Enhanced Expression And Activity of NAD(P)H Oxidase in Mouse Periaqueductal Gray Tissue During Morphine Antinociceptive Tolerance

Department of Pharmacology & Toxicology

Virginia Commonwealth University

Emily C. Wright
Background: Periaqueductal Gray (PAG)

- Area surrounding cerebral aqueduct in brain stem levels 9 and 10
- Contains receptors for opiate peptides which can eliminate the perception of pain
Background: Known Effect of Morphine on PAG

- Pain reduction takes place when opiates turn on inhibitory neurons in PAG.
- Antinociceptive tolerance may result from perpetual action of opiates on PAG.
- Morphine causes increase in intracellular [Ca+] in the PAG in chronic morphine treatment (CMT) mice.
Role of NAD(P)H Oxidase in Morphine Induced Tolerance

- Morphine
- NAD(P)H
- H+ → H2O2
- extracellular
- cytoplasm
- membrane
- analgesia
- ONOO-
- NO
- 2O2
- gP91
- P22
- P67
- P47
- Rac
- Na+
- H+
Question

- Is NAD(P)H oxidase (subunits p47 and NOX-2) present in the PAG?
  - Approach: Immunohistochemistry
    (process used to localize proteins in cells of tissue sections)
Hypothesis

NAD(P)H oxidase plays an important role in morphine-induced tolerance.
Western Blot Analysis of the NOX-2 subunit of NAD(P)H Oxidase in PAG

gp91\text{phox} protein expression (Ratio to $\beta$-actin)

Vehicle Morphine

PAG Cortex
Western Blot Analysis of the p47 subunit of NAD(P)H Oxidase in PAG

- **47kDa**
  - p47phox
  - β-actin

- **45kDa**

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>V</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Vehicle</th>
<th>Morphine</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cortex</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Gene Expression Level of the NOX-2 subunit of NAD(P)H Oxidase in PAG

Expression of gp91phox mRNA ($T_n$)
Gene Expression Level of the p47 subunit of NAD(P)H Oxidase in PAG

Expression of p47phox mRNA (Tn)
Protocol

- 3 groups of mice: naïve, placebo pellet, and morphine pellet (morphine tolerant)
- Performed a two-day immunohistochemistry protocol that included over-night incubation with the primary antibody
- Qualitatively analyzed results by taking pictures of images obtained by microscope
Results

Figure 1: Expression of the p47 antigen in the periaqueductal gray and cortex of placebo pellet mouse brain tissue. A) 400X magnification. B) 1000X magnification.
Figure 2: Expression of the NOX-2 antigen in the periaqueductal gray and cortex of placebo pellet mouse brain tissue. A) 400X magnification. B) 1000X magnification.
Conclusion

- NAD(P)H oxidase is present in the PAG of mice brain tissue
Future Direction

- Perform ESR to detect the levels of superoxide in the PAG
- Perform HPLC to assess the functioning of NAD(P)H Oxidase in the PAG
Results

Figure 3: Expression of the NOX-1 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification
Figure 4: Expression of the NOX-1 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification
Results

Figure 5: Expression of the NOX-2 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification.
Results

Figure 6: Expression of the NOX-2 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification.
Results

Figure 7: Expression of the NOX-3 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification
Figure 8: Expression of the NOX-3 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification
Figure 9: Expression of the NOX-4 antigen in the cortex and medulla of rat kidney tissue. A) 400X magnification. B) 1000X magnification
Results

Figure 10: Expression of the NOX-4 antigen in the cortex and medulla of mouse kidney tissue. A) 400X magnification. B) 1000X magnification
Conclusion

- There are some differences between rat and mouse kidney tissue in their expression of the NOX isoforms.
Future Direction

- Positive controls for NOX-3 and NOX-4 antigens in mice and rat kidney tissue
Acknowledgements

- Dr. Pin-Lan Li, M.D., Ph.D.
- Dr. William Dewey, Ph.D.
- Labs of Dr. Li and Dr. Dewey
- Program for Summer Research Experience of Undergraduates in Pharmacology & Toxicology
Bibliography