Short term of diabetes in the rat brain:

effects on neuroactive steroid, cholesterol homeostasis and mitochondrial functionality
Diabetic Disease

Chronic, endocrine and metabolic pathology
Elevated blood glucose levels

1980
108 million people

2014
422 million people

- Type I – insulin deficient
- Type II – insulin resistant
Chronic, endocrine and metabolic pathology

Elevated blood glucose levels

Affection of neuroactive steroid levels:
- Peripheral nerves
- Cerebellum
- Spinal cord
- Cerebral cortex (Pesaresi et al., - Horm Behav. 2010)

CNS complications:
- Risk of dementia
- Cognitive deficit
- Impairment in learning and memory
- Neurophysiological and structural changes

Long term Complications:
- Neuropathy
  - Peripheral Neuropathy
  - Diabetic encephalopathy

1980
108 million people

2014
422 million people

1980 vs 2014 increase of 314 million people

Diabetic Disease

Type I – insulin deficient
Type II – insulin resistant
Molecules that can exert their actions in the nervous system directly or after metabolization

Neuroactive steroids: peripheral hormonal steroids, neurosteroids, synthetic steroids
Highly compartmentalized in a sequence of reactions, which implies as first step the translocation of cholesterol from the cytoplasm to the inner mitochondrial membrane.
Brain Cholesterol

- Very abundant in the CNS (25% of total body cholesterol)
- Can’t pass the blood brain barrier
- De novo synthesis
**Brain Cholesterol**

- **Very abundant in the CNS (25% of total body cholesterol)**
- **Can’t pass the blood brain barrier**
- **De novo synthesis**

```
Acetyl-CoA + Acetoacetyl-CoA
   ↓
    HMG-CoA
    ↓
 HMG-CoA Reductase
    ↓
    Mevalonate
    ↓
    Lanosterol
    ↓
    Desmosterol
    ↓
    DHCR24
    ↓
    Cholesterol
```

**Cell Metabolism**

**Article**

Diabetes and Insulin in Regulation of Brain Cholesterol Metabolism

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Can Short-term diabetes alter the neuroactive steroids levels in the hippocampus?

**GOALS:**

1) Analysis of neuroactive steroid levels
2) Assessment of the steroidogenic machinery
3) Evaluation of the precursor of neuroactive steroids, the cholesterol
4) Analysis of the mitochondrial compartment

In a model of type 1 diabetes in rat raised diabetic by a single i.p. of streptozotocin after one month
Levels of neuroactive steroids in plasma and hippocampus of control and STZ rats

<table>
<thead>
<tr>
<th></th>
<th>Plasma</th>
<th>Hippocampus</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>STZ</td>
<td>Control</td>
</tr>
<tr>
<td>PREG</td>
<td>0.280 ± 0.063</td>
<td><strong>0.424 ± 0.145</strong></td>
<td>3.240 ± 0.576</td>
</tr>
<tr>
<td>PROG</td>
<td>0.347 ± 0.091</td>
<td>0.277 ± 0.070</td>
<td>0.999 ± 0.224</td>
</tr>
<tr>
<td>DHP</td>
<td>0.154 ± 0.042</td>
<td>0.143 ± 0.057</td>
<td>4.363 ± 0.571</td>
</tr>
<tr>
<td>THP</td>
<td>0.142 ± 0.022</td>
<td>&lt;0.100</td>
<td>1.104 ± 0.059</td>
</tr>
<tr>
<td>Isopregnanolone</td>
<td>0.876 ± 0.199</td>
<td>&lt;0.100 **</td>
<td>0.211 ± 0.031</td>
</tr>
<tr>
<td>DHEA</td>
<td>0.078 ± 0.021</td>
<td>0.066 ± 0.011</td>
<td>0.236 ± 0.044</td>
</tr>
<tr>
<td>T</td>
<td>4.149 ± 0.543</td>
<td><strong>0.950 ± 0.251</strong> ***</td>
<td>4.464 ± 0.755</td>
</tr>
<tr>
<td>DHT</td>
<td>0.090 ± 0.020</td>
<td>&lt;0.050</td>
<td>1.001 ± 0.143</td>
</tr>
<tr>
<td>3α-diol</td>
<td>0.690 ± 0.136</td>
<td><strong>0.156 ± 0.046</strong> **</td>
<td>0.279 ± 0.038</td>
</tr>
<tr>
<td>3β-diol</td>
<td>&lt;0.050</td>
<td>0.057 ± 0.005</td>
<td>0.057 ± 0.004</td>
</tr>
</tbody>
</table>

Data are expressed as pg/µl of plasma and pg/mg of tissue and are the mean ± SEM, n = 7 animals for each experimental group. Limit of quantification (LOQ) for THP and Isopregnanolone is 0.1 pg/mg tissue; for DHT and 3β-diol is 0.05 pg/mg tissue. Statistical analysis is performed by Unpaired Student’s t-test. * p < 0.05. ** p < 0.01. *** p < 0.001

Romano et al., 2016 J Steroid Biochem Mol Biol. [Epub ahead of print]
Effect of short-term diabetes in steroid synthesis

Student's t test * P < 0.05; ** P < 0.01

Romano et al., 2016 J Steroid Biochem Mol Biol. [Epub ahead of print]
## Effects of short-term diabetes on cholesterol levels

<table>
<thead>
<tr>
<th></th>
<th>Hippocampus</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>STZ</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td>µg/mg tissue</td>
<td></td>
</tr>
<tr>
<td>Free cholesterol</td>
<td>27.40 ± 1.34</td>
<td>29.23 ± 1.44</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>25.65 ± 1.26</td>
<td>31.30 ± 1.70 *</td>
</tr>
<tr>
<td>Desmosterol</td>
<td>1.28 ± 0.08</td>
<td>1.24 ± 0.07</td>
</tr>
<tr>
<td><strong>Oxysterols</strong></td>
<td>ng/mg tissue</td>
<td></td>
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<tr>
<td>24(S)-hydroxycholesterol</td>
<td>90.07 ± 1.80</td>
<td>84.25 ± 1.96 *</td>
</tr>
<tr>
<td>25-hydroxycholesterol</td>
<td>0.18 ± 0.01</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>24,25-epoxycholesterol</td>
<td>0.22 ± 0.03</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>27-hydroxycholesterol</td>
<td>0.28 ± 0.01</td>
<td>0.32 ± 0.02</td>
</tr>
<tr>
<td>7α-hydroxycholesterol</td>
<td>0.05 ± 0.01</td>
<td>0.06 ± 0.01 *</td>
</tr>
<tr>
<td>7β-hydroxycholesterol</td>
<td>0.15 ± 0.01</td>
<td>0.21 ± 0.01 ***</td>
</tr>
<tr>
<td>7-ketocholesterol</td>
<td>0.24 ± 0.01</td>
<td>0.33 ± 0.02 ***</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM, control (n = 8), STZ (n = 9). Statistical analysis is performed by Unpaired Student’s t-test. *p < 0.05. **p < 0.001.

Romano et al., 2016 J Steroid Biochem Mol Biol. [Epub ahead of print]
Effects of short-term diabetes on cholesterol bioavailability

Student's t test * $P < 0.05$; ** $P < 0.01$

Romano et al., 2016 J Steroid Biochem Mol Biol. [Epub ahead of print]
Effects of short-term diabetes on cholesterol bioavailability

Student’s t test * P < 0.05; ** P < 0.01

Romano et al., 2016 J Steroid Biochem Mol Biol. [Epub ahead of print]
Short-term diabetes induces reactive oxygen species and mitochondrial alterations

Student’s t test * P < 0.05; ** P < 0.01

Romano et al., 2016 J Steroid Biochem Mol Biol. [Epub ahead of print]
• Short-term diabetes affects the levels of PREG, PROG, THP, ISOPREG, T, DHT and 3α-diol and are associated with a decreased expression of steroidogenic molecules, such as StAR, P450sc and 5α-R type 1, suggesting that diabetes alters steroidogenesis and steroid metabolism in the hippocampus.

• Impaired hippocampal cholesterol homeostasis and mitochondrial dysfunction may contribute to the modification in steroid levels induced by diabetes.

• Our findings suggest that alterations in cholesterol synthesis and metabolism in the diabetic brain may be a relevant factor for the development of diabetic encephalopathy.
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