Endocannabinoidomics: “Omics” Approaches Applied To Endocannabinoids And Endocannabinoid-like Mediators

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The Endocannabinoid System

**CB₁ and CB₂ Receptors**

**Endocannabinoids**

**Biosynthetic and degradative enzymes**

**Bioactive N-acylethanolamides**

- N-palmitoylethanolamide (PEA)
- N-oleoylethanolamide (OEA)
- N-arachidonoylethanolamine (AEA)
- 2-arachidonylglycerol (2-AG)

**Endocannabinoids**

- **Anandamide**
  - FAAH
  - AA + ethanolamine

- **(-)-Δ⁹-Tetrahydrocannabinol (THC)**

**Biosynthetic and degradative enzymes**

- **FAAH**
  - AA + ethanolamine
- **MAGL**
  - AA + glycerol

**Bioactive N-acylethanolamides**

- **CB₁**
  - Anandamide
- **CB₂**
  - 2-arachidonylglycerol (2-AG)
  - N-oleoylethanolamide (OEA)
Detection of novel bioactive fatty acid derivatives as well as simultaneous monitoring of known members of endocannabinoid-like molecules and their biosynthetic precursors.

N-acyl-serotonin

N-acyl-amino acid

N-acyl-ethanolamine

Diacyl-glycerol (DAG)

N-acyl-phosphatidyl-ethanolamine

R=Alkyl chain
R₁=H or CH₃
R₂=-NH-CH₂-CH₂-OH

Fatty acids amino acids (FAAAs) and neuroprotection

N-arachidonoyl-L-serine is neuroprotective after traumatic brain injury by reducing apoptosis

Ayelet Cohen-Yeshurun, Victoria Tremblay, Alexander Alexandrovich, Erik Ryberg, Peter Gieselmann, Raphael Mechoulam, Esther Shohami and Rami R. Lekier

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AstraZeneca Research and Development Malmö, Malmö, Sweden

N-Arachidonoyl-L-Serine (AraS)

N-Arachidonoyl glycine (NAGly)

Fatty acid amino acids (FAAAs)

R=Alkyl chain
R₁=H or CH₂OH

Palmitoyl Serine: An Endogenous Neuroprotective Endocannabinoid-Like Entity After Traumatic Brain Injury

Anya Mann, Reem Smaoui, Victoria Tremblay, Alexander Alexandrovich, Aviva Brenner, Raphael Mechoulam, Esther Shohami

Cohen-Yeshurun et al., J Cereb Blood Flow Metab. 2011; Mann et al., J Neuroimmune Pharmacol. 2015
Traumatic Brain Injury (TBI)

TBIs occur when the impact of a rapid acceleration, deceleration, or collision causes a brain injury. TBIs are classified as mild, moderate or severe depending on the extent of the damage.

Traumatic brain injury (TBI) is a major cause of mortality and morbidity in the young age people (<40). At present, there are no effective drugs to treat brain trauma.
There is a large body of evidence showing that the endocannabinoid system is activated in response to pathogenic events, such as brain trauma.

In the closed head injury model (CHI), there is local and transient accumulation of 2-AG at the site of injury, peaking at 4 h and sustained up to at least 24 h.

Panikashvili and co-workers administered synthetic 2-AG to mice after CHI and found significant reduction of brain oedema, better clinical recovery, reduced infarct volume. This effect was dose-dependently and attenuated by SR-141761A, an antagonist of the CB₁ receptor.

Endocannabinoid degradation inhibition improves neurobehavioral function, blood-brain barrier integrity, and neuroinflammation following mild traumatic brain injury.

Pharmacological blockade or genetic deletion of cannabinoid CB₁ receptors reduces or eliminates many behavioral and neurochemical effects of nicotine that are related to its addictive potential.

A study from Simmonet and collaborators reported that selective blockade of CB₁ receptors in the ventral tegmental area (VTA) specifically decreased self-administration of nicotine, and this might occur in a physiopathological setting also through reduction of endocannabinoid levels.

1) To investigate the alterations of endocannabinoid levels in two different animal model of TBI

2) To discover new endocannabinoid-like molecules possibly involved in this process through the use of very sensitive and specific “targeted lipidomics” methods involving high resolution LC-ESI-IT-ToF (Liquid Chromatography-ElectroSpray Ionization-Ion Trap-Time of Flight).
Animal models of TBI

Moderate TBI
Lateral Fluid Percussion (LFP) model

24 h

Mild TBI
Weight drop model

Rats

Mice

mild TBI → weight=50 gr., height=20+2 cm
Tissues were dounce-homogenized and extracted with CHCl₃/CH₃OH/Tris-HCl 50 mM pH 7.5 (2:1:1, v/v) containing internal deuterated standards and quantificated by isotope dilution. Purification by open bed chromatography on silica gel and eluted with mixtures of CHCl₃/CH₃OH. 9/1 fractions. LC-APCI-MS (Shimadzu Corporation, Kyoto, Japan).
In this model of trauma the TBI did not lead to an elevation of the two main endocannabinoids in the brain.

* Sham ipsi vs. sham contra, * sham ipsi vs. injured ipsi, § injured ipsi vs. injured contra
One-way ANOVA + Bonferroni post hoc test
QIT and TOF are arranged linearly in a unique construction. The ion optical system in the hybrid IT-TOF-MS combines the DQ array, octopole and first lens to convert the continuous stream of ions into pulses for the introduction into IT QIT. The QIT is used to store ions before ejection into the TOF as well as to support MSn analysis with effective precursor ion selection capabilities. The TOF detects all ions in a draw-out pulse, thus producing a full mass spectrum with each pulse.

The LCMS-IT-TOF couples atmospheric pressure ionization with Ion-Trap (IT) and Time-of-Flight (TOF) technologies and delivers high mass accuracy and high mass resolution (10,000 at 1000 m/z), with high sensitivity.
New FAAAs identified by LC-ESI-IT-TOF: N-Oleoylglycine (OlGly)

In the PFC and Hip of the injured hemisphere in the LFP model, trauma is accompanied by a strong elevation of OlGly!
CPP is a learned behavior shown in many vertebrates, including humans. CPP occurs when a subject comes to prefer one place more than others because the preferred location has been paired previously with rewarding events. The CPP paradigm is widely used to explore the reinforcing effects of natural and pharmacological stimuli, including drugs of addiction.

**Conditioned place preference protocol:**
- **Habituation:** the mouse is allowed to explore freely the apparatus
- **Conditioning or Control:** the mouse is presented with a repeated uncontidioned stimulus ina single compartment or a control stimulus
- **Preference Testing:** the time spent in each compartment is measured.
In vivo treatment

- Oleoylglycine (100mg/kg i.p.) or vehicle (1:1:18) was administered 15 min before either vehicle or nicotine (0.5 mg/kg s.c.)

- Placed immediately into the paired box for 20 min
Oleoylglycine blocks nicotine preference
Weight drop model: EC levels

One-way ANOVA + Bonferroni post hoc test
Naive vs. Sham/TBI * p<0.05; ** p<0.01; *** p<0.001
Sham vs. TBI # p<0.05

Piscitelli et al., unpublished data
Detection of N-Oleoylglycine (OlGly)

Target proposed for NAGly:
QGPR18 (neuroprotection, anti-inflammatory and anti-nociceptive effects)
QT-type calcium channels (addiction)

NAGly is a natural ligand for the orphan receptor the $G_{i/o}$-coupled GPCR, GPR18 and at sub-nanomolar concentrations potently drives cellular migration in both BV-2 microglial and HEK293-GPR18 transfected cells. Very recently, Lu and co-workers demonstrated that application of NAGly on GPR18-expressing neurons did not inhibit calcium currents, but instead potentiated currents in a voltage-dependent manner.

NAGly strongly inhibits recombinant and native T-current in sensory neurons and another study showed that T-type calcium channel antagonists have potential for alleviating nicotine addiction by selectively decreasing the incentive motivational properties of nicotine

Olgly upregulated in injured hypothalamus also in the weight drop model
In vivo study: effects of OlGly

Mice (5-6 weeks)

Beginning of treatments

TBI

1h

Days

1 2 3 4 5 6 7 8 9 10 11 12 13 14

OlGly
50-100 mg/kg
(1 injection per day)

Locomotor activity
Pole test

The mouse is placed head-upward on the top of a rough-surfaced vertical pole and the time until it descends to the floor and the time to orient down is recorded to assess the mouse coordination and balance.

\[ \text{OlGly treatment (50 mg/kg and 100 mg/kg, i.p.) reduces the time to orient down (T-turn) and the total time to descend the pole (T-total) in the mild-TBI 3 days post-injury} \]
GPR18 expression level in primary hippocampal neurons

<table>
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<th>Gene name</th>
<th>Mean C(t) n=3</th>
<th>C(t) SEM</th>
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<tr>
<td>Neuron specific beta III Tubulin</td>
<td>27.16</td>
<td>0.11</td>
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<tr>
<td>GPR18</td>
<td>35.39</td>
<td>0.25</td>
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<tr>
<td>S16</td>
<td>23.15</td>
<td>0.07</td>
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GPR18 expression level in primary microglial cells

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Mean C(t) n=3</th>
<th>C(t) SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBA1</td>
<td>18.6</td>
<td>0.16</td>
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<tr>
<td>GPR18</td>
<td>24.59</td>
<td>0.15</td>
</tr>
<tr>
<td>S16</td>
<td>22.55</td>
<td>0.06</td>
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Conclusions

Both models of TBI did lead to a decrease of the main endocannabinoid AEA, especially in Hippocampus and Insula.

In the PFC and Hippocampus of the injured rats and in the Hypothalamus of injured mice, trauma is accompanied by a strong elevation of N-Oleoylglycine, a lipid that belongs to the FAAAs family.

OlGly treatment in TBI animals blocks nicotine preference and reverted locomotor impairment induced by the injury.

The proposed target for OlGly, GPR18, is highly expressed in microglia and not in neurons.
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