Essential Function of Ghrelin in Chronic Starvation

Guosheng Liang

UT Southwestern Medical Center at Dallas
Ghrelin: An Acylated Peptide

Kojima & Kangawa
1999
Ghrelin $O$-Acyltransferase (GOAT)

- Identified in our lab by combined approach of bioinformatics and expression testing of candidate cDNAs in cultured endocrine cells.

- Candidate cDNAs: Family of 16 Membrane-Bound $O$-Acyltransferases (MBOATs) that transfer fatty acyl groups to lipids or proteins (ACAT, DGAT, Porcupine)

- MBOAT4 transfers octanoate from octanoyl-CoA to serine-3 of Proghrelin

- Eli Lilly group also identified MBOAT4 as the acytransferase that octanoylates ghrelin in the same year
Processing of Preproghrelin

* Site of Octanoylation
Processing of Preproghrelin in Endocrine Cell Lines

AtT-20: Mouse pituitary tumor cell
INS-1: Rat insulinoma cell line
MIN-6: Mouse insulinoma cell line

<table>
<thead>
<tr>
<th></th>
<th>AtT-20</th>
<th>INS-1</th>
<th>MIN-6</th>
<th>HEK-293</th>
<th>CHO-7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin (Gh)</td>
<td>Mock</td>
<td>Prepro-Gh</td>
<td>Mock</td>
<td>Prepro-Gh</td>
<td>Mock</td>
</tr>
<tr>
<td>Proghrelin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghrelin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Expression Testing of 16 MBOAT cDNAs in Transfected INS-1 Cells
Hydropathy Plot of Ghrelin O-Acyltransferase (GOAT)

Hydrophobicity

Hydrophilicity

Two catalytic residues: Asn, His

Amino Acid Residue Position
Expression of GOAT in Mouse Tissues

- Stomach
- Duodenum
- Jejunum
- Ileum
- Colon
- Liver
- Kidney
- Brain
- Heart
- Lung
- Skeletal Muscle
- Testis
- White Adipose
- Brown Adipose
- Control

GOAT

- 1018 bp
- 517 bp
- 298 bp

Preproghrelin

- 298 bp

β-actin

- 517 bp
Co-localization of GOAT with Ghrelin in Gastric Mucosa

Generation of Goat^{−/−} Mice

Diagram showing the targeting construct and the relative mRNA levels of GOAT and Preproghrelin in WT and Goat^{−/−} mice.

Bar graphs showing the plasma levels of Ghrelin and Des-acyl Ghrelin in Fed and Fasted states for WT and Goat^{−/−} mice. Each bar represents the mean ± SEM, with n = 10 Mice.
Body Weights on Different Diets

- **WT** (black circles)
- **Goat** (-/-) (red triangles)

- **High Fat Diet**
- **Chow Diet**

$n = 8$ Mice

**Graph**: Over the weeks on diet, the body weights of WT and Goat (-/-) mice on different diets are compared. The graph shows a clear trend of weight gain over time for both diets, with the Goats (-/-) generally maintaining a lower body weight compared to WT mice on both diets.
Metabolic Response to Food Deprivation in GOAT -/- Mice

60% Calorie Restriction

● Each mouse fed 40% of its daily food intake for 7-9 days

● Food supplied at 6 pm each day and eaten within 1 hr

● Blood glucose measured every day at 5:30 pm
  (22.5 hr after eating and 30 min before next feeding)
Body Weight and Fat Mass in WT and Goat -/- Mice

**Body Weight**

- **WT**
- **Goat -/-**

**Fat Mass**

- **WT**
- **Goat -/-**

$n = 6$ Mice

Diet Treatment (days)
Calorie-restricted *Goat -/-* Mice Exhibit Severe Hypoglycemia Before Next Meal

Blood Glucose at 5:30 pm (mg/dl)

- **WT**
- **Goat -/-**

- Fed
- 60% Calorie Restriction

*n = 6 Mice*
Plasma Ghrelin and Growth Hormone in *ad lib* Fed or Calorie Restricted WT and Goat *−/−* Mice
Response to Day-Long Starvation After 7 days of Calorie Restriction in *Goat/-* Mice

**Blood Glucose**
- WT (black) and *Goat/-* (red) lines.
- Arrow indicating the time of food intake.

**Plasma Ghrelin**
- n = 4 mice.
- Arrow indicating the time of food intake.

**Plasma Des-acyl Ghrelin**
- Arrow indicating the time of food intake.

**Plasma Growth Hormone**
- Arrow indicating the time of food intake.

**Time during Day 8**
- 9 am, 12 pm, 2 pm, 4 pm, 6 pm, 8 pm.

*Significant differences indicated by asterisks (***p < 0.001, *p < 0.05).*
**Preproghrelin**/− Mice Show Same Defect as **Goat**/− Mice

![Graph showing changes in blood glucose, plasma ghrelin, plasma des-acyl ghrelin, and plasma growth hormone in WT and **Preproghrelin**/− mice.](image)

- **Blood Glucose**
  - Over time, the blood glucose levels decrease and then increase again upon feeding.
  - The graph shows a comparison between WT and **Preproghrelin**/− mice, with the latter showing a consistent decrease in blood glucose levels.

- **Plasma Ghrelin**
  - The levels of plasma ghrelin increase significantly in both WT and **Preproghrelin**/− mice after feeding.
  - The peak levels are indicated by asterisks (***), showing a statistically significant increase.

- **Plasma Des-acyl Ghrelin**
  - Similar to ghrelin, the des-acyl ghrelin levels also show a significant increase upon feeding.

- **Plasma Growth Hormone**
  - The levels of growth hormone are shown to increase in both groups, with a peak indicated by asterisks.

**Legend**
- **WT** (black circles)
- **Preproghrelin**/− (red circles)

**Note:**
- *n = 4 mice*
- Data points are shown with error bars to indicate variability.
Prevention of Hypoglycemia by Infusion of Ghrelin or Growth Hormone

Osmotic Minipump

Flow rate – 1.2 µl/hr
Ghrelin – 30 µg/24 hr
GH – 15 µg/24 hr

Ghrelin or GH
Calorie Restriction

Days

-3 0 2 4 6 8
Prevention of Hypoglycemia by Infusion of Ghrelin

![Blood Glucose Graph](Image)

**Blood Glucose (mg/dl)**

WT

Ghrelin

Saline

Goat -/-

Ghrelin

Saline

**Calorie Restriction (days)**

**n = 5 Mice**

![Growth Hormone Graph](Image)

**Growth Hormone**

**Plasma Level (ng/ml)**

WT

Goat -/-

Day 7

5:30 pm

Infusion

Saline

Ghrelin

**Day 7 5:30 pm**
Response to Infusion of Growth Hormone

**Graph 1:**
- **WT** vs. **Goat -/-**
- Blood Glucose (mg/dl) vs. Calorie Restriction (days)
- **n = 5 Mice**
- **GH** and **Vehicle**

**Graph 2:**
- Growth Hormone - Day 8
- Plasma Level (ng/ml)
- **Infusion**
  - **Vehicle**
  - **GH**
- Day 8
  - 5:30 pm
How Does Starvation-Induced Hypoglycemia Stimulate Ghrelin Secretion?
Generation of Transgenic Ghrelin-SV40-T Mice

[Diagram showing the preproghrelin gene (164 kb) with exons labeled 1 to 5 and an SV40 T antigen insertion.]
Markedly Elevated Ghrelin Levels in Ghrelinoma-bearing Mice

![Graph showing plasma ghrelin levels over age of mice. The y-axis represents plasma ghrelin (ng/ml) ranging from 0 to 6, and the x-axis represents age of mice (weeks) ranging from 6 to 20. Two lines are shown: one for TgGhrelin-SV40-T with n=17 and another for wild-type with n=13. The TgGhrelin-SV40-T line shows a marked increase in ghrelin levels over time, while the wild-type line remains relatively flat.](E-2420 slide 3 ppt)
Norepinephrine Stimulates Ghrelin Secretion in Ghrelinoma PG-1 Cells

Ghrelin in Medium (pg/μg protein)

None, Endothelin1, Secretin, Insulin, GRP, Somatostatin, Glucagon, Leptin, IGF-1, Growth Hormone, FGF-21

Red bar: Norepinephrine
Stimulation of Ghrelin Secretion in PG-1 Cells by β-Adrenergic Agents

Ghrelin in Medium (pg/μg protein)

Compound (μM)

Forskolin
Epinephrine
Norepinephrine
Carbachol
Muscimol

<table>
<thead>
<tr>
<th>Agonists</th>
<th>half-maximal stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norepinephrine</td>
<td>0.1 μM</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>0.6 μM</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antagonists</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol (β₁)</td>
<td>Yes</td>
</tr>
<tr>
<td>ICI 118,551 (β₂)</td>
<td>No</td>
</tr>
</tbody>
</table>
## Relative Amounts of mRNAs in PG-1 Cells and SG-1 Cells

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Stomach WT</th>
<th>Stomach Ghrelinoma</th>
<th>PG-1 Cells</th>
<th>SG-1 Cells</th>
<th>Fold-increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preproghrelin</td>
<td>1.0 (21)*</td>
<td>7.1</td>
<td>5.5</td>
<td>7.7</td>
<td></td>
</tr>
<tr>
<td>GOAT</td>
<td>1.0 (29)</td>
<td>8.3</td>
<td>13.8</td>
<td>13.4</td>
<td></td>
</tr>
<tr>
<td>Prohormone Convertase 1/3</td>
<td>1.0 (28)</td>
<td>36</td>
<td>231</td>
<td>188</td>
<td></td>
</tr>
<tr>
<td>$\beta_1$ Receptor</td>
<td>1.0 (33)</td>
<td>138</td>
<td>416</td>
<td>138</td>
<td></td>
</tr>
<tr>
<td>$\beta_2$ Receptor</td>
<td>1.0 (29)</td>
<td>1.1</td>
<td>0.5</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

*Threshold value for RT-PCR (cycles)*
Suppression of Ghrelin Secretion in Fasted WT Mice by Reserpine and Atenolol

![Graph showing suppression of ghrelin secretion in fasted WT mice by reserpine and atenolol.](E-2457_slide.ppt)
Summary: Metabolic Response to Chronic Starvation

Starvation

- Blood Glucose
  - Glucose Sensing Neurons
  - Sympathetic Neurons

Survival

- Blood Glucose
  - Growth Hormone
    - Arcuate Hypothalamus
      - GHS-R
    - Pituitary
      - GHS-R

Ghrelin

Norepinephrine

$\beta_1$ Receptor
How Ghrelin Releases Growth Hormone

Hypothalamic Arcuate Neurons

Growth Hormone Releasing Factor

Ghrelin

Ghrelin Receptor

Pituitary Somatotrophs

Growth Hormone

Stomach

Antagonism by Somatostatin
Summary

One Essential Function of Ghrelin Is to Maintain Blood Sugar by Stimulating Growth Hormone Release in Times of Famine
Acknowledgements

Tong-Jin Zhao
Robert Li
Jing Yang
Michael Brown and Joseph Goldstein

Ichiro Sakata and Jeff Zigman
Ghrelin-SV40 Tg
Regeneron Pharmaceuticals
Tamas Horvath
GOAT and Ghrelin Knockout