Interactions between bone and the central nervous system

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Estrogen  
Calcitonin  
...

Bone marrow cells

MCSF  
RANKL  
Ephrins  
IL  
SDF1

Estrogen  
Calcitonin  
...

Bone marrow cells

MCSF  
RANKL  
Ephrins  
IL  
SDF1

Osteoclasts  
Osteoblasts

Insulin  
IGF1  
PTH  
PTHrP  
BMP  
...

Bone resorption  
Bone formation
BONE REMODELING

NEURONAL CLUES

Estrogen
Calcitonin
...

Bone marrow cells

MCSF
RANKL
Ephrins
IL
SDF1
...

Insulin
IGF1
PTH
PTHRp
BMP
...

BONE RESORPTION
BONE FORMATION

Osteoclasts
Osteoblasts
THE BONE AND BRAIN CONNECTION

• Traumatic brain injury promotes ectopic bone formation and bone healing

• Bone formation and resorption markers display circadian patterns

• Bone are innervated

• Stroke, spinal injury and peripheral neuropathies provoke bone loss

• Obesity is associated higher BMD
BONE REMODELING IS AN HOMEOSTATIC PROCESS

REGULATED BY

THE CENTRAL NERVOUS SYSTEM
A model of hypothalamic dysfunction: The ob/ob mice

- Body weight
- Reproduction
- Immunity

WT ob/ob

Adipocytes → Leptin
Leptin is an inhibitor of bone formation

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>ob/ob</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Vol. (%)</td>
<td>14.0 ±0.4</td>
<td>19.4 ±0.6*</td>
</tr>
<tr>
<td>BFR</td>
<td>64.6 ±18.0</td>
<td>110.3 ±15.2*</td>
</tr>
</tbody>
</table>
Does leptin inhibit bone formation via a central relay?

Leptin intracerebroventricular infusion (ICV)
(8 ng/h, 28 days treatment)
Central versus peripheral?

Arcuate nucleus (Arc)

Ventromedial hypothalamus (VMH)

3rd Ventricle

Arcuate nucleus (Arc)
Central leptin delivery corrects the bone phenotype of ob/ob mice

<table>
<thead>
<tr>
<th>PBS</th>
<th>Leptin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>0</td>
</tr>
<tr>
<td>Bone Vol. (%)</td>
<td>18.3 ± 0.3</td>
</tr>
</tbody>
</table>
Leptin binds to ObRb in the hypothalamus.
Goldthioglucose destroys VMH neurons

CONTROL GTG

Nissle stain

SF1

NPY
VMH neuron destruction increases bone mass in WT mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bone Vol. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>14.0 ± 0.6</td>
</tr>
<tr>
<td>ob/ob</td>
<td>19.4 ± 0.6*</td>
</tr>
<tr>
<td>GTG</td>
<td>19.3 ± 0.6*</td>
</tr>
</tbody>
</table>
Lack of ObRb in neurons, not osteoblasts, increases bone mass

Y. Shi and al, PNAS 2008
Hypothalamic Y2R inhibits bone formation

P. Baldock and al, JCI 2002
How does the hypothalamus regulate osteoblast function?

Soluble factor → nerves → ? → bone
How does the hypothalamus regulate osteoblast function?
Leptin does not use a humoral pathway to control bone mass.
BONES ARE INNERVATED

- Neurons are detected in the bone micro-environment
- Retrograde viruses injected in bone label hypothalamic neurons
- Ob/ob mice have a low sympathetic tone
THE NEURONAL MODEL

SNS

Osteoblast

Bone mass

? Leptin

Bone mass
Lack of catecholamines increases bone formation

**WT**

<table>
<thead>
<tr>
<th>BV/TV (%)</th>
<th>10.2 ±0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFR</td>
<td>82.3 ±4.5</td>
</tr>
<tr>
<td>ObNb/BPm</td>
<td>9.6 ±0.7</td>
</tr>
</tbody>
</table>

**Dbh-/-**

<table>
<thead>
<tr>
<th>BV/TV (%)</th>
<th>13.2 ±0.8*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BFR</td>
<td>105.4 ±5.8*</td>
</tr>
<tr>
<td>ObNb/BPm</td>
<td>12.6 ±0.8*</td>
</tr>
</tbody>
</table>
THE NEURONAL MODEL

SNS → Osteoblast → Bone mass

Leptin

Bone mass?
The $\beta_2$-Adrenergic receptors is expressed by osteoblasts

Ob:  Primary osteoblasts  
C:  Positive control
Induction of cAMP production by βAR agonists in osteoblasts

- PBS
- Iso
- Iso + Pro
- NE
- PE
- PTH

cAMP levels Fold Induction

* Indicates statistically significant difference compared to control.
β-agonists decrease bone mass

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>Isoproterenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Vol. (%)</td>
<td>17.8 ±0.6</td>
<td>11.7 ±0.6 *</td>
</tr>
<tr>
<td>BFR/BS</td>
<td>143.4 ± 7.7</td>
<td>109 ± 11.1 *</td>
</tr>
<tr>
<td>ObNb/BPm</td>
<td>18.2 ± 0.7</td>
<td>12.2 ± 2.2 *</td>
</tr>
</tbody>
</table>
Lack of $\beta_2$AR increases bone formation

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>$\beta_2$AR-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV (%)</td>
<td>11.9 ±0.9</td>
<td>17.9 ±1.1*</td>
</tr>
<tr>
<td>BFR</td>
<td>253.8 ±20.3</td>
<td>354.1 ±1.1*</td>
</tr>
<tr>
<td>ObNb/BPm</td>
<td>13.6 ±0.8</td>
<td>20.5 ±1.1*</td>
</tr>
<tr>
<td>Body weight</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Insulin/leptin</td>
<td>normal</td>
<td>normal</td>
</tr>
</tbody>
</table>

*Significantly different from WT.
The SNS regulates osteoclastogenesis

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>Adrβ2-/-</th>
</tr>
</thead>
<tbody>
<tr>
<td>OcN/BPm</td>
<td>9.0 ±0.2</td>
<td>6.0 ±0.2*</td>
</tr>
<tr>
<td>OcS/BS</td>
<td>28.0 ±0.8</td>
<td>23.6 ±1.5*</td>
</tr>
<tr>
<td>Dpd</td>
<td>21.1 ±1.3</td>
<td>15.0 ±1.3</td>
</tr>
</tbody>
</table>
How does $\beta$2AR signaling stimulate osteoclast differentiation?

1. SNS

2. “Osteoclastogenic” cytokines

Osteoblast

Osteoclast
Adrenergic signaling in osteoblasts - not osteoclasts - favors osteoclastogenesis.

Clasts/well

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>Iso</th>
<th>PBS</th>
<th>Iso</th>
<th>PBS</th>
<th>Iso</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
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<td>wt</td>
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<td>0</td>
<td>wt</td>
<td>0</td>
<td>wt</td>
<td>0</td>
</tr>
</tbody>
</table>

Osteoblasts: wt wt wt wt -/- -/
Osteoclast progenitors: wt wt -/- -/- wt wt

* Significant difference.
Isoproterenol stimulates \textit{Rankl} expression

\textit{Rankl} expression (fold induction)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>ISO</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>1</td>
<td>++ -</td>
<td>- - +</td>
</tr>
<tr>
<td>2</td>
<td>*</td>
<td>- - +</td>
</tr>
<tr>
<td>4</td>
<td>*</td>
<td>- - +</td>
</tr>
<tr>
<td>6</td>
<td>*</td>
<td>- - +</td>
</tr>
</tbody>
</table>

\textit{WT Osteoblasts}

\textit{Adrβ2-/- Osteoblasts}

* indicates significant difference.
β2AR signaling in osteoblasts regulates *Rankl* expression via ATF4

Elefteriou & al., Nature 2006
β2AR signaling in osteoblasts regulates osteoblast proliferation via clock genes

Fu & al., Cell 2005
THE NEURONAL MODEL

SNS

Leptin

Osteoblast

Bone mass
Adrβ2 is required for leptin anti-osteogenic function

**Fat pad weight (mg)**

**BV/TV (%)**

PBS  Leptin

WT  WT  Adr  Adr
β2/-  β2/-  β2/-  β2/-

Fat pad weight (mg)

PBS  Leptin

WT  WT  Adr  Adr
β2/-  β2/-  β2/-  β2/-

* Indicates significant difference
Are $\beta$-blockers good for bones?

$\beta$-blocker

SNS

Osteoblast

Leptin

Bone mass
**β-blockers increase bone mass**

<table>
<thead>
<tr>
<th></th>
<th>PBS</th>
<th>Propranolol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone Vol. (%)</td>
<td>13.8 ±0.3</td>
<td>16.2 ±0.8*</td>
</tr>
<tr>
<td>BFR</td>
<td>116.8 ± 6.3</td>
<td>169.3 ± 8.6*</td>
</tr>
<tr>
<td>NbOb/BPm</td>
<td>19.0 ± 1.4</td>
<td>26.7 ± 2.4*</td>
</tr>
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</table>
Propranolol prevents bone loss following ovariectomy

<table>
<thead>
<tr>
<th>Group</th>
<th>Bone Vol. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sham</td>
<td>14.2 ± 0.8</td>
</tr>
<tr>
<td>OVX</td>
<td>12.1 ± 0.4*</td>
</tr>
<tr>
<td>OVX + β-blocker</td>
<td>14.1 ± 0.7</td>
</tr>
</tbody>
</table>
HUMAN CLINICAL DATA

• 2006 Meta-analysis: 28.5% reduction in hip fracture risk \( \text{(Wiens et al, 2006)} \)

• Some studies reporting no effects
  • Confounding factors
  • # of patients
  • Long-term vs short-term
  • \( \beta \text{AR selectivity/dose} \)
  • Skeletal sites

**Prospective studies are needed \( \text{($$$)} \)**
What is the role of $\beta$AR signaling in bone pathophysiology and diseases?

- **Bone Resorption**
  - $\beta$AR signaling
  - Elefteriou and al, Nature 2005

- **Bone Formation**
  - $\beta$AR signaling
  - Elefteriou and al, Nature 2005

- **Osteoblasts**
  - SNS outflow
  - Drugs
  - Beta AR

- **HSC Trafficking**
  - Katayama and al, Cell 2006

- **Insulin Secretion**
  - Hinoi and al, JCB 2009
• Major depression is associated with bone loss and increased fracture risk

• Associated with hypercorticolism and SNS activation
Stress/depression decreases bone mass through SNS activation

Yirmiya and al, PNAS 2006
POST-SYNAPTIC EFFECT:
The cannabinoid system

Tam and al, Mol Pharm 2006
CB1 STIMULATION IN PRE-SYNAPTIC NEURONS INHIBITS NE RELEASE

Traumatic brain injury → 2-AG → CB1

Tam and al., FASEB J., 2007
2-AG: 2-arachidonoyl glycerol
SUMMARY

✓ Diseases and clinical observations are great opportunities to unravel novel mechanisms

✓ Multiple genetic mouse models are critical to address hypotheses raised by such clinical observations and to demonstrate mechanisms

✓ Bone remodeling is regulated by the CNS and SNS

✓ Drugs/diseases affecting SNS outflow or β2AR signaling in osteoblasts alter bone remodeling and bone mass
Post-doctoral and student positions are open in the lab...

PROJECTS:
- SNS and bone
- Skeletal dysplasia in NF1
- Calorie restriction, sirtuins and bone
- Mechanism of cancer bone metastasis

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