Stress affects survival of adrenal gland stem cells through leptin in Sprague Dawley rats

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Background:
Adrenal gland involvement in stress
Introduction:
Stress and leptin

• The leptin is a hormonal product of ob gene which acts through leptin receptor (Ob-R)
• Secreted by adipose-tissue
• Leptin receptor is concentrated in the hypothalamus (but widespread through the brain)
• Satiety
• Ob-R is present in adrenal gland (glucocorticoid suppression during stress)
Adrenal gland structure

Functional zonation of adrenal gland: zona glomerulosa (zG), zona fasciculata (zF), zona reticularis (zR), undifferentiated cells zone (zU) and medulla(M). Mitani et al. 2003.

Aim of the Study

• To assess the changes of Ob-R expression during stress in Sprague Dawley rats

• In this study we hypothesize that leptin plays an important role in adrenal gland stem cells survival through its receptor (Ob-R) upon acute and chronic stress
Methods

- 22 male, 24 female (NON-OVX) and 24 ovariectomized female (OVX) of 19 weeks Sprague Dawley rats were used in the study. Animals were further divided in control (sham), acute and chronic stress groups.

- Acute stress was performed by 1 hour cold restraint in metal tubes at +4°C

### Protocol of chronic stress

<table>
<thead>
<tr>
<th>STRESS SESSION 1</th>
<th>STRESS SESSION 2 and STRESS SESSION 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day</td>
<td>Stressor</td>
</tr>
<tr>
<td>1</td>
<td>Food deprivation overnight (14-18h in total)</td>
</tr>
<tr>
<td>2</td>
<td>GTT test (baseline measurement)</td>
</tr>
<tr>
<td>3</td>
<td>60 minutes of restraint in metal tubes at +4°C and food deprivation</td>
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<tr>
<td>4</td>
<td>GTT test (acute stress reference)</td>
</tr>
<tr>
<td>5</td>
<td>Overnight lights on</td>
</tr>
<tr>
<td>6</td>
<td>60 minutes of cage rotation</td>
</tr>
<tr>
<td>7</td>
<td>3 minutes of forced swimming</td>
</tr>
<tr>
<td>8</td>
<td>Overnight lights on with irregular noise</td>
</tr>
<tr>
<td>9</td>
<td>Exposure to cat's odor and food deprivation</td>
</tr>
<tr>
<td>10</td>
<td>GTT test (chronic stress reference)</td>
</tr>
</tbody>
</table>

Examples of stressors
Methods

- Adrenal glands of 7-months-old rats were immunostained with leptin receptor (Ob-R) primary antibody and secondary FITC-conjugated fluorescent antibody
- DAPI was used to stain nuclei
- Images were photographed by confocal microscope and quantified by ImageJ software
Immunostaining of stem cells zone with Ob-R

Tukey test (▲ for males compared to NON-OVX, F=6.56; * for NON-OVX compared to OVX, F=6.2).

**Kruskal-Wallis test: males ($\chi^2=12.31; p=0.002$), NON-OVX ($\chi^2=12.11; p=0.002$) and OVX ($\chi^2=15.15; p=0.0005$).
Conclusion

• Leptin might be involved in general stress regulation at the level of adrenal gland
• Since leptin regulates glucose uptake and inhibits stress-induced apoptosis this finding might indicate its important role in stem cells survival and long-term effect on stress response by adrenal gland

• Future experiments: long term survival of adrenal gland stem cells labeled with Ob-R/BrdU
Thank you for your attention!

Marija Heffer
Srećko Gajovič
Irena Labak
Vedrana Ivić
Senka Blažetić