The Role of Agonal Factors in Human Postmortem CNS Research

Daniel W. McKeel, Jr., M.D.
ADRC Neuropathology Tissue Resource
Washington U. School of Medicine
St. Louis MO - 6/23/04
Category 4 citations were examined in detail for factors pertinent to using ADRC postmortem brain material for biochemical research
### Identified Agonal Factors

<table>
<thead>
<tr>
<th>Identified Agonal Factors</th>
<th>mRNA heterogeneous factor effects add to varying stability on yield and quality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coma, MOF, respiratory arrest, hypox.</td>
<td></td>
</tr>
<tr>
<td><strong>Brain pH</strong> <em>(need a standard method!)</em></td>
<td></td>
</tr>
<tr>
<td>PMI</td>
<td></td>
</tr>
<tr>
<td>Febrile state</td>
<td></td>
</tr>
<tr>
<td>Terminal medications</td>
<td></td>
</tr>
<tr>
<td>Age and Gender</td>
<td></td>
</tr>
<tr>
<td>Brain lobe <em>(regional)</em></td>
<td></td>
</tr>
</tbody>
</table>

- Low: depressed energy, proteolysis
- **High**: elevated stress, transcription factors

- We observed a remarkable degree of natural variation among 120 samples, which represented three brain regions in 40 subjects.

- Individuals who suffered prolonged agonal states, such as with respiratory arrest, multi-organ failure or coma, tended to have lower pH in the brain.

- Those who experienced brief deaths, associated with accidents, cardiac events or asphyxia, generally had normal pH.

- The lower pH samples exhibited a systematic decrease in expression of genes involved in energy metabolism and proteolytic activities, and a consistent increase of genes encoding stress-response proteins and transcription factors.

- Coma and hypoxia do affect RNA integrity and gene expression profiles more than age, gender & postmortem factors. Propose “Average Correlation Index” to reduce specimen variability.
Many factors affect mRNA


- TaqMan RT-PCR measured 7 mRNAs
- 90 AD and 81 control brains (lobar mRNA same)
- Females had less mRNA than males
- Brain pH & amount of RNA (+) corr. except GFAP
- “Agonal state a poor predictor of mRNA levels”

- Bryan ADRC Rapid Autopsy Program at Duke
- **10 AD + 9 Controls** (1 to 11 hr PMI)
- **19 brains** RNA integrity + mRNA gene expression (CSF pH, fever/sepsis, O₂, sudden?)
- “**All samples yield intact RNA without degradation**” (“successful gene expression may require enhanced procurement efforts”)

- Choline transporter degrades rapidly
- Brains acquired within 2 hours of death
- Choline transporter *increased* in AD cortex compared to non-AD controls
- Putamen used as a “spared” control region
What Can NACC/NIA Do? [1]

- Advertise its frozen brain resources!
- Broker tissue distribution requests that involve multiple ADRCs (clearing house)
- Encourage collaborative grants & symposia to standardize frozen tissue collection methods
- Explore feasibility of regional specialized brain banks (genomics, proteomics, laser capture microdissection analysis of single and pooled cells) -- *rigid acceptance criteria for specimens*
What Can NACC/NIA Do? [2]

- Gather and distribute center-specific specimen requests and distribution data!
- Tabulate center-specific practical experience with using agonal factor data to facilitate research.
- Agonal factor use and outcome research within the ADRC community - what factors matter?
- Add agonal factors to the NACC database and make this data widely available to investigators
What Can ADRC Pathologists Do?

- Use existing brain banking protocols to formulate a standard protocol for all centers.
- Develop standard tissue block label protocols to facilitate collaborative ADRC research.
- Adopt the McKeel-Gado visual stds-based system (*Brain Pathol 1994*) for scoring brain atrophy and ventricular dilatation at autopsy.
- Develop standard CSF collection protocols.
- Add banked CNS/CSF requests received and fulfilled to the NACC-reportable data.
Frozen Human Brain Protocols

  - Aluminum plates chilled with dry ice (CO2)
  - CNS suitable for LM, EM + biochemistry

  - Aluminum plates chilled with dry ice (CO2)
  - Top plate to flatten specimen (coronal slices)
  - Standardized block sampling protocol
WUSM ADRC Standard Blocks

1. Frontal cortex
2. STG + MTG
3. Inf. Parietal ctx
4. Primary visual
5. Hippocampus/ERC
ten levels
6. Striatum
7. Mamillary bodies
8. Thalamus
9. Nigra, rostral
10. Nigra, caudal
11. Pons, 3 levels
12. Medulla, 2 levels
13. Spinal Cord
14. Cbellum + Dent. N.
15. Cbellar vermis
16. Hypothalamus

17. Nucleus basalis
18. Orbitofrontal ctx
19. Ant. Cingulate
20. Inf. Temporal ctx
21. Primary motor ctx
22. Primary sensory ctx
23. Amygdala
24. Olfact. Tract & Bulb & ant. olf. nucleus
25. Optic chiasm & nerve
26. WM, deep frontal
27. WM, mid portion
28. WM, occipital
29. Caudate, putamen & globus pallidus
30. Posterior cingulate
31ff - Pathologic lesions
Standardized Immunohistochemistry

- At present no standardization exists in IHC methodology among ADRCs
- Includes fixation, embedding materials, pretreatment protocols, reagent sources, antibody working titers, substrates used, etc.
- Hence results vary non-systematically and adversely affect comparisons among results obtained at various centers.
CDR 0 Hipp: 10D5 Aβ + PHF1

Braak & Braak Neurofibrillary STAGE III
CDR 3 AD Hipp: 10D5 Aβ + PHF1

Braak Stage VI
There is lots of Work to do!

Standardization now will yield major dividends in the future.