Role of Animal Models

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Urinary Incontinence in the Elderly: A Translational Research Agenda for a Complex Geriatric Syndrome

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Disclosures

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  - NIDDK LURN Network
  - VA RR&D SPiRE
  - VA RR&D SPiRE

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  - Invited Speaker for Allergan
  - Consultant for Synergy Pharma and InVivo Pharma
  - Patent royalties from Lipella Pharma

• **Conflicts of interest**
  - None
Outline

• Animal models for basic and translational research
  – Species differences in LUT anatomy and physiology
  – Rodents as research models
  – Animal models of aging
  – Age-related changes in LUT function

• Measurement of LUT function
  – Cystometric Measurement of the Lower Urinary Tract
    • The Micturition Cycle
    • Open Cystometry
    • Closed Outlet
  – Metabolism Cage/VSOP
  – LPP

• Conclusions
Animal models for basic and translational research

- Species differences in LUT anatomy and physiology

  Muriform Rodents  
  - Female Urethra
  - Pelvis
  - Levator Muscles
  - Autonomic Ganglia
  - Sleep Patterns
  - Work the Tail
  - Extramural
  - Nocturnal

  Humans
  - Form Pelvic Floor
  - Intramural
  - Diurnal

Nocturnal
Animal models for basic and translational research

- Species differences in LUT anatomy and physiology

Muriform Rodents

Humans

- EUS EMG
- EO EMG
- BP

Q-Uflow1
EMG
Pdet
Pves
Animal models for basic and translational research

- Species differences in LUT anatomy and physiology

Special Circumstances - SCI
Animal models for basic and translational research

- Species differences in LUT anatomy and physiology

In quadrupedal animals, gravity directs urine to the ventral abdominal wall. In bipeds, gravity directs urine through the outlet.
Outline

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  – Age-related changes in LUT function

• **Measurement of LUT function**
  – Cystometric Measurement of the Lower Urinary Tract
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• Conclusions
Animal models for basic and translational research

• Rodents as research models
  – Rodents are more closely related to humans than dogs, cats or pigs !!!
Animal models for basic and translational research

- Rodents as research models
  - Rodents are more closely related to humans than dogs, cats or pigs
  - However, rodents are not always reliable as preclinical models for human disease and the scientific literature is littered with examples of drugs that worked well in animals but turned out to be ineffective in clinical trials on humans.
  - This is in part due to differences in
    - Anatomy and physiology
    - Drug metabolism
    - Structure Activity Relationships (SAR) between species-specific receptor modifications and a constant structure drug candidate
    - Off-target effects that may contribute to species-specific outcomes
  - It is also due, in part, to methodology and interpretation of results failing to account for species differences
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Animal models for basic and translational research

- Animal models of aging
  - Aging (health span) is similar across species when normalized to life span

Animal models for basic and translational research

- Animal models of aging
  - NIA Rodents Available for Aging Research

<table>
<thead>
<tr>
<th></th>
<th>% Survival</th>
<th>90%</th>
<th>75%</th>
<th>50%</th>
<th>25%</th>
<th>10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F344-cdf Males</td>
<td>14 mo.</td>
<td>19 mo.</td>
<td>22 mo.</td>
<td>25 mo.</td>
<td>27 mo.</td>
<td></td>
</tr>
<tr>
<td>F344-cdf Females</td>
<td>16 mo.</td>
<td>21 mo.</td>
<td>25 mo.</td>
<td>29 mo.</td>
<td>31 mo.</td>
<td></td>
</tr>
<tr>
<td>F344BN Males</td>
<td>25 mo.</td>
<td>29 mo.</td>
<td>34 mo.</td>
<td>37 mo.</td>
<td>38 mo.</td>
<td></td>
</tr>
<tr>
<td>F344BN Females</td>
<td>23 mo.</td>
<td>26 mo.</td>
<td>30 mo.</td>
<td>34 mo.</td>
<td>36 mo.</td>
<td></td>
</tr>
<tr>
<td>BN Males</td>
<td>22 mo.</td>
<td>27 mo.</td>
<td>32 mo.</td>
<td>34 mo.</td>
<td>36 mo.</td>
<td></td>
</tr>
<tr>
<td>BN Females</td>
<td>22 mo.</td>
<td>27 mo.</td>
<td>32 mo.</td>
<td>35 mo.</td>
<td>38 mo.</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57BL/6 Males</td>
<td>19 mo.</td>
<td>24 mo.</td>
<td>27 mo.</td>
<td>30 mo.</td>
<td>32 mo.</td>
<td></td>
</tr>
<tr>
<td>C57BL/6 Females</td>
<td>18 mo.</td>
<td>22 mo.</td>
<td>25 mo.</td>
<td>28 mo.</td>
<td>30 mo.</td>
<td></td>
</tr>
<tr>
<td>DBA/2 Males</td>
<td>16 mo.</td>
<td>22 mo.</td>
<td>25 mo.</td>
<td>28 mo.</td>
<td>29 mo.</td>
<td></td>
</tr>
<tr>
<td>DBA/2 Females</td>
<td>8 mo.</td>
<td>16 mo.</td>
<td>23 mo.</td>
<td>26 mo.</td>
<td>29 mo.</td>
<td></td>
</tr>
</tbody>
</table>

### Animal models for basic and translational research

- **Animal models of aging**
  - Comparison of results of interventions in mice and humans

<table>
<thead>
<tr>
<th>Interventions/genetic modifications</th>
<th>Mean lifespan extension (%) in the mouse</th>
<th>Effects on age parameters and age-related disease in the mouse</th>
<th>ref mouse</th>
<th>Reports in humans</th>
<th>ref humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloric restriction</td>
<td>35–65 both sexes</td>
<td>Delays the onset and/or slows the progression of most age-associated diseases including neoplastic, degenerative and immune diseases.</td>
<td>(Weindruch, 1996; Weindruch et al., 1986) (Liao et al., 2010)</td>
<td>No definite data that CR prolongs life span but data on the health benefits are promising.</td>
<td>(Roth and Polotsky, 2012)</td>
</tr>
<tr>
<td>Methionine restriction</td>
<td>30 females 17 males</td>
<td>Lower rate of age-related change in T-cell subsets and slower development of cataracts.</td>
<td>(Miller et al., 2005) (Sun et al., 2009)</td>
<td>Vegetal-based low methionine diets can increase life expectancy up to 10 years compared to people consuming typical Western diets.</td>
<td>(Singh et al., 2003) (Tognon et al., 2011)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>None</td>
<td>Shifts the physiology of middle-aged mice on a high-caloric diet towards that of mice on a standard diet and prevents their early mortality. Delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending lifespan.</td>
<td>(Baur et al., 2006) (Miller et al., 2011) (Pearson et al., 2008)</td>
<td>Reduced risk for coronary heart disease and a possible extension of life span was found in populations consuming wine with a higher amount of resveratrol. Reseveratrol-like compounds promote beneficial changes in health.</td>
<td>(Corder et al., 2006) (Chachay et al., 2011) (Smoliga et al., 2011)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>8 for males, no extension females</td>
<td></td>
<td>(Strong et al., 2008)</td>
<td>Aspirin use was associated with lower risks of cancer incidence and mortality.</td>
<td>(Bardia et al., 2007)</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>9 males and 14 females 10 males and 18 females</td>
<td></td>
<td>(Harrison et al., 2009) (Miller et al., 2011)</td>
<td>Possible anti-cancer effect.</td>
<td>(Sharp and Strong, 2010)</td>
</tr>
</tbody>
</table>

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Animal models for basic and translational research

- Animal models of aging
  - Affects of Aging on LUT Function – Conscious cystometry in F344 rats

<table>
<thead>
<tr>
<th></th>
<th>Y 6 moa</th>
<th>O+AL 25-28 moa</th>
<th>O+CR 25-28 moa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravesical pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voided volume</td>
<td>VV 0.81 ml</td>
<td>VV 0.59 ml</td>
<td>VV 0.78 ml</td>
</tr>
<tr>
<td>RV 0 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 cmH₂O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4 ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM Y</th>
<th>Mean ± SEM O+AL</th>
<th>p Value vs O+AL</th>
<th>p Value vs O+CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pressure (cm H₂O):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>5.20 ± 0.39</td>
<td>7.02 ± 1.56</td>
<td>0.547</td>
<td>5.29 ± 1.24</td>
</tr>
<tr>
<td>Threshold</td>
<td>10.43 ± 0.94</td>
<td>21.15 ± 3.57</td>
<td>0.018*</td>
<td>16.55 ± 2.14</td>
</tr>
<tr>
<td>Max</td>
<td>47.50 ± 2.94</td>
<td>52.32 ± 4.62</td>
<td>0.724</td>
<td>43.16 ± 4.86</td>
</tr>
<tr>
<td>Vol (ml):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Voided</td>
<td>0.71 ± 0.03</td>
<td>0.67 ± 0.068</td>
<td>0.869</td>
<td>0.77 ± 0.07</td>
</tr>
<tr>
<td>Residual</td>
<td>0.022 ± 0.007</td>
<td>0.11 ± 0.025</td>
<td>0.009*</td>
<td>0.035 ± 0.018</td>
</tr>
<tr>
<td>Bladder capacity (ml)</td>
<td>0.74 ± 0.03</td>
<td>0.78 ± 0.06</td>
<td>0.864</td>
<td>0.81 ± 0.07</td>
</tr>
<tr>
<td>Mean flow rate (ml/sec)</td>
<td>0.130 ± 0.008</td>
<td>0.126 ± 0.008</td>
<td>0.523</td>
<td>0.128 ± 0.007</td>
</tr>
<tr>
<td>Voiding efficiency (%)</td>
<td>97.21 ± 0.74</td>
<td>85.38 ± 3.11</td>
<td>0.014*</td>
<td>95.51 ± 2.68</td>
</tr>
<tr>
<td>Bladder compliance (ml/cm H₂O)</td>
<td>0.22 ± 0.05</td>
<td>0.08 ± 0.02</td>
<td>0.044*</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>NYCs:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No./min</td>
<td>0.27 ± 0.11</td>
<td>1.20 ± 0.36</td>
<td>0.034*</td>
<td>0.73 ± 0.18</td>
</tr>
<tr>
<td>Amplitude (cm H₂O)</td>
<td>5.57 ± 0.30t</td>
<td>5.89 ± 0.65</td>
<td>0.950</td>
<td>6.32 ± 0.78</td>
</tr>
</tbody>
</table>

Ito et al., 2016. J Urol 196:1575-1583
An animal model of aging: Affects of Aging on LUT Function – Conscious cystometry in F344 rats

- Combined results suggest more of an overactive bladder condition, as functional bladder capacity would be expected to be decreased, NVC are increased and compliance is low
- This is consistent with their gene expression studies which demonstrated increases immune and inflammation pathways in the bladder and DRG

Ito et al., 2016. J Urol 196:1575-1583
Animal models for basic and translational research

- Animal models of aging
  - Affects of Aging on LUT Function – Anesthetized cystometry in C57BL6 mice

These data are consistent with ageing-induced underactive bladder !!!

Species difference, conscious vs. anesthesia difference, cystometric technique difference ???
Animal models for basic and translational research

- Affects of Aging on LUT Function – Anesthetized cystometry in 18 moa SD rats
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• Conclusions
Cystometry - The Micturition Cycle

Common Descriptors

\[ \text{Compliance} = \frac{\Delta V}{\Delta P} \]

- Maximum Voiding Pressure
- Pressure Threshold
- Bladder Filling
- Micturition

Compliance = ΔV/ ΔP
Cystometry - The Micturition Cycle

Better Descriptors

Pressure at Volume Threshold

Compliance = $\Delta V / \Delta P$

Opening Pressure
Cystometric traces during conscious, restrained cystometry in a chronic SCI rat – The top trace is from the vehicle control period, while the bottom trace is from the period following 100 µg/kg of CL-316,243.
What is Maximal Voiding Pressure?

Conclusions about the actual voiding contraction are not so straightforward.

Need to understand the anatomy of the voiding contraction:

- **Phase I** – Isovolumetric Contraction
- **Phase II** – Entire LUT open to external environment during peak detrusor contraction
- **Phase III** – Isovolumetric Relaxation

Pressure-Flow relationships can be explored during Phase II

Maggi et al, 1986
Human OP and CP also Discernable
Easy Bladder Contraction

OP  VP  CP

Ph I  Ph II  Ph III

Easy Bladder Contraction
Ambiguous Bladder Contraction – Tonic EUS gives False OP*

OP
VP
CP
Ph I
Ph II
Ph III

*VP

Ambiguous Bladder Contraction – Tonic EUS gives False OP*
Ambiguous Bladder Contraction – “Missing” OP

OP  VP  CP

Ph I  Ph III
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Open Cystometry Protocol

Saline Infusion, Bladder Pressure

Carotid
Jugular

Transvesical Approach

EUS EMG

Saline Infusion, Bladder Pressure
What is Bladder Capacity

Continuous vs. Single Fill Cystometry

- Continuous open cystometry is the current method of choice by many researchers.
- Allows for the determination of functional bladder capacity (FBC), as defined as infusion flow rate x ICI or IMI.
- However, it often underestimates true bladder capacity (TBC), which is best determined by single fill cystometrograms.
- By combining the approaches, as shown above, one can determine voiding efficiency easily by the equation: \( %VE = \text{mean FBC/TBC} \times 100 \)
Response to Drugs

- FBC decreases with atropine
- TBC increases !!! Decreased FBC due to decreased voiding efficiency.

If had only performed continuous open cystometry, might misinterpret effect as mild irritation or sensitization of reflex voiding !!!
Response of the Bladder to Filling: Biomechanical Considerations

- **Rate dependency** – slow strain causes lesser increase in force than fast strain – or - rapid filling results in decreased compliance
- **Time dependency** – It takes longer to reach equilibrium pressure if strain is faster
- **Hysteresis** – the pressure-volume relationship (force curve) is different – Viscoelasticity!

Flow rate affects the compliance measurements!

Coolsaet 1985
Response of the Bladder to Filling: Measurement System Considerations

- Flow rates matter not only to tissue biomechanics, but also to recordings
  - Resistance of the filling and recording catheter affects the pressure baseline as well as the fidelity of recording during filling
  - Effects become worse with increased fill rate
Transvesical Filling

Transvesical (cm H2O)
-0 10 20 30 40 50 60

Transurethral (cm H2O)
0 10 20 30 40 50

Baselines

Detail

2/19/2010 2:54:39.992 PM
Transureteral Filling

Transvesical (cm H2O)
10
20
30
40
50
60

Transurethral (cm H2O)
0
10
20
30
40
50
60

2/19/2010 3:08:58.075 PM

3:09:00 PM 3:09:30 PM 3:10:00 PM 3:10:30 PM 3:11:00 PM 3:11:30 PM 3:12:00 PM 3:12:30 PM 3:13:00 PM 3:13:30 PM 3:14:00 PM 3:14:30 PM
Response of the Bladder to Filling: Measurement System Considerations

• Placement of catheters may affect dynamic active measurements
  – The top-down contraction of the dome may occlude the catheter tip in transvesical filling and recording
Transvesical Filling

Traces are from transvesical double-lumen catheters with a static internal lumen for pressure recording.

Arrows Point to Apparent Closing Pressures
Transvesical Filling – False CP

False closing pressures (red arrows) may be due to bladder contraction from top-down, creating transient seal around transvesical filling/recording catheter tip.
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Simultaneous Isolated Bladder and Urethra


**Bladder Pressure**

**Urethral Perfusion Pressure**

**Bladder Pressure**
Rat UPP (3-Way System)

- Urethral Saline Infusion
- UPP Recording
- Bladder Filling/Recording
- EUS EMG
- Ureteral Drainage
- Carotid
- Jugular
Isovolumetric IVP and UPP

- Allows for pharmacological dissection of Active State players in the physiology of LUT function – External Urethral Sphincter contribution

- Note no change in the dynamic active responses of the bladder to isovolumetric conditions (constant volume distension)
NO-Mediated Relaxation

- Allows for pharmacological dissection of active players in the physiology of LUT function – Parasympathetic NO relaxation of urethral smooth muscle.

- Note no change in the dynamic active responses of the bladder to isovolumetric conditions (constant volume distension)
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Metabolism Cage / VSOP

- Measurement of LUT function

Both measure functional bladder capacity, similar to a bladder diary for humans. VSOP suffers from short sampling window and need to fix time due to diurnal variation.

2 moa SD Rats, 3 days

7 woa ddy mice, 2 hr
Sugino et al. 2008 NUU 27:548-552
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Leak Point Pressure Measurements

- **Leak Point Pressure Measurement**
  - Developed a method whereby bladder pressure could be increased in an experimental animal without evoking a micturition reflex
    - Does not require rat to cough or sneeze, animal can be supine or vertical
    - Includes entire outlet (from bladder neck through meatus)
    - Incremental increases in pressure until bladder pressure exceeds outlet resistance → Leak!
    - Affected by both striated and smooth muscle surgical and pharmacological manipulations
Rat Leak Point Pressure

Jugular

Pressure Clamp, Pressure Transducer

EUS EMG

Graph showing Leak Point Pressure (cm H2O) for different conditions: Control, PudX, PudX+Sling, PudX+Coll.
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Conclusions

• Significant species differences in anatomy and normal physiology, drug metabolism and SAR
• These differences must be addressed, understood and accounted for in order to interpret experimental results properly
• All animal appear to age similarly (health span/life span)
• Species and/or approach differences may yield seemingly disparate results, only by head-to-head comparison can these seeming differences be parsed out
• A variety of techniques are available for measuring LUT function, proper interpretation depends on in depth consideration of LUT physiology and measurement technique interaction with it
End