Fellowship (Marie Curie IF).

Keywords

HO-1, carbon monoxide, microglia

References


L42

Carbon monoxide targeting mitochondria in astrocytes: modulation of cell metabolism, redox response and cell death

Helena Vieira, Ana Almeida, Cláudia Figueiredo-Pereira, Cláudia Queiroga Chronic Diseases Research Center, NOVA Medical School, Universidade Nova de Lisboa, Portugal

SpringerPlus 2015, 4(Suppl 1):L42

The endogenously produced gasotransmitter carbon monoxide (CO) has been studied as a factor involved in cytoprotection, homeostasis and anti-inflammation. Small amounts of reactive oxygen species (ROS) are described as signaling factors in CO's biological mode of action. Mitochondria are the main source of ROS and are also key organelles in orchestrating cell function: metabolism, cell death control and redox signaling. Astrocytes are most abundant glial cells and essential for neuronal function, namely metabolic and physical support, expression of neurotransmitters and promotion of neuroprotection. In this work it is shown that CO prevents astrocytic cell death and improves cell metabolism by targeting mitochondria, and some of the underlying molecular mechanism are disclosed. CO directly targets non-synaptic mitochondria and inhibits their mitochondrial membrane permeabilization, by preventing mitochondrial swelling, depolarization and inner membrane permeabilization. Thus, CO limits the release of cytochrome c into the cytosol and the activation of apoptotic cascade in astrocytes. All these events are ROS-dependent and involve glutathionylation of adenine nucleotide translocator (ANT), whose activity is ATP/ADP transport through mitochondrial inner membrane. In addition, low amounts of exogenous CO increase ATP production by improving oxidative metabolism. Mitochondrial population and specific cytochrome c oxidase activity are higher upon CO treatment. The CO-induced metabolic improvement is dependent on Bcl-2 expression. Dysfunctional mitochondrial can be eliminated by mitophagy, which is a crucial process for maintaining their function and quality control. In astrocytes, CO promotes mitophagy at 1 h of treatment, while following 24 h mitochondrial population is back to basal levels, indicating that CO contributes to mitochondrial turnover. Furthermore, CO limits astrocytic cell death in an autophagic dependent manner.

Keywords

Carbon monoxide, astrocytes, mitochondria

L44

Neuroprotective effect of carbon monoxide and Nrf2 in cerebral ischemia

Sylvain Doné

Center for Translational Research in Neurodegenerative Disease, University of Florida, College of Medicine, USA

SpringerPlus 2015, 4(Suppl 1):L44

Carbon monoxide (CO) is a gaseous second messenger produced when heme oxygenase (HO) enzymes catabolize heme. We have demonstrated that CO can be therapeutic in ischemia-reperfusion brain injury; however, it is unclear whether CO can also offer protection in permanent ischemic stroke or what mechanism underlies the effect. HO1 neuroprotection is shown to be regulated by Nrf2; therefore, we investigated whether CO might partially exert neuroprotection by modulating the Nrf2 pathway. To evaluate potential protective effects of CO, we exposed wildtype and Nrf2−/− mice to 250 ppm CO or control air for 18 h immediately after permanent middle cerebral artery occlusion. Infarct volume and neurological deficits were assessed on day 7. Molecular mechanisms of Nrf2 pathway activation by CO were also investigated. Mice exposed to CO after permanent ischemia had 29.6 ± 12.6% less brain damage than did controls at 7 days. Additionally, 18 h CO treatment led to Nrf2 dissociation from Keap1, nuclear translocation, increased binding activity of Nrf2 to HO1 antioxidant response elements, and elevated HO1 expression 6–48 h after CO exposure. The CO neuroprotection was essentially completely abolished in Nrf2−/− mice. Low-concentration of exogenous CO represents a neuroprotective agent for stroke combination treatment and its beneficial effect would be at least partially mediated by activation of the endogenous Nrf2 pathway.

Keywords

Medical gas, neuroprotection, Nrf2-regulated protein, stroke

L45

The lipogenic regulator Sterol Regulatory Element Binding Factor-1c is required to maintain peripheral nerve structure and function

Nico Mitro1, Gaia Cemenati1, Matteo Audano1, Silvia Giatti1, Maurizio D’Antonio1, Emma De Fabiani1, Maurizio Cestani1, Enrique Saez2, Inigo Azcoitia2, Guido Cavaletti2, Luis-Miguel García-Segura3, Roberto C. Melcangi1, Donatella Caruso1

1DiSFeB, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Italy; 2IRCCS San Raffaele Scientific Institute, DBIT, Italy; 3Scrpps Research Institute, La Jolla, CA, USA; 1University Complutense of Madrid, Spain; 2Università degli Studi Milano-Bicocca, Italy; 3Instituto Cajal, C.S.I.C., Spain

SpringerPlus 2015, 4(Suppl 1):L45

Myelin is a membrane characterized by high lipid content to facilitate impulse propagation. Changes in myelin fatty acid (FA) composition

References

have been associated with peripheral neuropathy [1], but the specific role of peripheral nerve FA synthesis in myelin formation and function is poorly understood. We explored the extent to which lack of the key regulator of FA synthesis as Sterol Regulatory Element Binding Factor-1c (Srebf-1c) could result in the development of peripheral neuropathy. We found that Srebf-1c null mice display a neuropathic phenotype consisting in hypermyelinated small caliber fibers, the result of changes in myelin periodicity. Unexpectedly, transcriptomics and metabolomics revealed activation of peroxisome proliferator activated receptor α (Ppara) signaling in Srebf-1c null peripheral nerve as a result of increased levels of two distinct phosphatidyldcholine-based Ppara ligands, PC-C16:0/C18:1 and PC-C18:0/C18:1 [2,3]. Ppara is a nuclear receptor that directs uptake, utilization and catabolism of FAs [4]. As a consequence of abnormal local Ppara activation, Srebf-1c null peripheral nerve exhibit increased fatty acid utilization, a detrimental condition leading to peripheral neuropathy. Treatment with a Ppara antagonist rescues the neuropathy of Srebf-1c null mice. These findings reveal the importance of FA synthesis to sustain peripheral nerve structure and function.

Acknowledgements This study was supported by the Giovanni Armenise-Harvard Foundation (N.M.), Fondazione CARIPLO 2014-0991 (N.M.) and 2012-0547 (R.C.M.), Italian Ministry of Health GR-2011-02346791 (M.D. and N.M) and Research Center for the Characterization and Safe Use of Natural Compounds – “Giovanni Galli” directed by D.C.

Keywords Peripheral neuropathy, Schwann cells, metabolism

References

L46

The molecular control of GnRH neuron development

Anna Cariboni1, Andreé Valentina1, Kathryn Davidson1, John Parnavelas1
University of Milan, Italy; 1UCL, UK

SpringerPlus 2015, 4(Suppl 1):L46

Fertility critically depends by a small number of hypothalamic neurons secreting the neurohormone GnRH. During development GnRH neurons migrate from the nasal placode to the hypothalamus by following the migratory path formed by the vomeronasal axons. Developmental defects of this process can cause congenital GnRH deficiency (GD), characterized by absent/delayed puberty and consequent infertility. The underlying mutated loci are for the majority of GD cases unknown, partially because of a poor understanding of the molecular mechanisms that control the development of these crucial neuroendocrine cells. Here we provide evidence of the importance of class 3 semaphorins and their receptors in this process. Specifically, I will explain how two different semaphorins play distinct roles during the migration of GnRH neurons. Thus, semaphorin3A affects the migration of GnRH neurons in the nasal compartment, via the co-receptors Neuregulin-1 and 2, whereas semaphorin3E via its receptor plexin1 controls the survival of GnRH neurons once positioned in the hypothalamus. Accordingly, disrupted semaphorin signaling may be involved in the aetiopathogenesis of genetic diseases characterized by GnRH deficiency.

Keywords GnRH neuron, semaphorin, migration

References

L47

The role of microvascular endothelial WNT signaling the formation of the blood brain barrier

Maria Grazia Lampugnani, Luca Bravi, Elisabetta Dejana
IFOM, Italy

SpringerPlus 2015, 4(Suppl 1):L47

We analyzed the pathological consequences of abnormal Wnt/β-catenin signaling in endothelial cells of brain vessels using a murine model of Cerebral Cavernous Malformation (CCM) disease that develops after endothelial-cell-selective ablation of the CCM3 gene. We report increased transcription activity of β-catenin in CCM3-knockout endothelial cells in in-vitro and in-vivo models. Such activation is cell-autonomous, independent of Wnt-receptor stimulation, does not induce canonical Wnt/β-catenin signaling and represents an early response to CCM3 ablation that initiates the expression of EndMT makers before the onset of Tgf-β/BMP signaling which is required for the progression of the pathology, as we have previously described. We also show that the NSAIDs sulindac sulfide and sulindac sulfone, which attenuate β-catenin transcription activity, significantly reduce the number and dimension of vascular lesions in the central nervous system of mice with endothelial cell CCM3 knockout. These NSAIDs thus represent pharmacological tools for inhibition of the formation of vascular lesions, particularly with a view to patients affected by the genetic variant of CCM, who continue to develop new malformations over time.

Keywords Beta-catenin, cerebral cavernous malformation, endothelium

L48

WNT signaling mechanisms in nociception and sensitization of afferent neurons

Manuela Simonetti
Heidelberg University, Germany

SpringerPlus 2015, 4(Suppl 1):L48

Wnt signalling represents an ancient and highly versatile signalling system, which plays diverse critical roles in embryonic development. Sensory neurons of the dorsal root ganglia require Wnt signalling for initial cell fate determination as well as patterning and synapse formation over later development. Recent studies have functionally linked misregulation of Wnt signalling to cancer, bone disorders and abnormal synaptic function in adult life. We will discuss a novel, functional role of Wnt signalling pathways in sensitization of peripheral adult sensory neurons in a pathophysiological context, and the underlying molecular mechanisms. Using an interdisciplinary approach, through different technique spanning molecular, genetic, and behavioural experiment in vitro as well as in vivo pain models in mice, we show that Wnt3a is able to recruit different Wnt-pathways, to alter pain sensitivity in a modality-specific manner, acting via intracellular kinases in peripheral nerves. We found evidence for an intriguing dichotomy of non-canonical signalling pathways in mediating mechanical and thermal hypersensitivity. Indeed, while the calcium pathway is mainly involved in thermal hypersensitivity, mechanical hypersensitivity is driven by the planar cell polarity pathway. Interestingly we did not find clear functional evidence for the canonical Wnt signalling pathway in Wnt3a-induced sensitization of nociceptors. Finally, we will provides evidence for a translational potential for targeting peripheral Wnt signalling in tumour-nerve interactions and pathological pain hypersensitivity, paving the way for therapeutic interventions.

Acknowledgements This work was supported by grants from the Association of International Cancer Research and the Deutsches Forschungsgemeinschaft to R.K. R.K. is a principal investigator in the Excellence Cluster CellNetworks of Heidelberg University and M.S. was supported by the CellNetworks Postdoctoral Fellowship program

Keywords Wnt signalling, DRG, nociception

L49

WNT signaling in midbrain dopaminergic neuron development and cell replacement therapies for Parkinson's disease

Ernest Arenas, Carmen Saltò, Carlos Villaescusa
Karolinska Institute, Sweden

SpringerPlus 2015, 4(Suppl 1):L49

Parkinson’s disease (PD) is a neurodegenerative disease characterized by the loss of midbrain dopamine (mDA) neurons. Clinical trials using human embryonic midbrain tissue for transplantation have provided proof of concept that cell replacement therapy (CRT) can lead to not only symptomatic relief, but also changes in the course of disease and
withdrawal of medication. Human pluripotent stem cells are currently regarded as the main candidate cell type for CRT because they are readily available, expandable, and can be standardized and differentiated into mDA neurons capable of inducing functional recovery in animal models of PD. However, protocols for mDA differentiation are still far from optimal and require further improvement. We previously found that members of the Wnt family of morphogens regulate multiple aspects of mDA neuron development [1]. Different branches of the Wnt signaling pathway, such as Wnt/B-catenin, activated by Wnt1, and Wnt/PCP activated by Wnt5a, have been shown to regulate separate or opposing functions. However, we found that Wnt5a cooperates with Wnt1 to promote mDA neurogenesis and that Wnt1 cooperates with Wnt5a to promote the differentiation of postmitotic mDA neuroblasts [2]. We are currently applying this knowledge to improve protocols for the differentiation of human stem cells into mDA neurons suitable for transplantation and functional recovery in animal models for PD [3].

Keywords WNT, dopaminergic neurons, Parkinson’s disease

References

L50
WNT signaling in microglia and the glial microenvironment
Gunnar Schulte
Karolinska Institutet, Sweden
SpringerPlus 2015, 4(Suppl 1):L50

WNT signaling in microglia and the glial microenvironment Gunnar Schulte WNT signaling is important during embryonic development and organogenesis having specific roles in the development of the CNS such as regulation of neural tube formation, axon guidance and CNS stem cell regulation. Our work has recently established a role of WNT signaling in the regulation of the brain’s macrophages, the microglia and thus WNTs emerge as novel regulators of CNS inflammatory responses. First of all, it appeared that b-catenin levels are elevated in microglia in Alzheimer disease (AD) brains as well as microglia cells in AD mouse models. Employing in vitro studies of primary mouse microglia isolated from newborn mouse pups indicated that both WNT-3A and WNT-5A induce diverse signaling routes in microglia leading to differential proinflammatory modulation of the cells. Interestingly, the net effect of WNT stimulation on the inflammatory potential of mouse microglia is context dependent. While WNTs increase inflammatory markers when giving to microglia alone, they are able to act in an anti-inflammatory manner when microglia are activated by prestimulation with lipopolysaccharides. Our findings thereby indicate that WNTs act on microglia as a homeostatic regulator, further underlined by yet unpublished data that suggest that WNT-5A is elevated in human glioma associated with a distinct inflammatory signature of the tumor as well as a substantial invasion of microglia.

Acknowledgements Jacomijn P Dijkstra-Huis, Elisa Arthofer,Voichita D Marinescu, Sven Nelander, Mathias Uhlen, Frederik Pontén, Jan Mulder, Carina Halleskog. The study was financially supported by grants from Karolinska Institutet, the Swedish Research Council, the Swedish Cancer Society, Swedish Childhood Cancer Foundation, the Knut & Alice Wallenberg Foundation and the KI-NIH Joint PhD program in Neuroscience, Czech Science Foundation, European Regional Development Fund.

Keywords WNT, CNS inflammation, brain tumor

L51
Translational repression in the pathogenesis of FUS- and C9orf72-dependent ALS
Mauro Cozzolino1, Simonetta Rossi2, Gianluca Cestra2
1Institute of Translational Pharmacology, CNR, Rome, Italy, 2Institute of Molecular Biology and Pathology, CNR, Rome, Italy
SpringerPlus 2015, 4(Suppl 1):L51

The major focus of ALS research has recently moved to RNA control of motor neuron functions, as most of the newly identified genes, that alone account for more than half of ALS familial cases, are clearly associated to RNA regulation. These include FUS and TDP43, two DNA-RNA binding proteins with a role in the regulation of RNA transcription, splicing, transport and translation, and C9orf72, a gene that is marked by the presence, in carriers, of an highly expanded GGGGCC repeat that is believed to provide the mutant gene of an acquired, toxic feature by an RNA-dependent gain of function mechanism. Thus, RNA dyst-metabolism is likely to represent a central issue in ALS pathogenesis. Yet, whether a specific step of RNA processing is particularly affected in ALS motor neurons is unclear. We have recently obtained evidence that a prominent effect of FUS and C9orf72 expression is the induction of stress granules-associated translational repression. In this presentation I will discuss our recent work on the molecular mechanisms underlying these effects and their potential relevance in ALS pathogenesis.

Keywords ALS, RNA trafficking, stress granules

L52
Post-transcriptional modifications caused by TDP-43 mutations in mouse and man
Pietro Fratta
UCL – Institute of Neurology and National Hospital for Neurology and Neurosurgery, UK
SpringerPlus 2015, 4(Suppl 1):L52

TDP43 is a ubiquitously expressed prevalently nuclear protein involved in RNA splicing, RNA stability and mRNA processing. Amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and inclusion body myopathy (IBM) are characterized by TDP43 being depleted from the nucleus and accumulating in cytoplasmic inclusions, which are the pathological hallmark of the disease – these diseases are also defined as “TDP43 proteinopathies”. Mutations in TDP43 have been found to be causative of a proportion of ALS cases reinforcing the primary importance of this molecule in disease pathogenesis. The pathogenic mechanism by which TDP43 acts is unclear, and both loss of nuclear function (LOF) and gain of function (GOF) mechanisms have been proposed. We here discuss how we used muscle tissue, which provides high quality RNA of patient disease tissue, to investigate the consequences of TDP43 mutations expressed at physiological levels in the mammalian central nervous system. One mutation (deltaRNA) strongly reduces the RNA-binding capacity of the protein; the second mutation (C-TERM) is in the glycine-rich C-terminal domain where the majority of human pathogenic mutations are found. Our results underline the importance of studying models with physiological expression levels of TDP43 mutations and shed light on the different effects on RNA metabolism caused by the TDP43 loss and gain of function.

Acknowledgements

L53
Sm-like proteins in the pathogenesis of Spinal Muscular Atrophy
Tilmann Achsel, Robin Doms, Claudia Bagini, Fabrizio Renzi
VIB Center for the Biology of Disease at the KU Leuven, Leuven, Belgium
SpringerPlus 2015, 4(Suppl 1):L53

Proximal Spinal Muscular Atrophy (SMA) is caused by an insufficient supply of the Survival of Motor Neurons (SMN) protein. It is characterized by the selective degradation of the alpha motor neurons in the spinal cord. Analysis of the mouse model, which faithfully mimics the SMN insufficiency and the motor neuron phenotype, shows that motor neuron degeneration starts in the axons, specifically at the neuromuscular junctions. SMN is a house-keeping factor that is necessary for the assembly of the seven-membered ring of Sm proteins around the spliceosomal snRNAs and that is therefore required for pre-mRNA splicing. Such a function cannot easily explain the selective motor neuron phenotype. It has been proposed that insufficient supply of the SMN protein affects alternative splicing, especially of U11/U12-dependent introns, in mRNAs that are crucially required in the transport of mRNAs out of the nucleus.
motor neurons. Details, however, remain elusive. In addition to the Sm proteins, there are the like-Sm (LSm) proteins that also form heptameric complexes and participate in various steps of mRNA metabolism. We have previously shown that one such LSm complex is involved in the transport of mRNAs to the neurites, especially the axons of motor neurons. mRNA transport and local protein synthesis is an important mechanism to bring about sudden changes in the proteome at distal regions of the cell. Here, we further explore the role of the LSm proteins in neuronal mRNA regulation and how this could be relevant for the SMA pathology. Interestingly, we find that one such LSm protein is significantly depleted in the SMA mouse model before the onset of the disease, possibly indicating a causal involvement.

Keywords: SMA, mRNA regulation, Sm-like proteins

L54 Diverse roles of FUS in Amyotrophic Lateral Sclerosis
Maria Teresa Carri1, Francesca Bozzo2, Ilaria Salvatori1, Cristiana Vallev3
1University of Rome Tor Vergata, Rome, Italy; 2Fondazione Santa Lucia, Rome, Italy; 3Institute of Cell Biology and Neurobiology, CNR, Rome, Italy

A number of different genes have been found mutated in patients with Amyotrophic Lateral Sclerosis (ALS). Several of these genes encode proteins involved in multiple steps of RNA processing, suggesting that mRNA dys-metabolism has a role in the degeneration of motor neurons. This is the case also for FUS-linked ALS. FUS (Fused in Sarcoma) is a DNA/RNA binding proteins with an established, yet not completely clear, role in the regulation of RNA transcription, splicing, transport and translation. However, recent evidence indicates that (similarly to mutant ALS-linked SOD1) the toxic function of this protein may lie also in its propensity to aggregate and sequester other proteins, and/or in its ability to induce mitochondrial damage and oxidative stress. In this presentation I will discuss our recent work on the molecular mechanisms underlying these effects and their potential relevance in the pathogenesis of ALS.

Acknowledgements This work is supported by ARISLA (Project OligoALS to M.T.C.)

Keywords: ALS, protein aggregation, mitochondrial damage

L55 The protein quality control system in motoneuron diseases
Angelo Poletti, Paola Rusmini, Valeria Crippa, Riccardo Cristofani, Maria Elena Cicardi, Marco Meroni, Rita Galbiati, Margherita Piccolella, Elio Messi
Università degli Studi di Milano, Italy

Spinobulbar muscular atrophy (SBMA) and amyotrophic lateral sclerosis (ALS) are two related motoneuron diseases (MNDs), both characterized by the presence of inclusions or aggregates of proteinaceous materials. In SBMA, aggregates contain mutant androgen receptors (AR) with an elongated polyglutamine tract (ARpolyQ), while in ALS aggregates contain TDP43, ubiquilin, optineurin, etc. Exceptions are familial ALS (ALS) forms linked to superoxide dismutase 1 (SOD1) mutations, in which aggregates are composed of mutant SOD1. Protein aggregation occurs when a large excess of proteins with aberrant conformations (misfolding) in produced and poorly cleared from the cells. Neurons contains an efficient protein quality control (PQC) system, but this may be insufficient to correctly remove misfolded proteins, especially during aging. The PQC system requires the activities of efficient chaperones and of the two major intracellular degradative systems: the ubiquitin-proteasome (UPS) and the autophagic systems. When misfolded protein are recognized by chaperones, they can be removed via autophagy by their entrance into autophagosomes which then fuse to lysosomes. We found that motoneurons may respond to misfolded species by activating the expression of a small HSP, HSPB8, which facilitate the clearance of misfolded species via autophagy, usually acting by restoring the proper autophagic flux, found altered in MNDs. HSPB8 requires its co-chaperone BAG3. BAG3 binds the protein 14-3-3 and with this it interacts with dynen in a complex which also includes HSC70-CHIP. Dynen moves this large complex on the microtubules organization center where autophagosomes are assembled. Here, CHIP ubiquitinated misfolded protein substrates allowing their recognition by p62 and clearance from the motoneurons. Thus, together, the PQC and the HSPB8 proteins help to protect motoneurons from damages associated to the presence of aberrant protein species accumulating in affected cells.

Keywords: Motoneuron diseases, protein misfolding, autophagy

L56 Neurochemical changes in different brain regions induced by PACAP – relations to neuroprotection
Dora Reglioli1, Gabor Maasz1, Zsolt Pirger1, Adam Rivnyak1, Dorottya Baloghy1, Adel Jungling, Balazs Fulop, Laszlo Mark, Andrea Tamas2
1Department of Anatomy, MTA-PTE “Lendulet” PACAP Research Team, University of Pecs, Pecs, Hungary; 2Centre for Ecological Research, HAS, Balaton Limnological Institute, Department of Experimental Zoology, Group of Chemical Ecology and Neurobiology, Tihany, Hungary

This work is supported by OTKAK104984, Arimura Foundation, MTA-PTE “Lendulet” Program, KTIA 13. NAP-A-III/S, NAP-B.

Keywords: PACAP, neuroprotection, proteomics

L57 Contribution of the galanin system to inflammation
Barbara Koller1, Susanne Brunner1, Andreas Koller1, Silke Wiensmayer2, Felix Locker1, Roland Lang1, Balint Botz3, Agnes Remény1, Zsuzsanna Helyes1
1Laura Bassi Centre of Expertise THERAPEP, Research Program for Receptor Biochemistry and Tumor Metabolism, Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria; 2Department of Pediatrics, Paracelsus Medical University, Salzburg, Austria; 3Department of Pharmacology and Pharmacotherapy, János Szentágothai Research Center and MTA NAP B Pain Research Group University of Pécs, Pécs, Hungary.

Neurogenic inflammatory components mediated by peptidergic sensory nerves have a crucial impact on the symptoms of inflammatory diseases. Galanin is a regulatory sensory neuropeptide, which was shown to attenuate neurogenic inflammation, but our current understanding about its endogenous targets, and physiologic importance is incomplete. Among the endogenous receptors of
galanin (GAL1, GAL2, GAL3) we found GAL3 to be the most abundantly expressed on the vasculature and GAL2 on different types of immune cells including polymorphonuclear neutrophils and natural killer cells. Therefore, we evaluated if galanin exerts direct or indirect effects on these immune cells. Our data revealed that galanin can be regarded as an immunomodulatory peptide as it can sensitize polymorphonuclear neutrophils and natural killer cells towards proinflammatory cytokines. Since there are only scarce in vivo data concerning the role of GAL3 in inflammatory disease conditions, we analysed its involvement in the K/Bi EB severe transfer model of autoimmune arthritis and the oxazolone-model of allergic contact dermatitis, employing GAL3 gene-deficient mice. After arthritis induction, GAL3-knockout mice demonstrated increased clinical disease severity and earlier hindlimb edema than wildtype mice. Vascular hyperpermeability was also elevated compared to wildtypes, but neutrophil myeloperoxidase activity and arthritic hyperalgesia were not significantly different. In contrast, disease severity, vascular, and immune components were not affected in allergic contact dermatitis in GAL3 knockouts in comparison with wildtypes. Our findings suggest GAL3 activation as a substantial effect in allergic contact dermatitis and consequent edema formation. However, the involvement of GAL3 in inflammatory disease conditions, we analysed its involvement in neutrophil-dominated autoimmune arthritis, modulating the early neurogenic vascular hyperpermeability anti-inflammatory pathway in neutrophil-dominated autoimmune arthritis, modulating the early neurogenic vascular hyperpermeability and consequent edema formation. However, the involvement of GAL3 activation in the T-cell dependent allergic contact dermatitis remains unsupported.

**Keywords** Galanin, inflammation, immune cells

---

**POSTER PRESENTATIONS**

**P1**

**Characterization of ligand binding to dopamine receptors with fluorescence anisotropy based assay**

Anni Allikalt, Ago Rinken

Department of Bioorganic Chemistry, Institute of Chemistry, University of Tartu, Competence Centre on Health Technologies, Estonia

SpringerPlus 2015, 4(Suppl 1):P1

Dopamine receptors are G-protein-coupled receptors (GPCRs), which are involved in a wide variety of physiological processes. Abnormalities in dopaminergic signal transduction are associated with many different diseases. Therefore, dopamine receptors are targets for variety of drugs involved in disorders like schizophrenia, Parkinson's disease, depression and many others. In order to develop drugs with less side effects and better efficacy it is necessary to understand and characterise receptor-ligand interactions in further detail. In addition, measuring the on- and off-rates of different ligands provides important information about the kinetic profiles of potential drug candidates. We have applied fluorescence anisotropy (FA) assay to investigate kinetic properties and affinities of different ligands for dopamine D1 receptor. For that we have implemented budded baculoviruses as a source of recombinant protein (Veiksina et al., 2014). As a result, we have seen that fluorescent ligand Bodipy-FL-SKF-83566 is suitable for the pharmacological characterization of non-labelled dopaminergic ligands. The obtained results are in good agreement with the data obtained from the radioligand [3H]SCH 23390 binding experiments with the same baculovirus preparations. In conclusion, by using fluorescence based detection assay, we are now able to perform real-time monitoring of ligand binding. Obtained results demonstrate that fluorescence anisotropy based assay is applicable for the study of dopamine receptors and their ligands.

**Acknowledgements** Supported by the Medical University of Lodz, Poland (no. 4524/B/P01/2010/38).

**Keywords** Orexins, neuroprotection, oxidative stress

---

**L58**

**Characterization of neuropeptides which control cerebellar granule cell survival, migration and differentiation**

David Vaudry1, Auriane Corbière1, Magalie Basille1, Seyma Bahdoudi1, Olfa Masmoudi1, Jérôme Leprince1, Delphine Burel1, Magalie Bénard1, Ludovic Galais1, France Hitoshi Komuro4

1INSERM U982, Rouen University, France; 2Tunis University, Tunisia; 3PRIMACEN, INSERM, France; 4Yale University, New Haven, USA

SpringerPlus 2015, 4(Suppl 1):L58

During cerebellar development, granule cell precursors are produced from a secondary germinative zone forming the external granule cell layer (EGL). Immature granule neurons from the inner part of the EGL then start a tangential migration followed by a centripetal inward radial migration across the molecular and Purkinje cell layers to reach their final destination at the bottom of the forming internal granule cell layer (IGL). This complex migratory process is highly regulated and takes about 2 days in rodents and it is essential for the proper formation of the cortical layers forming the mature cerebellum. In the IGL, granule cells differentiate to establish functional excitatory synapses with GABAergic neurons including Purkinje, basket, stellate and Golgi cells, or die. Some neurotrophins and neurotransmitters have been shown to be involved in the control of cerebellar granule cell survival, migration and differentiation. Initially, when I started my carrier as a researcher, we used to claim that very few neuropeptides were produced in the cerebellum. Nevertheless, we now know that this was wrong as we have recently identified by mass spectrometry over 70 peptides expressed in the cerebellum during development. Over the years, the involvement of some of these peptides such as somatostatin, PACAP or ODN, has been established in the control of cerebellar granule cell survival, migration and differentiation as will illustrate my presentation.

**Acknowledgements** Supported by INSERM, Rouen University, Interreg PelleNE and Region Haute-Normandie.

**Keywords** Cerebellar granule neurons, neuropeptides, brain development

---

**L59**

**A new face of orexins action – neuroprotection**

Jolanta Zawilska

Department of Pharmacodynamics, Medical University of Lodz, Poland

SpringerPlus 2015, 4(Suppl 1):L59

Orexins A and B (hypocretins 1 and 2) are two closely related peptides produced mainly by hypothalamic neurons that project to numerous brain structures. Orexins exert their biological activity by binding to two subtypes of GPCR receptors, OX1R and OX2R. The orexin system has been shown to orchestrate multifaceted physiological functions, including vigilance and the sleep/wake cycle, energy homeostasis, endocrine, visceral functions and pathological states, such as narcolepsy and drug abuse [1]. In addition, a neuroprotective potential of orexin A has been recently demonstrated in rats using a model of focal cerebral ischemia [2]. In our studies we investigated effects of orexins on survival of cultured neurons from the rat cerebral cortex. Quantitative real-time PCR revealed the presence of OX1R and OX2R in cortical neurons. Orexins and [Aa11-D-Leu1]Sprexin B (a selective agonist of OX2R), used at 0.001-1 microM concentrations, markedly increased neuronal cells viability, an effect associated with an attenuation of caspase-3 activity. Comparable potency of the three peptides suggests a predominant role of OX2R in the studied phenomenon. Under conditions of chemical hypoxia, orexins potently increased neuronal viability and protected cortical neurons from oxidative stress. The pro-survival effect of orexins was blocked by U0126 and 10-DEBC, inhibitors of MEKK and Akt, respectively. In addition, orexins A and B stimulated Akt and ERK1/2 in cortical neurons in a time- and concentration-dependent manner. It is suggested that both Akt and ERK1/2 play an important role in the pro-survival effects of orexins in neurons.

**Acknowledgements** Supported by the Medical University of Lodz, Poland (no. 503/3-011-01/503-01) and the National Research Centre of Poland (no. 4254/B/P01/2010/38).

**Keywords** Orexins, neuroprotection, oxidative stress

---

**References**

**P2**

The effect of P2Y12 receptor inhibition in chronic inflammatory pain

Katinka Bekő1,2, Ádám Dénès1, Gergely Horváth1, Beáta Sperlárgh1
1Laboratory of Molecular Pharmacology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Hungary; 2Department of Physiology, School of Medicine, University of Málaga, Spain

Previous studies of our group indicate that genetic deletion and pharmacological antagonism of P2Y12 receptors alleviate mechanical allodynia in acute inflammatory pain. In this project we investigated the role of P2Y12 receptors in chronic inflammation. For this study we have introduced a CFA-model in wild type and P2Y12R gene deficient (P2y12−/−) mice. By the injection of Complete Freund’s Adjuvant (CFA) in the plantar surface of the right hind paw, we could induce local inflammation persisting at least 14 days. During this 2 week period, mechanical sensitivity was evaluated at several time points using dynamic plantar von Frey aesthesiometer. Mechanical allodynia could be observed 3 days after CFA injection. In P2Y12 receptor gene deficient mice the decrease in the paw withdrawal threshold (PWT) was significantly lower than in wild type mice. Both intrathecal and intraplantar administration of PSB-0739, a selective and potent P2Y12 receptor antagonist had robust antihyperalgesic effect in wild type mice, whereas treatment did not affect the PWT in the P2y12−/− group. Intraperitoneal injection of A-803467, a potent and selective NaV1.8 sodium channel blocker evoked antihyperalgesic effect similar to the PSB-0739. When these two compounds added together, no additive effect was observed, i.e. A-803467 occluded the effect of PSB-0739. To investigate whether P2Y12 receptors on thrombocytes contribute to this effect, we used anti-mouse CD41 antibodies to induce depletion of platelets. Mice were submitted to intraperitoneal injection of this antibody at the end of the first week and measurement of the mechanical allodynia was performed on the second week. Our findings indicate that P2Y12 receptor inhibition is a potential therapeutic approach in chronic inflammatory pain, although its exact mechanism of action needs further investigation.

**Keywords** Inflammation, pain, purinergic signaling.

**P3**

Acute stimulation of glycolytic flux in cultured primary astrocytes by the tyrosine kinase inhibitor tyrphostin 23

Eva-Maria Blumrich1, Reshma Kadam1, Ralf Dringen1,2
1Centre for Biomolecular Interactions Bremen (CBIB), University of Bremen, Germany; 2Centre for Environmental Research and Sustainable Technology, University of Bremen, Germany

Tyrophostin 23 (T23) is a well-known inhibitor of protein tyrosine kinases. To investigate potential effects of T23 on the viability and the glucose metabolism of brain cells, we exposed cultured primary rat astrocytes to T23. While the viability and the morphology of the cells were not acutely affected during an incubation of the cultures for up to 4 hours with T23 in concentrations of up to 200 μM, the presence of T23 rapidly stimulated glycolytic flux as demonstrated by a time- and concentration-dependent increase in glucose consumption and lactate release. Maximal effects were observed for incubations with 100 μM T23 which caused a doubling of glucose consumption and lactate production. The stimulation of glycolytic flux by T23 was fully reversible upon removal of the compound. In contrast to T23, the structurally related tyrosin kinase inhibitor tyrphostin 25 did not affect glycolytic flux, nor was the stimulation by T23 substantially affected by the triflucoracetate-induced activation of pyruvate dehydrogenase. Further experiments are now required to elucidate the mechanism of T23-induced stimulation of astrocytic glycolysis.

**Keywords** Tyrophostin 23, astrocytes, glycolysis.

**P4**

Increase of the FGFR1 signaling in the FGFR1-S-HT1A heteroreceptor complex in midbrain raphe 5-HT neuron systems via astrocytic receptor-receptor interaction

Daiši O Borroto-Escuela1, Manuel Narvaez2, Antonio Jiménez-Beristain1, Julia Oflijan1, Luca Pinton1, Michael Di Palma1, Alexander O Tarakanov3, Giuseppe Muddó4, Luigi F Agrati1, Natale Belluardo4 and Kjell Fuxe1
1Department of Neuroscience, Karolinska Institutet, Sweden; 2Department of Physiology, School of Medicine, University of Málaga, Spain; 3Russian Academy of Sciences, St. Petersburg Institute for Informatics and Automation, Russian Federation; 4Department of Experimental Biomedicine and Clinical Neurosciences, Laboratory of Molecular Neurobiology, University of Palermo, Italy

The ascending midbrain 5-HT neurons known to contain 5-HT1A autoreceptors may be dysregulated in depression due to a reduced trophic support. New findings show existence of FGFR1-S-HT1A heteroreceptor complexes in the rat hippocampus with a partial characterization of their interface and in midbrain raphe 5-HT nerve cells. With in situ Proximity Ligation Assay (PLA) and supported by co-location of the FGFR1 and S-HT1A immunoreactivities in midbrain raphe 5-HT cells, evidence is provided for the presence of FGFR1-S-HT1A heteroreceptor complexes that were obtained in the dorsal and median raphe nuclei of the Sprague–Dawley rat. Their existence in the rat medullary raphe RN33B cell cultures was also established. After combined FGF-2 and 8-OH-DPAT treatment, a marked and significant increase in PLA positive clusters was found in the RN33B cells. Synergistic receptor-receptor interactions in these heteroreceptor complexes indicated their enhancing role in hippocampal plasticity. The existence of FGFR1-S-HT1A heteroreceptor complexes also in midbrain raphe 5-HT nerve cells open up the possibility that antidepressant drugs by increasing extracellular 5-HT levels, can cause an activation of the FGF-2/FGFR1 mechanism in these nerve cells as well. Therefore, the agonist modulation of the FGFR1-S-HT1A heteroreceptor complexes and their specific role is now determined in rat medullary raphe RN33B cells and in the caudal midline raphe area of the midbrain rich in 5-HT nerve cells. The combined icv treatment with FGF-2 and the 5-HT1A agonist 8-OHDPAT synergistically increased FGFR1 and ERK1/2 phosphorylation in the raphe midline area of the midbrain and in the RN33B cells. Cotreatment with FGF2 and the 5-HT1A agonist induced RN33B cell differentiation as seen from development of the increased number and length of extensions per cell and their increased 5-HT immunoreactivity. These signaling and differentiation events were dependent on the receptor interface since they were blocked by incubation with TMV but not by TMI of the 5-HT1A receptor. Taken together, the 5-HT1A autoreceptors by being part of a FGFR1-S-HT1A heteroreceptor complex in the midbrain raphe 5-HT neuron systems besides playing a key role in reducing the firing of these neurons.

**Keywords** Fibroblast growth factor receptor, serotonin 5-HT1A receptor, heteroreceptor complexes.

**P5**

Copper oxide nanoparticles: Synthesis, toxic potential and modulation of astrocytic metabolism

Felix Bulcke1, Ralf Dringen1, Karsten Thiel1
1Centre for Biomolecular Interactions Bremen (CBIB), University of Bremen, Germany; 2Fraunhofer Institute for Manufacturing Technology and Advanced Materials, Germany

To test for potential consequences of an exposure of brain cells to copper oxide nanoparticles (CuO-NPs), we have synthesized dimercaptosuccinic acid-coated CuO-NPs. These particles had a diameter of around 5 nm as determined by transmission electron microscopy but were dispersed as aggregate as demonstrated by their average hydrodynamic diameter in aqueous dispersion of 136 ± 4 nm. Exposure of cultured primary astrocytes to CuO-NPs increased the cellular copper levels and compromised the cell viability in a time- and concentration-dependent manner. CuO-NPs in concentrations above 100 μM (6.3 μg
### P6

**Alteration of the protein quality control system in motor neuron and muscle expressing mutant proteins causing ALS and SBMA**

Maria Elena Cicardi, Valenìa Crippa, Riccardo Cristofani, Paola Rusmini, Angelo Poletti

Università Degli Studi di Milano, Italy

**SpringerPlus 2015, 4(Suppl 1):P6**

Motor neuronal system and muscle tissue are two districts differently affected at onset and during the progression of diseases like amyotrophic lateral sclerosis (ALS) or spinal and bulbar muscular atrophy (SBMA). The bases of these diseases are linked to mutated proteins: fALS is commonly caused by mutations in the SOD1 gene and the TDP43 genes and SBMA is caused by a of CAG repeat in the AR gene. A fraction of these proteins cannot reach a mature conformation and misfold. The Protein Quality Control (PQC) system is responsible for the correct protein homeostasis: the chaperones maintain proteins in their correct conformations. If they fail, mutated proteins are directed to the proteasome or the macroautophagy. When misfolded proteins are not correctly removed, they aggregate in nucleus and cytoplasm. To understand cellular behavior in presence of misfolded toxic proteins we investigate the different activation of the PQC system in the two major tissues involved. Initially we investigated the differences in PQC activation between NSC34 motor neuronal and C2C12 muscular cell models. Using RTq PCR, western blot and immunocytochemical analysis for p62 and LC3 expression, localization, and turnover we demonstrated that C2C12 cells have a more active autophagic system than NSC34 cells. Then, we compared the two models in presence of misfolded protein inhibiting degradative systems. With Filter Retardation Assay, we observed that these proteins tend to aggregate when PQC system is impaired. Then, we potentiates the PQC response to reduce the insoluble species. By overexpressing the small heat shock protein B8 in both systems we demonstrated that AR polyQ and SODG93A insoluble species were reduced. Also autophagy activation by trehalose caused a reduction in protein aggregation in both cell models. In conclusion misfolded protein aggregates can be reduced by modulating macroautophagy and this could represent a new therapeutical strategy for disease like SBMA and ALS.

**Keywords** Neurodegeneration, misfolded proteins, PQC system

### P7

**Neuroprotection by Nerve Growth Factor (NGF) involves modulation of neuronal autophagy**

Anna Maria Colangelo1, Michele Papa2, Miluscia Berbenni1, Marco Pizzocri1, Vita M Ippolito1, Valentina Riganti, Lilia Alberghina

Lab. of Neuroscience “R. Levi-Montalcini”, Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Italy; 1Lab. of Morphology of Neuronal Network, Dept. of Public Medicine, Second University of Napoli, Italy

**SpringerPlus 2015, 4(Suppl 1):P7**

Nerve Growth Factor (NGF) plays a key role in development and function of specific neuronal populations of the CNS. Decreased NGF availability is also responsible for neuronal vulnerability in neurodegenerative disorders, such as Alzheimer’s disease (AD). We used PC12 cells and primary neurons to investigate the potential role of NGF in modulating neuronal autophagy, a cellular process whose deregulation has been linked to the loss of neuronal. The mammalian target of rapamycin has been recently proposed as a therapeutic target for AD due to the role of the autophagy pathway in improving cognitive function by reducing Aβ and Tau pathology. We here show that NGF treatment induces LC3 conversion (LC3-I to LC3-II), a marker of autophagy, through the activation of AMP-activated protein kinase (AMPK). We show that NGF increases the autophagic flux (the dynamic process of autophagosome synthesis, delivery to the lysosome and degradation) as indicated by western blot and fluorescence microscopy analyses in the presence of autophagy inhibitors. These data were confirmed by RT-PCR array analysis and RNA interference-mediated knockdown of autophagy-related genes: Atg9b, Atg12 and AMBRA1, a positive regulator of the BECLIN 1-dependent program of autophagy. In addition, flow cytometry analysis by DCF-DA showed that treatment with autophagy inhibitors determined a strong increase of reactive oxygen species (ROS) followed by decreased cell survival. These changes were fully reversed by NGF treatment, suggesting its potential role in clearing dysfunctional mitochondria. This hypothesis was further confirmed by fluorescence microscopy studies using LC3 and Cox-IV antibodies, showing co-localization of autophagosomes and mitochondria. Overall these data identify a novel aspect of the neuroprotective function of NGF in promoting the clearance of dysfunctional mitochondria (or mitophagy), thus further supporting its therapeutic potential in neurodegenerative pathologies.

**Keywords** NGF, Alzheimer disease, autophagy

### P8

**Brain extracellular levels of dimethylarginines (ADMA, SDMA) and cGMP and their modulation by exogenous S-adenosylmethionine in rats with acute liver failure**

Anna Czarnecka, Jan Albrecht, Magdalena Ziełińska

Department of Neurotoxicology, Mossakowski Medical Research Centre, Polish Academy of Sciences, Poland

**SpringerPlus 2015, 4(Suppl 1):P8**

Acute liver failure (ALF) instantly evokes symptoms of hepatic encephalopathy (HE), mainly attributed to hyperammonemia. Ammonia neurotoxicity is related to disturbances in the nitric oxide (NO)/cGMP pathway. The methylated derivative of L-arginine (MDALs) – asymmetric dimethylarginine (ADMA) but not symmetrically methylated arginine (SDMA) is an endogenous inhibitor of nitric oxide synthase. Elevated blood and brain ADMA was reported in HE patients and experimental animals. The question arose whether ALF induces changes in the incorporation of the substrate into S-adenosylmethionine (SAM) to MDALs, and how they affect cGMP accumulation. To this end, we investigated the effect of intracortical perfusion of SAM on the brain extracellular levels of ADMA, SDMA and cGMP in rats with ALF in the thioacetamide (TAA) model. Sprague Dawley rats (250–270 g) received three i.p. injections of TAA (300 mg/kg) at 24 h intervals. Bilateral microdialysis of the prefrontal cortex was carried out 24 h after the last TAA administration. SAM at 2 mM concentration in artificial cerebrospinal fluid was infused for 40 min and then the medium was changed back. The extracellular levels of ADMA and SDMA were analyzed using positive mode electrospray LC–DMS–MS/MS and cGMP was determined with cGMP enzyme immunoassay. ALF resulted in the increased extracellular levels of ADMA (by ~800%) and SDMA (by ~250%). Moreover, in ALF rats infusion of SAM decreased the ADMA and SDMA (~30%) as compared to the basal value. It seems reasonable that an excessive amount of substrate inhibited the enzymes synthesizing MDALs. On the other hand, the cGMP level did not differ between control and TAA rats. SAM increased by ~90% cGMP only in the control group, what indicates affected NO/cGMP pathway in TAA model. The study demonstrates, that ALF modulates methylation of arginine to MDALs in a manner affecting cGMP accumulation.

**Acknowledgements** This study was supported by Grant N 2013/09/B/ N24/00536 from the National Science Centre.

**Keywords** Asymmetric dimethylarginine (ADMA), S-adenosylmethionine (SAM), hepatic encephalopathy
P9
Exogenous carbon monoxide improves neuronal differentiation: a near-death experience
Ana S Almeida, Melissa Vieira, Diogo Fortunato, Helena LA Vieira
CEDOC – Chronic Diseases Research Center, Portugal
Cerebral ischemic injuries and neurodegenerative disorders lead to death or impairment of neurons in the central nervous system. Application of stem cell based therapies, namely stimulation of endogenous neurogenesis or cell transplantation, are promising strategies and currently under investigation. Carbon monoxide (CO) is an endogenous product of heme degradation by heme oxygenase. Although there is no published data reporting CO as a factor involved in stem cell differentiation, several evidences support this hypothesis. This gasotransmitter induces mitochondrial biogenesis, which is also broadly described to be involved in metabolic shifts during neuronal differentiation process. Likewise, CO-induced pathways can occur via generation of small amounts of ROS, which are also important signaling molecules in neuronal differentiation. The CO effect on modulation of neuronal differentiation is assessed in three different models with increasing complexity: human neuroblastome SH-SYSY cell line, human teratocarcinome NT2 cell line and hippocampal organotypics slice cultures (HOSC). CO does increase the final yield of post-mitotic neurons. During neuronal differentiation, CO promotes an increase on precursor cell proliferation and in parallel CO inhibits cell death. Furthermore, cell mitochondrial population is increased by CORM-A1 supplementation. Further work is needed for assessing the mechanisms underlying CO effects in neuronal differentiation, namely by targeting modulation of cellular metabolic pathways, redox alterations and autophagy related pathways. In conclusion, CO appears as a promising therapeutic molecule to stimulate endogenous neurogenesis or to improve in vitro neuronal production for cell therapy strategies.

Keywords Carbon monoxide, neuronal differentiation, neuroregeneration

P10
sRAGE plasma level in association with multiple sclerosis risk in Slovak population
Dusan Dobrota1, Daniel Cierny1, Jozef Michalik2, Egon Kurca2, Jan Lehotsky1
1Department of Medical Biochemistry, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Slovakia; 2Clinic of Neurology, University Hospital and Jessenius Faculty of Medicine in Martin, Slovakia
SpringerPlus 2015, 4(Suppl 1):P10
Receptor for advanced glycation end products (RAGE) triggers an intracellular signalling pathways of various proinflammatory ligands. Soluble form of RAGE (sRAGE) in human plasma can hinder the function of RAGE and can have the role in the pathogenesis of inflammatory diseases. In patients with multiple sclerosis (MS), altered level of sRAGE in plasma and cerebrospinal fluid has been found. In our study, we tried to identify possible association of sRAGE serum level with MS. Serum levels of sRAGE were detected by ELISA in 44 MS patients (22 patients with rapid progression of MS and 22 patients with slow progression of MS according to the MSSS score) and 32 healthy control subjects. We found significantly increased level of sRAGE in serum of MS patients when compared to controls. No significant differences in serum level of sRAGE where found between rapidly progressing and slow progressing subgroup of MS patients. Our results suggest for the role of sRAGE in MS ethiopathogenesis, but we did not find any association of sRAGE in serum with the rate of MS disability progression.

Acknowledgements This work was supported by the grant MZ SR No2012/30-UKMA-7 Biological and molecular markers of MS and by the grant MZ SR No2012/31-UKMA-8

Keywords Multiple sclerosis, sRAGE, ELISA

P11
Characteristics of adenine nucleotides content in acute hypoxia by mathematical analysis methods
Elena Erlykina1, Albina Moshkova1
1Nizhny Novgorod State Medical Academy, Russian Federation; 2Nizhny Novgorod State Technical University n.a. R.E. Alekseev, Russian Federation
Oxygen starvation is observed in a variety of pathological states and serves as one of the urgent problems in medicine. A decrease in oxygen supply to tissues is accompanied by the inhibition of metabolic processes (primarily of energy metabolism), which impairs functional activity of the brain. The main source of energy for brain is adenosine triphosphate (ATP). It was shown that the components of adenylate pool can be used as early predictors of hypoxia.

Aim of the study The aim of our study was analysis of adenine nucleotide stock indicators for the assessment and prognostication of brain energy state in extreme conditions were used.

Results and area of their application The use of empirical dependencies methods allowed to create multiregression models, subtly enough to unite experimental indicators ATP and AMP in hypobaric hypoxia and ischemia with different-term exposure. Obtained models can be used for prognostication of ATP and AMP concentrations in disturbances of the oxygen regime in a short or over a long period of time, as well as to receive information of indicator K changing depending on brain hypoxia.

Conclusions Functional dependencies are presented in this study to analyze shape, closeness and stability of relations between adenine nucleotides characterizing coupling of production and utilization energy processes, and also to predict the direction of these processes under hypoxic conditions.

Keywords Adenylate pool, hypoxia, multiregression model

P12
Extracellular acidification leads to mitochondrial depolarization with following free radical formation in rat brain synaptosomes
Sergei Fedorovich, Tatjana Pekun, Svitlana Hrynevich, Tatjana Waseem
1Nizhny Novgorod State Medical Academy, Russian Federation; 2Nizhny Novgorod State Technical University n.a. R.E. Alekseev, Russian Federation
Brain ischemia is accompanied by lowering of pHo and pHi. We investigated an influence of of acidosis on free radical formation in synaptosomes. Three models were used: 1) Strong extracellular acidification down to pHo 6.0. 2) Moderate extracellular acidification down to pHo 7.0. 3) Intracellular acidification induced by addition of 1 mM amiloride corresponding to pHi decrease down to 6.65. We have shown that both types of extracellular acidification, but not intracellular acidification, increase DCFDA fluorescence by calcium-independent pathway on high pHo and by decrease of pHo. Moderate extracellular acidification down to pHo 7.0 was almost completely eliminated by CCCP. Furthermore, using fluorescent dyes JC-1 and rhodamine-123, we confirmed that decrease of pHo leads to mitochondria depolarization. Low pHi was not effective. Iron chelator deferoxamine and antioxidant ionol are inhibits pH-induced increase of DCFDA fluorescence, but does not influence mitochondria depolarization. We are failed to found sodium influx monitored by fluorescent dye Sodium Green.
in case of low pHo. Involving of plasma membrane receptor which is distinct from acid-sensitive ion channels (ASIC) and electron transport chain of mitochondria for moderate acidification can be suggested. Action of strong acidification seems to be mediated by release of iron from proteins. We have shown that low pHo led to oxidative stress in neuronal presynaptic endings that might underlie the long term irreversible changing in synaptic transmission.

Acknowledgements This work was supported by Belorussian Republican Foundation of Basic Investigation (grant B13-066).

Keywords Synaptosomes, ischemia, free radicals

P13
Oxidation of Methionine-35 in Alzheimer’s amyloid-beta peptide and the aggregation of the oxidized peptide
Merlin Friedemann, Eneken Heik, Ann Timan, Kairnt Zovo, Peep Palumaa, Vello Töigü
Tallinn University of Technology, Estonia

Alzheimer’s disease (AD) is a neurodegenerative disease characterized by progressive loss of brain tissue and accumulation of amyloid-β (Aβ) and tau. Aggregation of Aβ peptides into amyloid plaques is considered to be a causative factor in AD, however the precise mechanism behind the AD onset has remained elusive. Oxidative stress (OS) is also characteristic to AD, but it is not known whether OS is a risk factor or a consequence of AD. It is assumed that Aβ aggregates generate free radicals in the presence of copper ions by participation of the Met35 residue, which can increase the OS levels. The aim of our study was to establish the role of Met35 residue in the oxidation of Aβ and peptide aggregation processes. Oxidation of Aβ was studied in the presence of two redox-active compounds: H2O2 and copper ions. In the absence of copper ions the Met35 residue was readily oxidized by H2O2 in a two electron process. The fibrilization of Aβ with Met35 oxidized to sulfone was threefold slower compared to that of the native peptide. TEM analysis showed that the fibrils of native and oxidized peptides are similar. The relatively small inhibitory effect of Met35 oxidation on the fibrilization suggests that the possible variation in the Met oxidation state should not affect the in vivo plaque formation. In the presence of copper ions (one-electron process) the oxidation was more complex: addition of the first oxygen was still the fastest process, however, it was accompanied by multiple unspecific modifications of several amino acid residues. Addition of copper ions to the already oxidized Aβ Met35 by H2O2, resulted in a similar pattern of nonspecific modifications, suggesting that the one-electron oxidation processes in Aβ do not depend on the oxidation state of Met35. Thus, it can be concluded that Met35 residue is not a part of the radical generating mechanism of Aβ-Cu(I) complex.

Keywords β-Amyloid, copper(I)ion, methionine oxidation

P14
The effects of optical, electrical and chemical stimulation on serotonin release from median raphe and hippocampus of mice
Flora Goloncser1,*, Romero Ando1, Dora Zelena1, Jozsef Haller1, Beata Sperlagh1
1Institute of Experimental Medicine of the Hungarian Academy of Science, Hungary; 2Semmelweis University School of PhD Studies, Hungary

The present study has examined several characteristics of the release of [(3H)5-HT from the median raphe nucleus (MN) and hippocampus in terms of its dependence of nerve impulse. We used electrical stimulation and the sodium channel opener veratridine, which excite all of the neuronal processes in the stimulation field, and optogenetics to selectively stimulate those terminals which express channelrhodopsin-2 (ChR2) and compared 5-HT release evoked by electrical and chemical depolarization and by light. We injected an adeno-associated virus containing DNA construct encoding ChR2 into the MN of mice and investigated [(3H)5-HT release from MN and hippocampal slices. Serotogenic nerve terminals was locally stimulated with 473 nm light (blue laser diode) and electrically by bipolar electrode and by veratridine and transmitter release was monitored by collecting the effluent in a fraction collector. Electrical filed stimulation and veratridine resulted in a significantly increase in the efflux of 5-HT, whereas optical stimulation of ChR2 expressing nerve terminals at various frequencies (10, 20, 50, 100 Hz) elicited only a negligible increase in 5-HT release either from the hippocampus or from the MN itself. The electrically induced release of radioactive neurotransmitter was completely inhibited by perfusion with tetrodotoxin. We have also applied the 5-HT transporter inhibitor, fluoxetine and the GABA blocker bicuculline to relieve released 5-HT from re-uptake and any endogenous inhibition. Nevertheless, the effect of optical stimulation remained closed to the detection limit under these condition. In conclusion, whereas our method is suitable to detect [(3H)5-HT efflux in response to ongoing neuronal sodium channel activity its sensitivity is too low to detect transmitter efflux evoked by focal optogenetic stimulation. The most likely reason for the failure of detection of 5-HT efflux is that ChR2 is expressed only by a small subpopulation of nerve terminals.

Keywords Channelsrhodopsin-2, median raphe nucleus, serotonin

P15
Identification of the antigen recognized by rHlgM22, a remyelination-promoting human monoclonal antibody
Sara Grassi1, Simona Proni1, Yana Zorina2, Livia Cabitta1, Sandro Sonnino1, Alessandro Pinetti1
1Department of Medical Biotechnology and Translational Medicine, University of Milano, Italy; 2Acorda Therapeutics, Inc., Ardsley, USA
SpringerPlus 2015, 4(Suppl 1):P15

Recombinant human IgM22 (rHlgM22) binds to myelin and to oligodendrocytes, and promotes remyelination in a mouse model of multiple sclerosis [1]. rHlgM22 preferentially reacts with sulfatide-positive (O4-positive) oligodendrocytes [1]. Moreover, binding of rHlgM22 is abolished in CNS tissue slices from Cst(+/−) mice [2], suggesting that its binding to myelin requires the presence of a product of cerebroside sulfotransferase, possibly sulfatide, abundantly expressed in oligodendrocytes and in myelin. However the exact identity of the antigen recognized by this antibody remains to be elucidated. We have tested the binding of rHlgM22 to purified lipids and to lipid extracts prepared from mouse brain, brain myelin, mixed glial cultures, and O4-positive oligodendrocytes using TLC immunostaining and ELISA using liposomes and lipid monolayers with different composition. Our preliminary results show that rHlgM22 binds to sulfatide in vitro, while it does not bind to other myelin sphingolipids, including galactosylceramide and sphingomyelin, suggesting that sulfatide at the oligodendrocyte surface might be important for the binding of rHlgM22 to the surface of these cells and to myelin. However, IgM22 does not bind structures expressing sulfatide outside the nervous system, thus additional factors are likely relevant for the immunoreactivity of IgM22 in CNS. Indeed, we have observed in lipid extracts from different sources another lipid molecule selectively recognized by rHlgM22, whose identity is still under investigation. Remarkably, this lipid is also present in the extracts from mixed glial cultures, which do not contain mature O4-positive oligodendrocytes, suggesting that other glial cells in addition to oligodendrocytes might be important in the response to rHlgM22.

Acknowledgements This work was supported by Acorda Therapeutics.

Keywords Multiple sclerosis, remyelination, sulfatide

References

P16
Investigation of P2X7R involvement in maternal poly(I:C) exposure evoked autistic features in mice
Gergely Horváth, Lilla Ottokocs, Agnes Kettel, Mária Baranyi, Beáta Sperlágh
Hungarian Academy of Sciences Institute of Experimental Medicine, Hungary
SpringerPlus 2015, 4(Suppl 1):P16

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition with core symptoms of unusual social interaction and communication
Alzheimer’s disease (AD) is a neurodegenerative disorder. The pathohistological features in AD are intracellular accumulation of neurofibrillary tangles and extracellular senile plaques. Plaque deposition leads to recruitment and activation of microglial cells, which induces neuroinflammation and drives neurodegeneration. Recent evidence show that soluble pre-fibrillar Aβ species, rather than insoluble fibrils, are highly neurotoxic and correlate with disease severity. Hence, preventing formation of soluble Aβ and its interaction with neurons is a major goal in AD. Despite massive efforts, how extracellular factors regulate assembly of Aβ peptide and neurotoxic activity of Aβ species is still largely undefined. Recent studies indicate that Extracellular Vesicles (EVs), including exosomes and PM-derived microvesicles (MVs), may influence Aβ neurotoxicity. Our findings reveal that production of microglial MVs (m-MVs) is strikingly high in patients with mild cognitive impairment and as a result of glia with Aβ-ergic neurons of the hypothalamus is a focus of attention. It was demonstrated earlier that this could result from the decreased activity of monoaminergic and peptidergic systems that control the GnRH preovulatory secretion surge initiation, specifically from the loss of the signal coming from the suprachiasmatic nuclei (SCN) of the hypothalamus. This signal is critical for the emerging of the GnRH regular cyclic secretion, which is mediated prominently by vasoactive intestinal peptide (VIP). We have studied age-related changes in the biogenic amines and VIP content in the hypothalamic structures responsible for the GnRH synthesis and secretion. It has been shown by us that the GhRH level in the median eminence with the arcuate nuclei (ME-Arc) of the hypothalamus of 22-month-old rats is half as high compared to that of 7–8-month-old animals. Beside that, the VIP level in the SCN tended to decrease, with the norepinephrine, dopamine, and 5-hydroxyindoleacetic acid levels decreased significantly in the median preoptic area of the hypothalamus responsible for the GnRH synthesis and in the ME-Arc exercising its secretion into the portal vein of the pituitary. It has been shown that the initial phase of the reproductive failure with 13–14-month-old animals having irregular estrous cycles is characterized by gradual disappearance of the normal biogenic amine diurnal dynamics in the studied hypothalamic structures, which could be due to the loss of regulatory signals coming from the SCN.

Keywords 
GnRH, biogenic amines, hypothalamus

P18

Effect of extracellular vesicles derived from distinct brain cells on Aβ toxicity and assembly: focus on microglia derived vesicles

Pooja Joshi1, Elena Turola1, Ana Ruiz, Alessandra Bergami1, Dacia Libera1, Luisa Benussi1, Clemens Falk1, Paola Giussani2, Giuseppe Magnani1, Giancarlo Comi1, San Raffaele Scientific Institute, Italy; 2Department of Biotechnology and Translational Medicine, University of Milano, Italy; 3INSPE, Division of Neuroscience, San Raffaele Scientific Institute, Italy; 4Proteomics Unit IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Italy; 5Institute of Neuropathology, University Medical Center, Germany; 6Scientific Institute, Italy; 7SISSA, Department of Neuroscience, Italy; 8IRCCS Humanitas, Italy

To verify if the overall effect of exosomes and MVs on Aβ neurotoxicity may vary depending on parental cell type we are currently studying the influence of EVs (exosomes & MVs) derived from distinct brain cells on Aβ toxicity and as a result of glia with Aβ-ergic neurons of the hypothalamus.

Keywords 
Extracellular vesicles (EVs), Alzheimer’s disease (AD), neurotoxicity

P19

Toxicity of amyloid beta 1-40 and 1-42 on SH-SY5Y cell line

Jekaterina Krishtal, Olga Bragina, Kristel Metsla, Peep Palumaa, Vello Töugu Tallinn University of Technology, Estonia

To verify if the overall effect of exosomes and MVs on Aβ neurotoxicity may vary depending on parental cell type we are currently studying the influence of EVs (exosomes & MVs) derived from distinct brain cells on Aβ toxicity and as a result of glia with Aβ-ergic neurons of the hypothalamus.

Keywords 
Extracellular vesicles (EVs), Alzheimer’s disease (AD), neurotoxicity

Objectives
Amyloid beta plaques are primary hallmark of Alzheimer’s disease, which is characterized by specific neurodegeneration. Amyloid beta peptide – the main plaque component- was shown to be neurotoxic in animal models, primary neuronal cultures and immortalized cell lines. However, the results are often controversial and there is no good human cell line model for evaluation of the toxicity of amyloid peptides. Here we studied the effect of amyloid beta 1-40 and 1-42 on undifferentiated and differentiated human neuroblastoma cell line SH-SY5Y.

Results
Undifferentiated cell culture was too diverse and unstable to reveal a toxic effect of amyloid beta peptides quantitatively. Differentiated cells established more neuron-like phenotype and were more identical and stable in culture suggesting potential susceptibility to amyloid beta as a neurotoxic agent. Amyloid beta peptides are prone to form different aggregates with diverse toxic properties, in current study, monomeric amyloid beta 1-40 and 1-42 were applied to the cells. Viability test WST-1 and propidium iodide (PI) uptake tests showed that undifferentiated cells are not susceptible to amyloid beta, however, differentiated cells showed reduced viability and increased PI uptake in case of amyloid beta 1-42, but not in case of amyloid beta 1-40.
Conclusions: Current study revealed that amloid beta has no remarkably toxic effect on undifferentiated SH-SYSY cell line whereas viability of the neuron-like differentiated cell culture is significantly decreased by the amyloid beta 1-42 peptide that is known to form spontaneously toxic aggregates.

Keywords: Amyloid beta, toxicity, SH-SYSY

P20 Arsenic induced oxidative stress and mitochondrial dysfunction in rat brain
Vijay Kumar, Chandra Prakash
Maharshi Dayanand University, India

The present study was undertaken to reveal the effects of chronic arsenic exposure (25 ppm intragastrically for 12 weeks) on mitochondrial functions and oxidative stress in male Wister rats. Chronic arsenic exposure resulted in decrease in the activities of the mitochondrial complexes. There was increased generation of ROS followed by decrease in MnSOD activity. The generation of oxidative stress was associated with increased protein oxidation and lipid peroxidation in rat brain as evident by FTIR spectra. The RT-PCR analysis of NRF 1, NRF 2 and PGC 1a revealed decrease in gene expression suggesting decreased biogenesis following chronic exposure in rat brain. Thus, the findings of the present study reveal that arsenic induced decrease in mitochondrial biogenesis may be responsible for the decreased metabolic response that may be further involved in the generation of oxidative stress and neurodegeneration in rat brain.

Keywords: Arsenic, oxidative stress, mitochondria

P21 Neuroprotection exerted by ischemic preconditioning in rat hippocampus involves extracellular signal receptor change
Jan Lehotsky, Maria Kovaliska, Barbara Tothova, Zuzana Tatarkova, Peter Kaplan
Comenius University, Jessenius Faculty of Medicine in Martin, Slovakia
SpringerPlus 2015, 4(Suppl 1):P21

Ischemic-reperfusion injury induced by four vessel occlusion affects vulnerable hippocampal CA1 (cornus ammonis 1) pyramidal neurons. Ischemic tolerance evoked by preconditioning (IPC) represents a phenomenon of CNS adaptation to any subsequent ischemia. We refer here for the changes in the external signal receptor protein kinase pathways of the hippocampal area following by IPC. Ischemia was induced by a 4-vessels occlusion (4VO) and the rats were preconditioned by a non-injurious ischemia. Apoptotic markers were used to follow the degeneration process. Western blot and immunohistochemistry revealed phosphorylated extracellular signal-regulated protein kinase and p38 proteins in injured hippocampal areas. P-ERK quantification increased after IPC and reached the highest level at 24 h after ischemia. Interestingly, neuroprotection induced by IPC lead to the opposite effect on MAPK/p38, where the level was lowest at 24 h after ischemia. The study clearly shows that phosphorylated form takes part in complex cascades triggered by IPC in the selectively vulnerable hippocampal region. In addition, study reveals an interplay between p-ERK and p-p38 which participates in the tolerance mechanisms induced by preconditioning.

Acknowledgements: Study was supported by VEGA 213/12, MZ SR 2012/30-UKMA: 7

Keywords: Ischemic/reperfusion injury, preconditioning, ERK, rat

P22 Wolfram syndrome 1: from ER stress to impaired mitochondrial dynamics and neuronal development
Mailis Liiv, Michal Cagalinicz, Zuzana Hodurova, Annika Vaarmann, Merle Mandel, Akbar Zeb, Malin Kuum, Miriam Ann Hickey, Dzhamila Safullina, Vinay Choubey, Eero Vasar, Vladimir Vekler, Allen Kaasik
Department of Pharmacology, Centre of Excellence for Translational Medicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia
SpringerPlus 2015, 4(Suppl 1):P22

Wolfram syndrome 1 (WFS1) is a genetic disorder which has been associated both with impaired early brain development and neurodegeneration. Recent studies have suggested regulation of Ca2+ homeostasis by wolframin (WFS1) and have demonstrated the involvement of endoplasmatic reticulum (ER) stress in WFS1 deficiency. Despite the ER dysfunction WFS1 shows several characteristics of pathologies related to mitochondrial dynamics. Therefore our aim was to examine the hypothesis that WFS1 deficiency could disturb mitochondrial dynamics contributing to impaired neuronal functioning. First we show that WFS1 deficiency induces mild ER stress leading to Inositol 1,4,5-Trisphosphate Receptor (IP3R) dysfunction and disturbed cytosolic Ca2+ homeostasis, which, in turn alters mitochondrial trafficking, inhibits mitochondrial fusion and augments mitophagy. The overexpression of the active IP3R fragment restores IP3R-mediated Ca2+ release and corrects all perturbations in mitochondrial dynamics suggesting that these events are causally linked. We further demonstrate that suppressing the expression of two Parkinson disease-related proteins, Pink1 and Parkin, leads to reduced WFS1 deficiency-induced mitophagy and also to the correction of the fusion-fission dynamics and mitochondrial motility. These data suggest that WFS1 deficiency may over-activate Pink1 and Parkin pathways. Our most important discovery is that WFS1 deficiency delays neuronal survival and axonal growth in primary rat cortical neurons. According to our data, the link between WFS1 deficiency and delayed neuronal development appears to be mediated by impaired mitochondrial dynamics because suppression of the Parkin- Parkin pathway corrected also the developmental delay. Our data shed light on the mechanisms of neuronal abnormalities in WFS1 and point out potential therapeutic targets. This work may have broader implications for understanding the role of mitochondrial dynamics in neuropsychiatric diseases.

Keywords: Mitochondrial dynamics, ER stress, neuronal development

P23 Novel peptides for kinetic studies of ligand binding to melanocortin-4 receptors using fluorescence anisotropy
Reet Link, Sergei Kopanchuk, Ago Rinken
1 Institute of Chemistry, University of Tartu, Estonia, 2 Competence Centre on Reproductive Medicine and Biology, Estonia

Melanocortin receptors (MCRs) are seven transmembrane G protein-coupled receptors that are known for their broad physiological relevance. The subtype 4 melanocortin receptor (MC4R) has emerged as a central element in the regulation of energy homeostasis, eating behavior and regulation of sexual functions. MCRs are governed by a complex dynamic homotropic regulation [1]. There is an increasing trend towards using fluorescence anisotropy (FA) for studying the aforementioned complex receptor-ligand interactions. FA allows the characterization of ligand binding dynamics [2]. The quality of the FA assay can be greatly increased with budded baculovirus particles that display the receptors of interest on their surfaces [3]. The use of a fluorescent ligand Cy3B-NDP-α-MSH has made it possible to study MC4Rs with higher precision and sampling rate [3]. However, this ligand has relatively slow kinetics. Modification of the structure of a MC4R antagonist revealed two new reporter ligands (UBTC101 and UBTC102) for fluorescence labeling. These new reporter ligands selectively bind to MC4Rs and exhibit improved kinetic properties. The association and dissociation rate constants of UBTC101 and UBTC102 are kon = ((2.0 ± 0.6) × 107 M–1 min–1), koff  = ((4.6 ± 0.3) × 10 –3 min–1) and kon = ((1.9 ± 0.5) × 107 M–1 min–1), koff  = ((1.0 ± 0.2) × 10 –1 min–1), accordingly. UBTC101 and UBTC102 enable the characterization of both labelled and non-labelled ligand binding dynamics in regard to the MC4R. UBTC102 could be especially valuable for ligand screening, because of its very high dissociation rate, which makes it possible to achieve equilibrium conditions.

Acknowledgements: This work was financed by the Estonian Research Council (IJ20-17) and by the European Union (TK114, 30020).

Keywords: Melanocortin-4 receptor, fluorescence anisotropy, kinetic studies
Mitochondrial biogenesis is rate limiting-factor for axonal growth

Merle Mandel, Annika Vaarman, Akbar Zeb, Przemyslaw Warsi, Joanna Liiv, Malle Kuum, Eva Antsov, Mailis Liiv, Vinay Choubey, Allen Kaasik
Department of Pharmacology, Centre of Excellence for Translational Medicine, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

During early development neurons undergo complex morphological rearrangements. This active axo-dendritic growth must be supported by sufficient cellular energy, thus, cellular energy status could be a key factor in initiating this process. However, the precise mechanisms of how cells adapt their energetic status to physiological demand and upcoming energy needs and how they are coupled to adaptive energy generation, e.g., increased ATP production by mitochondria, remain unclear. Previous studies have shown that peroxisome-proliferator-activated receptor gamma co-activator 1 (PGC-1α) is a master regulator of mitochondrial biogenesis and cellular energy metabolism. Our aim was to examine whether neuronal growth depends on mitochondrial biogenesis and whether activation of cell growth pathways could promote mitochondrial biogenesis to support the energetic need of neuronal development. Over-expression of PGC-1α in cortical neurons increased mitochondrial density in the periphery of axonal tree and was two-fold higher in axonal tips compared to control group. Moreover, induction of mitochondrial biogenesis by PGC-1α facilitated the axonal growth and neuronal development. Activation of PGC-1α upstream kinases such as Ca2+/calmodulin-dependent protein kinase kinase 2 (CaMKKβ), transforming growth factor-β-activated kinase (TAK1) and STE-related adaptor (STRAD) increased PGC-1α transcriptional activity and mitochondrial density in axons. Most importantly, they promoted neuronal development through mitochondrial biogenesis. This study shows that mitochondrial biogenesis itself is limiting factor for axonal growth and AMPK-PGC-1α upstream pathways sensing cellular energy status could signal not only the energy deficit but also upcoming energy need to generate new mitochondria.

Keywords Mitochondrial biogenesis, PGC-1α, neuronal development

GPR37 is a G protein-coupled receptor that is abundantly expressed in the brain and has been implicated in dopaminergic signaling [1]. The receptor has been identified as a substrate of the ubiquitin ligase parkin and it has been linked to the autosomal recessive juvenile parkinsonism (AR-JP), an early onset familial Parkinson's disease. The loss of parkin function and deficits in the ubiquitin proteasome pathway were proposed to cause intracellular accumulation of unfolded GPR37 leading to the AR-JP pathogenesis [2]. Here, we found that while GPR37 appears to mature normally in a heterologous expression system, the receptor is subject to proteolytic cleavage at its large N-terminal extracellular region. To study this proteolytic processing, we used stably and transiently transfected human embryonic kidney (HEK) 293 and SH-SY5Y neuroblastoma cells that express N- and C-terminally epitope-tagged human GPR37. N-terminal sequencing of the cleaved C-terminal receptor fragment revealed that GPR37 is cleaved between Glu187 and Gln188 and the metabolic pulse-chase data suggests that receptor cleavage is a rapid and efficient process. Moreover, our results indicate that the receptor N-terminus is released from the cells by shedding, a phenomenon rarely described for GPCRs. Immunofluorescence microscopy with subcellular markers indicates that GPR37 is still in the full-length form in the trans-Golgi network but is predominantly expressed in the cleaved form at the cell surface. Additionally, experiments with various proteinase inhibitors imply that the receptor is cleaved by a metalloproteinase. As proteolytic processing is involved in the regulation of many cell surface receptors, our findings provide valuable information about GPR37 that help to understand its function and role in AR-JP at the molecular level.

Keywords GPR37, AR-JP, shedding
place during ALS. Main objectives of this work were to investigate 1) if the expression of S100B protein and RAGE change during the course of the disease in rodent models of ALS, 2) if the expression of mutant SOD1 protein per se is sufficient to modify S100B levels in astrocytic cultures. We observed that S100B levels and localization are modulated in the spinal cord and in the brain cortex of rat and mouse models of ALS. We also demonstrated a differential expression of RAGE subunits in SOD1-G93A-derived CNS tissues. Moreover, we showed that the overexpression of mutant SOD1 in astrocytic cell line is sufficient to induce the intracellular levels and release of S100B, while it is not enough to induce a differential expression of RAGE. Thus, the expression of mutant SOD1 interferes with the physiological expression of S100B and RAGE and reveals that in astrocytes S100B modulation is an early event related to the mere expression of mutant SOD1, while the dysregulation of RAGE might be a phenomenon possibly requiring a more complex interplay between different cell types and pathways. Overall, these data suggest that S100B might be a toxic mediator released by astrocytes in the ALS-linked neuroinflammatory process.

**Keywords** S100B, RAGE, ALS

P29

Synapse and dendrite deficits induced by mutations in the X-linked intellectual disability gene I11rapl1

Caterina Montanna1, Mariana Ramos-Brossier2, Mariana Ramos-Brossier2, Paola Corno, Carlo Sala2

1CNR Neurosciences Institute, Italy; 2Institut Cochin, INSERM, Université Paris Descartes, France


Synapse and dendrite deficits induced by mutations in the X-linked intellectual disability gene I11rapl1. Caterina Montanna, Mariana Ramos-Brossier, Pierre Billuart, Carlo Sala. Mutations and deletions of Interleukin-1 receptor accessory protein like 1 (IL1RAPL1) gene, localized on X chromosome, are associated to intellectual disability (ID) and autism spectrum disorder (ASD). IL1RAPL1 protein is localized at the postsynaptic compartment of excitatory synapses and plays a role in synapse formation and stabilization. Our project was to characterize IL1RAPL1 mutants identified in patients with ID and ASD and to perform a behavioral and neuronal morphology analysis on IL1RAPL1 KO mice. Specifically, we studied the function of three novel mutations of IL1RAPL1 gene in patients presenting ID. We found that two of the studied mutants lead to a partial loss of function of IL1RAPL1 and we pointed out the important function of the extracellular domain for the trans-synaptic PTP6/IL1RAPL1 interaction in synaptogenesis. We also characterized the role of IL1RAPL1 wild type and mutants in regulating dendrite morphology using in vitro neuronal cultures and IL1RAPL1 KO mice. We identified, associated to hippocampal cognitive impairment an increased number of dendrite branching points in CA1 and CA2 hippocampal neurons of IL1RAPL1 KO mice. In transfected hippocampal neurons the expression of full length IL1RAPL1 and mutants lacking part of C-terminal domains leads to a simplification of neuronal arborisation. This effect is abolished when we overexpressed mutants lacking part of N-terminal domains. Our results indicate the importance of IL1RAPL1 extracellular domains not only in synaptogenesis but also in dendrite development. We also concluded that for this activity PTP6 interaction is not required, suggesting that an unknown IL1RAPL1 binding partner is involved in the effect on dendrite morphology.

**Keywords** Synapse, intellectual disability, autism spectrum disorder

P30

Organic cation/carnitine transporter (OCTN2) interaction proteome in rat astrocytes: Role of phosphatase PP2A

Katarzyna Nałęcz, Barbara Juraszek

Nencki Institute of Experimental Biology, Poland


L-carnitine is essential for translocation of fatty acids for their mitochondrial oxidation, a process shown in the brain to take place in astrocytes. However, it is not synthesized in the brain and has to be transported to brain cells. Organic cation/carnitine transporter novel family member 2 – OCTN2 (SLC22A5) presence was shown in endothelial cells forming the blood-brain barrier, as well as in neurons and astrocytes. We showed that OCTN2 activity in astrocytes and its presence in plasma membrane are higher upon activation of protein kinase C (PKC), but no phosphorylation of OCTN2 was detected. Therefore, we aimed to identify OCTN2-interacting partners and to define their role in transporter regulation. Mass spectrometry analysis identified several cytoskeletal, ribosomal, mitochondrial, and heat shock proteins as well as the proteins involved in signaling pathway and trafficking. We focused on protein phosphatase PP2A subunits identified in OCTN2 proteome and we observed co-precipitation of OCTN2 with PP2A structural (A) and catalytic (C) subunits, as well as with two regulatory subunits – striatin and SG2NA. Activation of PKC with phorbol ester (PMA) did not change the amount of co-precipitating subunits A and C but significantly lowered the amount of

**Keywords** Synaptic dysfunction, PP2A, OCTN2, ALS

References

co-precipitating SG2NA. Immunocytochemistry analysis of astrocytes showed OCTN2 co-localization with PP2A C subunit and with SG2NA in vesicular structures in the cytoplasm. PMA treatment did not change this co-localization, although an augmented amount of OCTN2 was detected in plasma membrane. We postulate that interaction of OCTN2 with PP2A arrests the transporter in cytoplasm in dephosphorylated state, while PKC activation releases SG2NA subunit from the complex, resulting in transporter trafficking to the cell surface. 

Acknowledgements This work has been financed by grant 2012/07/B/ NZ3/00225 from National Science Centre in Poland.

Keywords Astrocytes, membrane transporter, protein phosphatase

---

**P31**

Evidence for the existence of the A2A-A1 heteroreceptor complex in the rat brain, and comparison of its distribution to that of the A2A-A2A homoreceptor complex

Julia Oflıjan1,2, Dasiel Borroto-Escuela1, Miles Woolfenden1, Michael Di Palma1, Luca Pinton1, Ismel Brito, Manuel Naváez, Fidel Corales, Antonio Jimenez-Berstein, Luigi F. Agnati and Kjell Fuxe

Karolinska Institutet, Sweden; 2University of Tartu, Estonia

SpringerPlus 2015, 4(Suppl 1):P31

Adenosine receptors play critical roles in cellular processes and signaling and have been shown to form heteromers with diverse biochemical and/or pharmacological activities that are different from those of the corresponding homomers.1 However, despite extensive experimental results supporting the formation of adenosine heteromers in heterologous systems, the existence of such heteroreceptor complexes in the brain remains largely unknown, mainly because of the lack of appropriate methodology. Also no systematic study was carried out on heteromers form by adenosine receptor subtypes alone. In this study, we used several experimental approaches2 to investigate whether adenosine receptor A2A and A1 subtypes can form heteromers among themselves. In situ PLA clearly demonstrated that adenosine receptors (A2A-A2A, A2A-A1) exist as homo/heteroreceptor complexes in rat brain. In the hippocampus, A2A-A1 heteroreceptor complexes are mainly localized to the pyramidal cell layer of the Ammon’s horn and the hilo (PoDG). The complex was also observed throughout the piriforms layer. Several distinct differences were apparent between the distribution of the A2A-A1 heteroreceptor complexes and that of the A2A-A2A homoreceptor complex, which could have important functional consequences. Furthermore, bioluminescence resonance energy transfer analysis of adenosine A2A receptors established that they can physically interact in HEK293T27 cells, as both homomers and heteromers. In addition, static/non-dynamical human GPCR data derived from this study and other interaction studies were integrated in a large scale graph, called the GPCR heterodimer network (http://www.iiia.csic.es/~ismel/GPCR-Nets/index.html), which provides global insight into adenosine heteromer connectivity, topology and organization in the context of the adenosine receptor subfamily and the GPCR network as a whole.

References


---

**P32**

Validation of astrocytic reference genes for qRT-PCR in CO treatment studies

Sara Oliveira1, Helena Vieira2, Carlos Duarte1

1Center for Neuroscience and Cell Biology, University of Coimbra, Portugal; 2Chronic Diseases Research Center FCM, Portugal

SpringerPlus 2015, 4(Suppl 1):P32

Quantitative real-time reverse transcription-polymerase (qRT-PCR) is a widely used technique to characterize changes in gene expression in complex cellular and tissue processes, such as the cytoprotection or inflammation. The selection of an adequate internal reference gene for accurate and consistent analysis of gene expression is of major importance. Carbon monoxide (CO) affects several metabolic pathways and de novo protein synthesis is crucial in the cellular responses to the gas although an aurora a selection of commonly used reference genes was analyzed to identify the most suitable internal control genes to evaluate the effect of the CO on gene expression in cultured cortical astrocytes. The cells were exposed to CO by incubation with CORM-A1 (CO releasing molecule A1) and four different algorithms (geNorm, NormFinder, Delta Ct and BestKeeper) were applied to better evaluate the stability of eight putative reference genes. Our results indicate that Gapdh (glyceraldehyde-3-phosphate dehydrogenase) and Pia (peptidyl)propyl isomerase A) is the most suitable gene pair for normalization of qRT-PCR results under the experimental conditions used. Pdgk1 (phosphoglycerate kinase 1), Hprt1 (hypoxanthine guanine phosphoribosyl transferase I), Sdha (Succinate Dehydrogenase Complex, Subunit A), Tbp (TATA box binding protein), Actg1 (actin gamma 1) and Rn18s (18S rRNA) genes presented less stable expression profiles in cultured cortical astrocytes exposed to CORM-1 for up to 60 min. Analysis of the effects of CO on the expression of Bdnf and P2rx7, in contrast with the results obtained when the other genes were used as reference. This study highlights the need for proper and accurate usage of reference genes in quantification of qRT-PCR results in studies on the effect of CO in gene expression.

Keywords Carbon monoxide, astrocytes, reference genes

---

**P33**

Learned helplessness paradigm in P2rx7 wild type and knockout mice and its effect on synapses in the dentate gyrus

Lilla Otrokovcs, Flóra Gölöncsér, Beáta Sperlágh, Ágnes Kittel

Institute of Experimental Medicine, Hungary

SpringerPlus 2015, 4(Suppl 1):P33

Although depression is one of the prevailing central nervous system disorders, there is still not enough knowledge about the exact alterations underlying its pathophysiology and the treatment is often inefficient. Our study aims at exploring a potential relationship between major depressive disorder and the purinergic P2X7 receptor (P2X7R), since in previous behavioural tests P2x7 knock out mice displayed an antidepressant-like phenotype. Among many other known symptoms, the loss of hippocampal spine synapses is a revealing feature of the disorder. Therefore we wanted to measure the density of spine synapses in the molecular layer of the dentate gyrus in the learned helplessness paradigm and later see if genetic inhibition of P2X7R could have influence on this condition. Wild type C57Bl/6 and P2rx7 knock out male mice were exposed to inescapable footshocks (IES) in shuttle boxes during 2 training days and on the 3rd day learned helplessness was tested, where helpless animals usually failed to escape. Control animals were also placed in shuttle boxes but did not receive footshocks until testing. Escape failures and the latency to escape were measured to determine helpless behaviour. Electron microscopy analysis was performed to determine spine synapse density in the different groups. In wild type mice both average escape latency and the number of failed escapes were significantly higher in the IES treated group, however, we could not find such dergency in P2x7 knockout mice. Electron microscopy analysis confirmed alterations in spine synapse density in the molecular layer of the dentate gyrus subsequent to learned helplessness experiments, as results indicated a significant decrease in spine synapse density in wild type mice, but not in knockout animals. These findings may lead to presume a role of the P2X7 receptor in this disorder and further experiments will hopefully help get a better understanding and thus more effective treatment of major depression.

Keywords p2x7, depression, spine synapse
P34

Trophic support following peripheral axotomy show different behaviour of reactive microglia and astrogliosis in the ventral horn

Michele Papa1, Anna Maria Colangelo2, Leonilde Savarese3, Ciro De Luca1, Giovanni Crillo1, Lilia Alberghina3
1Lab. of Morphology of Neuronal Network, Dept. of Public Medicine, Second University of Napoli, Italy; 2Lab. of Neuroscience “R. Levi-Montalcini”, Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Italy; 3SYSBIO, Centre of Systems Biology, University of Milano-Bicocca, Italy

SpringerPlus 2015, 4(Suppl 1):P34

Maintaining a neurotrophic support after peripheral nerve injury can be a key point in reducing the plastic changes which neurons, microglia, and astrocytes in the ventral horn undergo due to the loss of afferent synaptic and neurotrophic stimuli originating from the periphery. Therefore, intrathecal administration of trophic factors or the inhibition of the mechanisms responsible for their degradation could help prevent these changes. The purpose of our study was to analyze the changes in the ventral horn produced by gliopathy determined by the suffering of the motor neurons after peripheral nerve injury following spared nerve injury (SNI) of the sciatic nerve and how the administration of NGF or its synthetic analogue BB14, as well as the increase of endogenous NGF levels by i.t. infusion of GM6001, a MMPs inhibitor modulating events. Immunohistochemical analysis of spinal cord sections revealed that SNI was associated with increased microglial (Iba1) and astrocytic (GFAP) responses, indicative of reactive gliosis. These changes were paralleled by decreased glial aminooacid transporters GLT1 and Glt1, and increased levels of neuronal glutamate transporter EAAC1, this maladaptive behavior of neuronal and glial EAATs is paralleled by a net increase of the Glutamate/GABA ratio as measured by HPLC analysis. These molecular changes were found to be linked to an alteration of endogenous NGF metabolism, as demonstrated by decreased levels of mature NGF. The continuous i.t. NGF infusion or of its analogue BB14, or of the generic MMPs inhibitor GM6001 reduced reactive astrogliosis and normalized the expression of neuronal and glial glutamate and glycine transporters, restoring the reduction of the Glutamate/GABA ratio but it showed to be absolutely ineffective in modifying the reactivity of microglia, demonstrating that the two glial populations have different mechanisms of modulation associate to neuronal damage.

Keywords: Gliopathy, motoneuron axotomy, NGF

P35

LRRK2 modulates neuronal vesicles cycle through protein interactions

Giovanni Piccoli1, Francesca Pischedda4, Daniela Cirnaru1, Elisa Greggio2, Franco Onofri3, Anna Maria Colangelo2, Leonilde Savarese3, Ciro De Luca1, Giovanni Crillo1, Lilia Alberghina3
1Università Vita-Salute San Raffaele, Italy; 2Università di Padova, Italy; 3Centre of Systems Biology, University of Milano-Bicocca, Italy; 4SYSBIO, Centre of Systems Biology, University of Milano-Bicocca, Italy


Mutations in Leucine-rich repeat kinase 2 gene (LRRK2) are associated with familial and sporadic Parkinson’s disease (PD). LRRK2 is a complex protein that consists of multiple domains executing several functions, including GTP hydrolysis, kinase activity and a C-terminal WD40 domain devoted to protein binding. Robust evidence suggests that LRRK2 acts at the synaptic site as a molecular hub connecting synaptic vesicles to cytoskeletal elements via a complex panel of protein-protein interactions. We have investigated the impact of pharmacological inhibition of LRRK2 kinase activity on synaptic function. Acute treatment with LRRK2 inhibitors reduced the frequency of spontaneous currents, the rate of synaptic vesicle trafficking and the release of neurotransmitter from isolated synaptosomes. The investigation of complementary models lacking LRRK2 expression allowed us to exclude potential off-side effects of kinase inhibitors on synaptic functions. Next we studied whether kinase inhibition affects LRRK2 heterologous interactions. We found that the binding among LRRK2, presynaptic proteins and synaptic vesicles is affected by kinase inhibition. Interestingly, a sequence variant (G2385R) within the WD40 domain has been implicated as a risk factor in PD, but its physiological and pathological function has not been systematically addressed yet. We analyzed molecular features of the WD40 domain and we addressed the functional implication of the G2385R variant. Our results suggest that LRRK2 WD40 domain serves as a hub for protein interactions setting LRRK2 as part of a protein network involved in synaptic vesicle trafficking. Furthermore we showed that the G2385R mutation influences WD40 domain features in terms of domain folding and binding properties and has an impact on synaptic vesicle dynamic.

Our results suggest that different PD mutation might influence synaptic vesicle release via modulation of LRRK2 macro-molecular complex.

Keywords: LRRK2, synaptic vesicle, Parkinson’s disease

P36

GABA agonist muscimol ameliorates learning/memory deficits in streptozocin-induced Alzheimer’s disease non-transgenic rat model

Vladimirs Pilipenko, Jolanta Pupure, Juris Rumiaks, Ulrika Beinre, Zane Dzirkale, Raimonds Skumbins, Vija Klusa
University of Latvia, Faculty of Medicine, Department of Pharmacology, Latvia


Background: GABAergic inhibitory action regulates learning/memory processes and contributes to neurotransmission (Gong et al., 2009). Existing evidence suggests GABAergic system is involved in pathophysiology of Alzheimer’s disease (AD) via inhibitory interneuron deficits (Verret et al., 2012) and decrease in functional GABAA receptors (Limon et al., 2012). In vitro, GABA and muscimol (GABAa receptor agonist) blocked neuronal death induced by Aβ in rat hippocampal and cortical neurons (Paula-Lima et al., 2005). Our concept: low doses of muscimol may prevent learning/memory deficits in intracerebroventricular (icv) streptozocin (STZ)-induced AD non-transgenic rat model.

Methods: Wistar male rats (260 ± 20 g) were pre-treated with saline (control) or muscimol (0.01 and 0.05 mg/kg) for 3 days. On day 4, rats received icv STZ (100 µg/ml) or aCSF. From day 18, rats received muscimol or saline for 4 days; rat spatial learning and memory were assessed in water maze test (4 trials/day) by recording the time to reach the hidden platform (escape latency). A probe trial without platform was carried out 24 h after the training trials, and the number of platform zone crossings has been recorded.

Results: STZ statistically increased the escape latency vs. control group (p < 0.0001). Muscimol at both doses significantly decreased the escape latency in STZ rats vs. STZ, reversing STZ effect by about 90% on days 3 and 4 (p < 0.0001). In probe trial, the number of platform crossings in muscimol+STZ rats was significantly increased vs. STZ rats. Muscimol at both doses per se showed values comparable to control.

Conclusions: Obtained data suggest that icv STZ significantly decreased rat spatial learning and memory and learning ability. Muscimol at both low doses significantly improved rats’ learning and memory abilities in both normal and AD-type rats. One may suggest that intensification of GABAergic processes may be a useful pharmacotherapeutic strategy to halt early memory decline in AD.

Keywords: Memory, muscimol, streptozocin

P37

Evidence for the existence of dopamine D2R and Sigma 1 allosteric receptor-receptor interaction in the rat brain: role in brain plasticity and cocaine action

Luca Pinton1, Daniël O Borroto-Escuela1, Manuel Narváez1, Julia Oflijan1, Luigi F Agnati1, Kjell Fuxe2
1Department of Neuroscience, Karolinska Institutet, Sweden; 2Department of Physiology, School of Medicine, University of Malaga, Spain

SpringerPlus 2015, 4(Suppl 1):P37

Drug addiction is regarded as one of the most important neuropsychiatric diseases afflicting our society today. A prototypic drug of abuse is cocaine which directly acts on the brain reward system. In this work we present evidence on the existence of dopamine D2R-Sigma1R heteroreceptor complexes which may play a role in the etiology of cocaine addiction. By means of BRET D2R-Sigma1R heteromers were
demonstrated in HEK293 cells after receptor cotransfection. The existence of D2R-Sigma1R heteroreceptor complexes was demonstrated also in discrete regions of the ventral and dorsal striatum with in situ proximity ligation assay. Through saturation binding assay it was clearly demonstrated that in membrane preparations of HEK293 cells co-expressing D2R-Sigma1R, cocaine (1 mM) significantly increased the D2R Bmax values (998 ± 40 fmol/mg protein) over D2R alone cells (664 ± 37 fmol/mg protein). This effect was counteracted by the Sigma1R selective antagonist PD144418 (Bmax value: 726 ± 39 fmol/mg protein). Furthermore, CREB reporter luc gene assay indicated that the presence of D2R-Sigma1R significantly reduced the potency of the D2R like agonist quinpirole to inhibit the forskolin induced increase of the CREB signal. In contrast, the presence of a low concentration of cocaine (100nM) was found to markedly increase the quinpirole potency to inhibit the forskolin induced increase of the CREB signal in the D2R-Sigma1R cells. These dynamic changes in D2R-Sigma1R signalling produced by cocaine maybe explained by synergistic allosteric receptor-receptor interactions in the D2R-Sigma1R heteroreceptor complexes at the plasma membrane level. An antagonistic allosteric receptor-receptor interaction between the dopamine D2R and the Sigma1R in absence of cocaine instead of can explain the reduced potency of quinpirole. These dual conformational changes in the D2R-Sigma1R heteroreceptor complexes could be associated with the redistribution of both protomers from the intracellular compartment to the plasma membrane as indicated by means of confocal analysis of agonist induced D2R-Sigma1R trafficking and internalization. Overall, the dynamic of D2R-Sigma1R heteroreceptor complexes may represent a mechanism that shapes neuronal and addictive responses to cocaine.

Keywords: Dopamine D2 receptor, Sigma 1 receptor, heteroreceptor complexes

P38 The role of Negr1 in cortical development via NCAM-FGFR2 signalling
Francesca Pischedda1, Joanna Szczurkowska2, Maria Daniela Cirnaru3, Laura Cantedda, Giovanni Piccoli
1University of Milan, Italy; 2Italian Institute of Technologies Genova, Italy; 3Università Vita-Salute, Italy
SpringerPlus 2015, 4(Suppl 1):P38

Autism spectrum disorder (ASD) affects 0.9% of children and it is recognized as the most genetic of all developmental neuropsychiatric syndromes. Mutations in NGR1 and FGFR2 genes have been recently identified as ASD candidates. Negr1 is a member of IgLON adhesion protein family but its functions are largely unknown. Our original approach has identified Negr1 as a developmentally regulated synaptotagmin-like protein. Thus we examined the consequences of Nanog1 acute downregulation. Strikingly, we found that Negr1 ablation impairs neuronal maturation in vitro. A combination of biochemical and imaging investigation has demonstrated that Negr1 influences neurites outgrowth via MAPK signaling organizing trans-synaptic heterodimer. In detail, we demonstrated that ectopic Negr1 is sufficient to improve neurite arborization and rescue the morphological phenotype observed in Negr1 KD cells. This function is dependent on the activation of MAPK pathway through tyrosine kinase receptors. In fact, we found that Negr1 physically and functionally interacts with NCAM and FGFR2, modulates FGFR2 response to FGF and consequently influences MAPK pathway. FGFR2/NCAM pathway plays an important role during brain development. Not surprisingly, our investigation of the radial migration of newly generated cortical neurons, revealed that Negr1-FGFR2-NCAM cross-talk controls cortical organization in vivo. Connectivity dysfunctions have been suggested as causative alterations in ASD. Given the functional, physical and genetic correlation among Negr1 and FGFR2 and NCAM, the study of Negr1/FGFR2/NCAM molecular cross talk may offer new therapeutic opportunities.

Keywords: Autism, Negr1, MAPK

Reference

P39 Cortical synapses in acute hepatic encephalopathy: morphology and expression of proteins involved in synaptic transmission
Mariusz Popek, Małgorzata Frontczak-Baniewicz, Barbara Zabłocka, Jan Albrecht, Magdalena Zielińska
Mossakowski Medical Research Centre Polish Academy of Sciences, Neurotoxicology, Poland

Hepatic encephalopathy (HE) is manifested by impaired glutamatergic transmission. The current view is that HE spares the glutamatergic synapse, its dysfunction being primarily due to impaired astrocytic control. Here we show that in acute HE, the glutamatergic synapse by itself presents discrete changes in the ultrastructure (electron microscope) and expression of functionally critical synaptic proteins (Western blot). We used C57Bl6 mice subjected to a hepatotoxic insult in the azoxymethane (AOM) model. Cerebral cortex of AOM mice was ultrastructurally characterized by the presence of increased numbers (~by 15%) of enlarged synapses showing abundance of synaptic vesicles in the presynaptic zone. The expression of synaptophysin and synaptotagmin in S2 fraction was increased by ~80% and ~30%, respectively. The expression of the NR1 subunit of NMDA receptor, but not of NR2A, was slightly increased. The amount of PSD-95 in P2 fraction and mNOS in S2 fraction, were elevated by ~40% and ~30% respectively. The expression of PKCβ was increased by ~30% and ~40% at the mRNA and protein level, respectively. PKCα protein level was reduced by ~20% in P2 membrane fraction and elevated by ~30% in S2 cytosolic fraction suggesting its altered intracellular trafficking. This report is to our knowledge the first to demonstrate distinct changes in the synaptic ultrastructure and composition of synaptic proteins in the acute stage of HE. Except for PKCα, all the other changes are indicative of a compensatory response of as yet unknown functional implications.

Acknowledgements Supported by the National Centre for Research and Development (Program “Core”) grant no Pol-Nr/196190/26/2013 and the Leading National Research Centre (KNOW)

Keywords: Ammonia, synapses, synaptic proteins

P40 Proteasome stress triggers differential cellular responses of neural cells
Peter Racay, Ivana Pilečková, Katariina Klačanová, Dušan Dobrotová
Jesenní ROTUMA Faculty of Medicine, Martin, Slovak Republic
SpringerPlus 2015, 4(Suppl 1):P40

Ubiquitin-proteasome system (UPS) represents important intracellular system controlling protein quality and intracellular signalling. Overload or dysfunction of UPS leads to proteasome stress that is implicated in mechanisms of neurodegeneration associated with neurodegenerative diseases, e.g. Parkinson and Alzheimer disease. Proteasome stress is also considered as the main cause of delayed neuronal death observed after transient global brain ischemia. Despite significant progress made to date, the exact mechanism and selectivity of cell death induced by proteasome stress after global brain ischemia is still not completely understood. The aim of our work was to study effect of proteasome stress on cell viability, stress response as well as on mechanism of death of neuroblastoma SH-SYSY and glioblastoma T98G cells. Proteasome stress was induced by treatment of cells with bortezomib, inhibitor of proteasome 26S complex. Neuroblastoma cells were more sensitive to bortezomib than glioblastoma cells and death of neuroblastoma cells occurred significantly faster than death of glioblastoma cells. With respect to cellular response, treatment of both SH-SYSY and T98G cells with bortezomib was associated with accumulation of polyubiquitylated protein aggregates and increased expression of HSP70. With respect to cell death mechanism, we have documented bortezomib-induced release of cytochrome c from mitochondria and activation of caspase 3 in SH-SYSY cells. In T98G cells, bortezomib induced activation of caspase 4 but not caspase 3 and did not induce release of cytochrome c from mitochondria. Our results indicate that proteasome stress affects neural cells in different way but does not answer the question about selectivity and delay of cell after global brain ischemia.

Acknowledgements Supported by APVV grant no. 0245-11 to PR.

Keywords: ischemia, cell death, proteasome
P41
Miro1 overexpression protects against α-synuclein-induced mitochondrial loss in neuronal culture
Dzhamila Safitulina, Vinay Choubey, Miriam Ann Hickey, Allen Kaasik
Department of Pharmacology, Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia
SpringerPlus 2015, 4(Suppl 1):P41

Mitophagy is a selective degradation of mitochondria that promotes the turnover of mitochondria and prevents the accumulation of damaged organelles. Pink1 and Parkin proteins are crucial in the removal of damaged mitochondria. Mutations in the corresponding PINK1 and PARK2 genes are associated with Parkinson’s disease (PD), and mitochondrial impairments are central to PD pathogenesis. Pink1 has been shown to interact with the atypical Rho GTPases Miro, the outer mitochondrial membrane proteins involved in mitochondrial trafficking. Miro1 is degraded shortly after mitochondrial damage in a Parkin-dependent manner. α-synuclein is a major component of Lewy bodies, the characteristic cellular inclusions in PD. Mutations of α-synuclein, including A53T, are linked to familial PD. α-synuclein has been shown to bind to the mitochondrial membrane, and increased membrane-bound α-synuclein in PD contributes to the functional distance of mitochondria. Overexpression of A53T-mutated α-synuclein has been shown to induce mitophagy in vitro and in vivo. We hypothesized that Miro1 function is disturbed in an α-synuclein (A53T)-overexpressing model, and the aim of this study was to elucidate the involvement of Miro1 in α-synuclein-induced mitophagy. We have found that α-synuclein is one component of the Miro1 interactome. Moreover, co-expression of Miro1 restored mitochondrial length and density in primary neuronal culture overexpressing A53T-mutated α-synuclein. Miro1 overexpression did not change the basal mitophagy, but decreased significantly α-synuclein-induced mitochondrial removal. Together, our results suggest that Miro and α-synuclein may interact in the mitophagic pathway.

Keywords Mitochondria, autophagy, Parkinson’s disease

P42
Glycophospholipids in the central nervous system: the role of GBA2 in the neuronal differentiation
Maura Samarani, Nicoletta Loboerto, Valentina Murdica, Domitilla Schiumarini, Simona Prioni, Alessandro Prinetti, Sandro Sonnino, Rosaria Bassi, Massimo Aureli
Department of Medical Biotechnology and Translational Medicine, Università degli Studi di Milano, Italy
SpringerPlus 2015, 4(Suppl 1):P42

Mammalian neurodevelopment is characterized by qualitative and quantitative changes in plasma membrane glycosphingolipids due to a fine regulation of their metabolic pathways. While the biosynthetic pathway is largely studied scant is the information available on the catalytic one. For this reason, I studied the activity of the main glycohydrolases expressed in the central nervous system during neuronal differentiation. To this purpose the activities of the principal glycohydrolases involved in glycosphingolipid catabolism have been evaluated in different experimental models such as brains and cerebella of mouse at different ages and neuronal cell cultures (immortalized mouse neuronal cell lines Gn11 and Gt1-7, primary cultures of mouse cerebellar granule cells and human neuroblastoma cells SH-SYSY). The results obtained indicate that the process of neuronal differentiation is associated with a marked increase in the activities of all the glycohydrolases evaluated, in particular, the activity of the non-lysosomal β-glucosylceramidase GBA2 undergos the most relevant increase representing the prevalent form of β-glucocerebrosidase in mature neurons. In order to evaluate the possible role of GBA2 in the neuronal differentiation, SH-SYSY cells have been stably transfected for GBA2 overexpression. Cells overexpressing GBA2 acquired a neuronal phenotype and showed a significant increase in ceramide levels. These results are in line with literature data that demonstrate the involvement of ceramide in the neuronal differentiation. Therefore, it is possible to hypothesize that the hydrolysis of glucosylceramides to ceramide, catalyzed by GBA2 at the plasma membrane level, has a functional role in the neuronal differentiation process. Collectively these findings suggest that GBA2 may represent a possible neuronal marker and demonstrate for the first time its direct involvement in the neuronal differentiation.

Keywords Sphingolipids, neuronal differentiation, GBA2

P43
A new potential mechanism of action of tianeptine – the effect on microglial cell activation
Joanna Ślusarczyk, Ewa Trojan, Anna Piotrowska, Katarzyna Glombik, Joanna Mika, Agnieszka Basta-Kaim
Institute of Pharmacology, Polish Academy of Sciences, Poland
SpringerPlus 2015, 4(Suppl 1):P43

Tianeptine is an atypical antidepressant drug with proven efficacy, but still not fully understood mechanism of action. Recently it has been suggested that tianeptine may modulate inflammatory processes, however there is a lack of data on its influence on microglia – the main source of pro-inflammatory cytokines in the brain. Therefore this project aimed to investigate whether tianeptine can influence activation of microglial cells. We conducted our study in two experimental models: in vivo – in the hippocampus and frontal cortex of adult rats and in vitro in microglial cultures. Pregnant rats were subjected daily to 3 stress sessions from 14th day of pregnancy until delivery. Control pregnant females were left undisturbed in their homecages. Microglial cells were pre-treated for 30 min with different concentrations of tianeptine and stimulated with LPS (100 ng/ml). Next, expression of microglial activation markers and pro-inflammatory cytokines were evaluated. In the second part of experiments at 3 months of age, after behavioral verification, control and prenatally stressed rats were injected with tianeptine (10 mg/kg i.p.) for 14 days. Next, biochemical studies were carried out on hippocampus and frontal cortex. We observed that in microglial pre-treatment with tianeptine (1–10 μM) reduced the expression of microglial activation markers (CD40 and MHCII) and production of pro-inflammatory cytokines. Moreover, in adult animals subjected to prenatal stress (an animal model of depression) chronic tianeptine treatment inhibited microglial activation (decreased CD40 and CD68 expression) in both examined structures. In conclusion, our results show that tianeptine exerts anti-inflammatory properties suppressing microglial activation in both in vitro and in vivo experimental models.

Acknowledgements This research was supported by grant no. 2013/09/B/NZ7/04096, NCN, Poland. Joanna Ślusarczyk and Ewa Trojan are holders of scholarships from the KNOW.

Keywords Tianeptine, microglia, inflammation

P44
The effects of STZ-induced diabetes on cognition and brain amyloid in 5XFAD mouse model of Alzheimer’s disease
Katrin Sonn, Alexander Zharkovsky
University of Tartu, Institute of Biomedicine and Translational Medicine, Department of Pharmacology, Estonia
SpringerPlus 2015, 4(Suppl 1):P44

Introduction Alzheimer’s disease (AD) is an incurable neurodegenerative disease characterized by progressive dementia. Main neuropathological features of AD include extracellular β-amyloid (Aβ)-containing plaques, intraneuronal aggregates of hyperphosphorylated τ-protein and neurofilaments, microglial activation and clustering around Aβ plaques and synaptic loss. 5XFAD transgenic mice are a model of AD, exhibiting rapid brain accumulation of Aβ and microgliosis. The aim of the study was to characterize the effects of streptozocin (STZ)-induced diabetes on learning and memory of 5XFAD and wild-type (WT) mice in Morris water maze (MWM) at ages 2 and 6 months and on brain amyloid load.

Methods and results Mice were injected with STZ 90 mg/kg or vehicle i.p., once daily for 2 consecutive days. MWM was performed on week 9 and histological analysis of brains of mice injected with STZ or vehicle at 2 months of age was performed on week 16. STZ treatment did not affect locomotion or vision of mice in MWM. At both 2 and 6 months of age, STZ treatment impaired memory of both 5XFAD and WT. Learning
was significantly impaired in STZ-treated 5XFAD mice at 2 months. Surprisingly, Congo Red-positive area fraction (%) of hippocampus and amygdala was decreased 5XFAD mice treated with STZ at 2 months. Plaque diameter was not different between STZ treated and vehicle treated 5XFAD mice.

Conclusions Insulin deficiency could affect cognition through mechanisms unrelated to Aβ metabolism. Also different mechanisms may underlie effects of STZ treatment on learning and memory in different age groups, possibly including enhancement of brain amyloid deposition and inhibition of neural cell precursor proliferation. We also hypothesize that STZ treatment might increase the soluble brain amyloid fraction in this model, since it is currently acknowledged that oligomeric (soluble) rather than fibrillar Aβ species disrupt cognitive function in AD.

Keywords Amyloid, diabetes, Alzheimer's

P45

The effect of hypoxic postconditioning after severe hypobaric hypoxia on the expression of Cu, Zn-SOD in hippocampus and neocortex of rats

Sergei Alexandrovich Stroev1, Tatiana Sergeevna Glushchenko2, Ekaterina Isosifovna Tjul’kova3, Mikhail Olegovich Samoilov1
School of Medicine, University of Tampere, Finland; 1,F.P. Pavlov Institute of Physiology of RAS, Russian Federation
SpringerPlus 2015, 4(Suppl 1):P45

Oxidative stress is a key mechanism of cellular damage during and after severe hypoxia. Accordingly, up-regulation of expression and activity of endogenous antioxidants is an important mechanism of cellular adaptation to hypoxia. Do endogenous antioxidants take part in postconditioning-induced neuroprotective mechanisms similarly to their participation in preconditioning-induced ones? In the present work the effect of postconditioning by 3-trial mild hypobaric hypoxia (360 Torr; 2 h, once a day) after 1 session of severe acute hypobaric hypoxia (180 Torr; 3 h) on the expression of Cu, Zn-superoxide dismutase (Cu,Zn-SOD) was studied by immunocytochemical analysis in areas CA1, CA2, CA3, CA4 and DG of hippocampus and in frontoparietal neocortex (NC) of male Wistar rats. Two time points were examined: 3 h after the last session of postconditioning that was 3 days after severe hypoxia and 24 h after the last session of postconditioning that was 4 days after severe hypoxia. It has been shown that postconditioning significantly increases the total number of Cu, Zn-SOD-immunoreactive cells (NT) at least in two areas of hippocampus studied (CA2 and DG) compared to non-postconditioned rats at 3 days but not at 4 days after severe hypoxia. In contrast, in NC of postconditioned rats, NT tends to increase compared to non-postconditioned animals at 4 days but not at 3 days after severe hypoxia. The effect of postconditioning on the number of intensely expressing Cu, Zn-SOD neurons differs in various areas and at various time points. The modification of Cu, Zn-SOD expression in some areas of hippocampus and NC, induced by 3-trial hypoxic postconditioning, correlates with the prevention of massive delayed apoptotic neuronal death and amelioration of functional disorders caused by severe hypoxia. Thus, Cu, Zn-SOD and other endogenous antioxidants may play, apparently, an important role in the treatment of severe hypoxia/ischemia stroke by postconditioning in brain neurons.

Keywords Postconditioning, hypoxia, Cu, Zn-superoxide dismutase

P47

Janus-faced taurine: protection or toxicity?

Andrey Taranukhin, Pirjo Saramaa, Simo Oja
School of Medicine, University of Tampere, Finland
SpringerPlus 2015, 4(Suppl 1):P47

Taurine is a simple sulfur-containing amino acid ubiquitously distributed in the tissues of most animals. It is involved in a wide range of physiological processes including osmoregulation, lipid metabolism, intracellular calcium regulation, neuronal development, neuromodulation and cell protection. Using in vivo model of acute ethanol intoxication (5 g/kg) in 7-day-old mice we have found that taurine treatment at total dose 2 g/kg has saved about 50% of dying neurons from ethanol-induced apoptosis in the internal (Taranukhin et al, 2009, 2010) and the external (Taranukhin et al, 2012) granular layers of developing cerebellum. However, any further increase in taurine doses (4–6 g/kg) aiming to protect more neurons against alcohol-induced apoptosis poses to threat to the whole organism and kills 7-day-old mice thus treated. Since the high doses of taurine alone or ethanol alone did not lead to animal death, lethality appears to be due to ethanol and taurine combined toxicity. We reveal the 50% and 100% lethal doses of taurine and ethanol combination for developing (7-day-old), adult (5–6-month-old) and old (12–13-month-old) mice and can conclude that the toxicity of ethanol and taurine combination is age-dependent. A dramatic drop in blood glucose levels observed in 25% of 7-day-old and 40% of elderly mice treated with taurine and ethanol suggests that one of the reason of animal death may be hypoglycemia (Taranukhin et al, 2013, 2015). Based on the results obtained we conclude that taurine may have beneficial effects in protecting brain cells against the apoptosis induced by alcohol. However, our finding on the toxicity of combined taurine and ethanol prompts serious concern particularly for young people mixing taurine-containing energy drinks with alcohol.

Acknowledgements This research was supported by the competitive research funding of Pirkkamaa Hospital District and the Finnish Foundation for Alcohol Studies.

Keywords Taurine, ethanol, hypoglycemia

P48

Peptidomic characterization of peptide processing in the hippocampus of Wfs1 knockout mice

Karlin Tein, Sergo Kasvandik, Eero Vasar, Anton Terasmaa
Institute of Biomedicine and Translational Medicine, University of Tartu, Estonia

Mutations in WFS1 gene cause Wolfram syndrome, which is a rare autosomal recessive disorder, characterized by diabetes insipidus,
diabetes mellitus, optic nerve atrophy and deafness (DIDMOAD). WFS1 gene product wolframin is located in the endoplasmic reticulum. Mice lacking this gene have disturbances in processing and secretion of peptides, such as vasopressin and insulin. In the brain, high levels of wolframin protein are observed in the hippocampus, amygdala and limbic structures. The aim of this study was to investigate the effect of Wfs1 invalidation on the peptide processing in hippocampus of mice. Peptidomic approach was used to characterize individual peptides in the hippocampus of wild type and Wfs1 knock-out mice. We identified 126 peptides in the hippocampal extracts and levels of 10 peptides were different in Wfs1 and wild type mice at \( p < 0.05 \). Largest alteration was found in the level of peptide little-LEN, which is processed (cleaved) from pro-SAAS (Pcsk1n) in prohormone convertase 2 (PC2) dependent ways. Results of this study reveal alterations of peptide processing in the hippocampus of Wfs1 deficient mice.

**Keywords** Peptide processing, Wfs1, pro-SAAS

**P50**

Corticosterone induces DNA methyltransferases expression in rat cortical neurons

Mari Urb1, Kaili Anier1, Terje Matsalu1, Aru Aonurm-Helm1, Tõnis Timmusk2

1University of Tartu, Estonia; 2Tallinn University of Technology, Estonia

SpringerPlus 2015, 4(Suppl 1):P50

Corticosterone (CORT) is the main glucocorticoid hormone involved in stress responses in rodents. It is established that CORT exerts its effects via glucocorticoid receptor (GR) and mineralocorticoid receptor that regulate downstream gene expression during development and adulthood. In our previous study, we have shown that maternal separation on postnatal day 15 increases DNA methyltransferase (DNMT) 1, 3A and 3B expression levels in rat nucleus accumbens lasting into adulthood (Anier et al. 2014). However, the exact mechanism how maternal separation alters DNMT expression is unclear. We hypothesize that stress-induced GR stimulation may increase the expression levels of DNMTs and alter long-term DNA methylation-demethylation balance in rat brain. Our aim is to evaluate the effect of CORT and maternal separation on the expression levels of DNMTs in rat cortex. In wild type mice at cortical neurons, CORT treatment increased mRNA levels of DNMT3A and DNMT3B. GR antagonist mifepristone significantly decreased CORT-induced DNMTs mRNA levels indicating GR stimulation-dependent upregulation of DNMTs expression. Higher mRNA levels of DNMT1, DNMT3A and DNMT3B in rat cortex at postnatal day 15 and increased plasma CORT levels suggest that elevated CORT upregulates DNMTs expression. Our results indicated that DNMTs are downstream targets of GR-dependent CORT stimulation and early life stress may induce aberrant DNA methylation pattern that could facilitate long-term changes in gene expression.

**Keywords** DNA methyltransferase, maternal separation, corticosterone

**Reference**


**P51**

Novel positive allosteric modulators of sigma-1 receptor

Edijis Vavers1, Liga Zvejniece1, Grigory Veinberg1, Baiba Svalbe1, Ilona Domracheva1, Reins Vilskersts, Maija Dambrova

1Latvian Institute of Organic Synthesis, Latvia; 2Riga Stradins University, Latvia

SpringerPlus 2015, 4(Suppl 1):P51

**Background** The Sigma-1 receptor is a chaperone protein that modulates intracellular calcium signalling of the endoplasmatic reticulum and is involved in learning and memory processes. The aim of the present study was to compare in vitro Ca2+ concentration modulating activity and in vivo behavioural effects of enantiomers of methylphenylpiracetam, a novel positive allosteric modulator of Sigma-1 receptors.

**Methods** The activity of enantiomers was compared in the model of electrically stimulated rat vas deferens and bradykinin-induced intracellular calcium signalling of the endoplasmatic reticulum and is emerging approach for cognition enhancement. The R-configuration is a promising positive allosteric modulator of Sigma-1 receptor. The Behavioural side effects were slightly more expressed for the enantiomers with S-configuration at the C-5 chiral centre. The enantiomers of methylphenylpiracetam did not induce any significant effects on the locomotor and depressive condition in the mice.

**Acknowledgements** The study was supported by the Latvian Science Council grant No. 108/2013.

**Keywords** Sigma-1 receptor, positive allosteric modulation, cognition
Implementation of fluorescence methods in studies of ligand binding to TRP channels opens up new possibilities to characterize these processes. One of the potential approaches is the detection of changes in the fluorescence anisotropy (FA) and/or total fluorescence intensity (TFI) signals upon binding reaction. However, to achieve significant changes in the FA/TFI signal, some requirements need to be met – the concentration of receptor binding sites as well as the dissociation constant of the interaction should be in the same order as the fluorescent ligand’s concentration. We have used FA assay to investigate ligand binding properties to Melanocortin 4 (MC4) receptor [1]. Implementation of budded baculoviruses (BBV) that display G protein-coupled receptors on their surfaces significantly increased sensitivity and temporal stability of this assay [2]. For the first time we demonstrate the applicability of BBV experimental setup to study Y1 receptor system. Here we used TAMRA-PYY, an Y1 receptor specific fluorescent peptide ligand, as a reporter ligand. Besides real-time monitoring of FA signal changes, up to 5 fold decrease in TFI signal was observed within TAMRA-PYY binding to the Y1 receptor. Pharmacological characterization of Y1 receptors with receptor-specific unlabelled ligands gave the rank order of potencies consistent with previously reported values. Additionally, allosteric heterogeneous interactions were revealed as koff values of TAMRA-PYY differed more than 7 times depending on the nature of dissociation initiated ligand. These observations provide evidence for similar allosteric receptor-ligand binding mechanism as previously shown for MC4 receptors [3].

**Acknowledgements** This work was financed by Estonian Research Council (IUT20-17) and the European Union (TK114, 30020).

**Keywords** Allostery, fluorescence assay, neuropeptide Y receptor Y1

**References**


**P52**

**Allosteric modulation of peptide ligand binding to Neuropeptide Y receptor Y1 revealed by fluorescence-based assay**

Santa Veiksina1, Sergei Kopanchuk1,2, Ago Rinken1

1Neuroscience Institute Cavalieri Ottolenghi, Italy; 2Istituto Mario Negri, Italy

**SpringerPlus** 2015, 4(Suppl 1):P52

Hypoxia postconditioning is an effective method of protection from severe hypoxia induced lipid peroxidation and neuronal apoptosis in rats

Oleg Vetrovoy, Ekaterina Tyulkova, Elena Rybnikova

Pavlov Institute of Physiology, Russian Academy of Sciences, St. Petersburg

State University, Russian Federation

**SpringerPlus** 2015, 4(Suppl 1):P54

**Acknowledgements** This work has been supported by RFBR (No. 13-04-00532).

**Keywords** Hypobaric hypoxia, hypoxic postconditioning, neuronal apoptosis, lipid peroxidation

**P53**

**Neuroprotection by MKK7 inhibition in excitotoxicity**


1Institute of Chemistry, University of Tartu, Estonia; 2Competence Centre on Health Technologies, Estonia

**SpringerPlus** 2015, 4(Suppl 1):P53

**Effect of brevican deficiency on neuroplasticity mediating molecules**

Solveig Weigel1, Juliane Meisner1,2, Alexander Zharkovsky1, Thomas Arendt1, Steffen Rossner2, Markus Morawski2

1Paul Flechsig Institute for Brain Research, University of Leipzig, Germany; 2Department of Pharmacology (Biomedicum), University of Tartu, Estonia

**SpringerPlus** 2015, 4(Suppl 1):P55

**Extracellular matrix (ECM) forms the pericellular part of the tissue. Due to the ECM’s constitution it defines the biophysical and biochemical properties of the tissue. In vertebrates’ brain the neuronal ECM mainly consists of negatively charged chondroitin sulfate proteoglycans (CSPG), hyaluronan and glycoproteins. CSPG are supposed to be involved in cell adhesion, axonal pathfinding and receptor binding. Therefore they are considered to play a role in neural development and plasticity as well as in several neurological and psychiatric disorders (e.g. schizophrenia and depression). One member of the CSPG family is brevican which was previously demonstrated by Blosa et al. (2013) to...**
The protective and toxic role of neuromelanins in brain aging and Parkinson’s disease

Luigi Zecca1, Chiara Bellei1, Pierluigi Mauri1, Luigi Casella2, Carolina Cebrían3, David Sulzer4, Fabio A Zucca1
1Institute of Biomedical Technologies – National Research Council of Italy, 20090 Segrate (Milano), Italy; 2Department of Chemistry, University of Pavia, Italy; 3Department of Neurology, Columbia University Medical Center, USA; 4Departments of Psychiatry, Neurology and Pharmacology, Columbia University, USA

SpringerPlus 2015, 4(Suppl 1):P56

Neuromelanins (NM) are a family of compounds occurring in all brain regions of human brain. These pigments are contained in special lysosomes together with lipid bodies, protein matrix, and accumulate in aging (Zucca et al. Neurotox Res. 2014). The synthesis of NM is a protective process because the melanin component is generated through the removal of reactive/toxic quinones that would otherwise cause neurotoxicity (Sulzer et al. PNAS 2000). NM serves an additional protective role through its ability to chelate and accumulate metals, including environmentally toxic metals such as mercury and lead. Other metals like Fe, Zn, Al, Cr and Mo are also accumulated by NM (Zecca et al. PNAS 2000). NM can play also a toxic role in Parkinson’s disease (PD), when it is released by dying neurons of substantia nigra and its polyisylated form PSA-NCAM. Both of them are known to be involved in the establishment and modulation of neuropalecticy which are essential for memory formation. Furthermore we analysed the expression of the prolyl endopeptidase PREP, the matrix metalloproteinase 9 (MMP9) and tissue inhibitors of MMPs (TIMP). Immunohistochemical and western blot analyses of hippocampus tissue of brevican knockout mice compared with wild type littermates did not reveal changes in the expression of MMP9, PREP, NCAM nor PSA-NCAM, but showed a reduction of TIMP1 and 3 by trend. Consequently, changes of LTP and local plasticity in brevican deficient mice do not seem to be mediated by an alteration of NCAM and PSA-NCAM, but might be supported by different expression levels of TIMP 1 and 3 modulating several MMPs’ activity.

Keywords Extracellular matrix, brevican deficiency, tissue inhibitor of matrix metalloproteinase

P57

Role of caspase-3 in development of neuronal plasticity and memory

Igor Zhuravin1,2, Nadezhda Dubrovskaya1,2, Dmitrii Vasilev1,2, Darya Kozlova1,2, Natalia Tumanova1,2, Anthony Turner2, Natalia Nalivaeva1,3
1I.M. Institute of Evolutionary Physiology and Biochemistry, RAS, Russian Federation; 2Saint-Petersburg State Pediatric Medical University, Russian Federation; 3School of Molecular and Cellular Biology, University of Leeds, United Kingdom

SpringerPlus 2015, 4(Suppl 1):P57

Caspases are known to play an important role in apoptosis and their increased activity in pre- and postsynaptic terminals leads to proteolysis of synapse-associated proteins, resulting in disruption of synaptic functions. Moreover, caspases degrade the C-terminal fragment of amyloid-precursor protein intracellular domain (AICD) which regulates expression of a variety of genes including an amyloid-degrading enzyme neprilysin (NEP). As such, inhibition of caspases is considered as a tool for prevention and compensation of various synaptic pathologies leading to cognitive deficit and Alzheimer’s disease pathogenesis. In this study we have evaluated the role of prenatal hypoxia on the activity of caspases and neuronal network characteristics in rat brain and the effect of caspase inhibitors on these parameters. We have found that the brain of rats subjected to prenatal hypoxia (E14, O2 7%, 3 h) is characterised by an increased number of caspase-3-positive neurones and higher activity of this enzyme in the neocortex and hippocampus in the period of intensive synaptogenesis (P20-30) compared to controls. Subsequently, in later life these animals had a reduced number of synaptotagmin-positive dendritic spines and reduced activity of NEP accompanied by disruption of cognitive functions. Single i.v. injection of caspase-3 inhibitors (AC-DEVD-CHO) to hypoxic rats on P18-23 led to a decrease in caspase-3 activity and increased NEP expression. In these animals we have also observed restoration of synaptotagmin levels and distribution of the labile synaptic spines in the neocortex and hippocampus which were accompanied by improved memory. The effects of inhibitors on memory was observed within one month after administration but not detected 2.5 months later. These data testify to the involvement of caspase-3 in normal brain development and indicate an important role of this enzyme in neuronal plasticity and regulation of cognitive functions.

Acknowledgements Supported by RFBR (13-04-00388), ARUK.

Keywords Caspase-3, brain development, cognitive functions.