Leptin-Insulin Signaling in the Brain

BY TEAM CEPHALIC
Aman Hamdard, Kevin Artiga, Megan Imreh, Ronald Baldonado, and Sharri Mo
Agenda

- Leptin in the Hypothalamus: Pathways and Roles
- Cross-talk between Insulin and Leptin
- Leptin & Obesity
- Is Insulin really necessary for survival? An experiment
- Signaling in other brain areas
- Clinical Implications
Leptin’s Role in the Hypothalamus

- **Main role: Energy Homeostasis**
  - Control food intake by inhibiting feeding behavior
  - Done through regulating orexigenic and anorectic peptide production
  - Regulation done through two main pathways: JAK/STAT and PI3K
- **Insulin also involved in Energy Homeostasis**
  - Leads to cross-talk between signaling pathways
Orexigenic and Anorexigenic Pathways

- Leptin is involved in both pathways
- Orexigenic
  - Increase appetite
  - Neurons involved in pathway use GABA, neuropeptide Y, and agouti-related peptide
- Anorexigenic
  - Decrease Appetite
  - POMC neurons → α-MSH released → agonist at MC4R → appetite decrease
The JAK/STAT pathway

- Mechanism for regulating peptide creation
- Ultimate goal is to regulate the genes responsible for the creation of anorexigenic/orexigenic peptides
- Used in the Central Melanocortin (Anorexigenic) Pathway
**SOCS3 in JAK/STAT**

- STAT3 translocation -> activation of SOCS3 (Suppressor of Cytokine Signaling 3)
- Provides negative feedback regulation on both leptin and insulin
  - Leptin: JAK2 negative feedback
  - Insulin: IRS (Insulin Receptor Substrate) negative feedback
  - Increase of SOCS3 occurs in obesity and states of leptin resistance
The PI3K pathway

- Leptin activity → anorexigenic effect
- AMPK (AMP-activated protein kinases) enhances food intake
  - Akt activated by PI3K inhibits AMPK → anorexic effect
- Akt also deactivates FOXO1 transcription factor through phosphorylation
  - Overexpression of FOXO1 can help cause obesity and glucose intolerance
- Insulin also participates in this pathway
The Cross-Talk between Insulin and Leptin
Both the Insulin Receptor and the Leptin Receptor recruit IRS2

- IRS2 -> Low-abundance
- Lack of IRS2 for IR -> may result in defective leptin signal transduction
- Insulin induces the phosphorylation of JAK2 -> increasing activation of STAT3
- Leptin -> JAK2 -> PI3K
- Both suppress food intake, both involve in decrease of cortical activity (in obese rate in presence of food).
Binding of Leptin to its receptor

- SHP2 (SH2-containing phosphatase 2) -> help to dephosphorylate the STAT3 -> maintain leptin sensitivity
- SOCS3 inhibits the action of SHP2 -> represses STAT3 pathway
- PTP1B (Protein tyrosine phosphatase 1B) -> reduces POMC-leptin sensitivity
- SOCS3 activates PTP1B -> further repress leptin signaling through STAT3?
- GRB2 -> ERK -> neural morphology, synaptic plasticity, and modulating activity of some NT
Activation of the Receptor

- Leptin depolarizes POMC neurons through PI3K (leptin activated PI3K - neuron specific) and nonspecific cation channel activation (TRP)
- Leptin hyperpolarize AgRP neurons by PI3K-mediated opening of K\textsubscript{ATP} channels $\rightarrow$ Potassium outflow
- Insulin hyperpolarize both POMC and AgRP neurons by PI3K -mediated activation of K\textsubscript{ATP} channels
- Insulin and Leptin both stimulate the phosphorylation and nuclear exclusion of FOXO1

https://www.researchgate.net/figure/Leptin-and-insulin-intracellular-signaling-mechanisms-in-hypothalamic-neurons-Activation_fig5_229433055
Insulin and Leptin signaling in Energy Expenditure and Glucose Homeostasis

- Tanycytes (Specialized glial cells)
- POMC (proopiomelanocortin) neuronal activation represses food intake and promotes expenditure
- AgRP/NPY (Agouti Related Peptide) stimulate food intake
- PVN (Paraventricular nucleus of hypothalamus)
- ARC (Arcuate Nucleus)
- These signals -> travel to the rest of the brain and peripheral tissue (WAT, BAT, pancreas, muscle, and...
Leptin effect on β cells

- Leptin inhibit glucose transport through GLUT2 transporters
- Leptin Activates PI3K-dependent reorganization of actin cytoskeleton -> opening K<sub>ATP</sub> -> hyperpolarization
- PI3K-dependent activation of PDE3B -> reduces cAMP -> inhibit PKA -> inhibit exocytosis
- Leptin inhibit PLC/PKC pathway

[Diagram showing the effect of leptin on β cells with specific pathways highlighted]
**Leptin effect on β cells**

- Leptin activate of STAT3 and STAT5B → SOCS3
- SOCS3 inhibit the production of proinsulin

---

[http://ime.endocrinology-journals.org/content/49/1/R9/F4.expansion.html](http://ime.endocrinology-journals.org/content/49/1/R9/F4.expansion.html)
Participation of SOCS3 in leptin resistance

- Prolonged receptor stimulation by leptin. Inhibition of JAK2 and ERK phosphorylation by SOCS3 independent of Tyr 985 of OB-Rb
Leptin in individual with normal body weight vs obese

- In normal individuals, LepRb receive inhibitory signals from multiple negative feedback loops like COCS3, PTPB1, PTPN2, PTPε and EPAC1
- In obese individuals, circulating leptin level increases → diminished leptin transport across BBB → activation of the inhibitory feedback systems → diminished LepRb signaling
- Chronic overnutrition + increase fatty acids → lipotoxicity and ER stress → inflammatory responses → may contribute to reduction in response to leptin (resistance)

https://www.nature.com/articles/nrendo.2016.222
Hypothalamus-mediated glucose and insulin resistance

- WAT expand, leptin increase in proportion to adipose expansion -> central leptin resistance -> favoring NPY (orexigenic peptide) in hypothalamus -> result in altered sympathetic and parasympathetic outflow.
- As insulin level rise -> adipose, liver, pancreas and hypothalamus become insulin-resistant -> exacerbate metabolic dysfunction

Leptin and insulin signaling pathways in hypothalamus

- MAPK (mitogen-activated protein kinase); AKT (thymoma viral proto-oncogene 1/protein kinase B)
- PI3K (phosphoinositide 3-kinase); PDE3B (phosphodiesterase 3B); POMC (proopiomelanocortin)
- AgRP (Agouti-related protein), EPAC (Exchange protein directly activated by cAMP)

Co-existence of leptin levels and obesity have lead many to conclude that this is the evidence for leptin resistance.

There are many other important factors at play, such as genetics, the environment, diet.
Leptin Action & “gleptin resistance”

- Leptin binds/activates long form of its proper receptor: LEPR-B.
  - Receptor exists in the brain; decrease in food intake while increasing usage of energy.
- It has been noted that the phenotypes of humans and rats lacking leptin or its receptor show signs of starvation, lower metabolic function, infertility, immune dysfunction, insulin resistance, etc.
- Leptin replacement has shown promising results with reversing the modified low-leptin states:
  - Genetic leptin deficiency, lipodystrophy syndrome.
  - Key regulator of metabolic neuroendocrine responses.
- Arriving at the key term: leptin resistance
LEPR- B Signaling
Attenuation of LEPR- B Signaling

1. Disrupting SOCS3
2. Effects of SHP2 (PTP1B)
3. Endoplasmic Reticulum (ER) Stress
4. Inflammation

https://www.nature.com/articles/nrendo.2016.222
Leptin Resistance in Genetic Models

- Assessment of LEPR-B signaling:
  - Detection of leptin stimulated pSTAT3 in hypothalamus
  - Response to food intake and body weight/ fat content to leptin administration.
- To analyze these models, 4 classes of genetic obesity are considered:
  1. Alterations in LEPR- B (and signaling)
  2. Disruption of neural pathways participating in leptin action
  3. Alteration in peripheral tissues that operate independently of food intake
  4. Alterations in CNS pathway with no clear link to leptin
Cellular Leptin Resistance in context-dependent obesity (including DIO)

- Genetics vs Environment
- Why use DIO (diet induced obesity)?
- Understanding the mechanisms
- Is cellular leptin resistance the primary factor in weight gain?
- Relationship between food intake and associated adiposity
Returning to the notion of “gsselective” leptin resistance

- DIO and leptin resistance complicates the model.
- DIO- leptin specific model differences.
  - Interference with feeding but effect on energy expenditure is unclear.
  - ARC in the major site of cellular leptin resistance in DIO, ARC leptin still modulates energy expenditure, glucose homeostasis, etc. associated with food intake.
  - Decreases of weight loss and normal chow.
    - Predictable novel diet effect.
    - Increased hunger
    - Decreased thyroid
    - Sympathetic tone
- Effect of weight loss from an obese state to lack of control of eating is due to leptin resistance, then other conditions are equally true.
Obesity in the Overall Picture

- Major differences between DIO and genetic altered impairments.
- Mechanisms that contribute to maintenance of obesity with dietary interventions.
- Implications in the modern world.
Is Insulin really necessary for survival? An Experiment

The idea that we can live without insulin has been challenged.

In everyday life, those with type I diabetes inject insulin to control blood sugar.
The Experiment

They tested rodents that did not have insulin in their bodies and then gave them leptin monotherapy.

The outcome of intracerebroventricular (i.c.v) administration of leptin to rodents without insulin were the ones being tested.

Mice without insulin/ mice that do not re-express leptin receptors (LEPRs) were accessed when they had I.C.V administration of leptin.
continued

They found re expression of leptin receptors in hypothalamic GABA and POMC neurons.

When the rodents have GABA and POMC neurons, it's enough to mediate anti-diabetes.

However, if you have a lot of glucose uptake and hepatic metabolism, these effects of leptin are decreased.
In insulin deficient rodents, leptin administration is also very effective in improving diabetes.

These latter findings may represent the groundwork for developing improved therapies for diseases of insulin deficiencies.

A comprehensive understanding of molecules and cell types underlying the lifesaving and metabolic improving actions of leptin in insulin deficiency is needed.
Different models of insulin

In this study they used two different models of insulin deficiency:

1. The streptozotocin (STZ)

1. The diphtheria toxin (DT)
- STZ + DT

-STZ treated mice retain miniscule amount of pancreatic insulin; therefore, it is unclear whether the residual insulin is required for the beneficial effect induced by leptin treatment.

-DT injected mice displayed overly high hyperglycemia, reduced body weight, hyperphagia, and hyperglucagon.
The results

Altogether, the results demonstrated that concomitant expression of leptin receptors only in GABA and POMC neurons is sufficient to fully mediate the lifesaving and anti diabetic effects of leptin administration in context of insulin deficiency.

Beta adrenergic receptors have been indicated as important components of the hypothalamic dependent pathway governing glucose metabolism.

To determine the fate of glucose in insulin deficient mice undergoing leptin administration, they used glucose training analysis.
continued

The results showed the I.C.V leptin administration does NOT affect glucose uptake in the brain or gastrocnemius and vastus skeletal muscle, but it significantly enhances glucose uptake in soleus muscle.

The results were focused on glucose and glycogen metabolism because lower circulating glucagon levels brought on by I.C.V leptin administration may affect pathways (those relevant to glucose metabolism)
Results continued

The results suggest that increased glucose uptake and utilization by soleus and improved hepatic metabolism are components of the mechanisms underlying the anti diabetic action of leptin in the context of insulin deficiency.

Rodents that are able to produce insulin, hypothalamic mediated control of glucose metabolism relies on the activation of the sympathetic nervous system.
Signaling in Other Brain Areas

These areas are still linked to energy homeostasis in that are involved in the regulation of eating behavior.

- Cerebral Cortex
- Cerebellum
- Hippocampus
Cerebellum - locomotor activity

- Leptin/Insulin are involved in the cerebellar control of motor activity through STAT3
- After leptin administration:
  - $\uparrow$ p-Tyr705-STAT3 levels
    - Activation of STAT3 promotes locomotor activity
    - Vs. leptin-deficiency contributes to reduced mobility
- IR levels are seen as reduced in experimental ataxia
Cerebral Cortex - metabolic control

- Cytokines in this area also exert effects for homeostatic regulation
- Leptin/insulin can overlook neurotransmitter activity and synaptic plasticity in this region through the ERK pathway
- Obese rats:
  - are hyperleptinemic, and may have high serum insulin levels
  - in the presence of food:
    - activation of JAK2/STAT3
    - decreased activity in the cortex
Hippocampus - learning & memory

- **Leptin signaling can facilitate synaptic plasticity**
  - When mice are infused with leptin, short-term potentiation shifts to long-term potentiation
    - Caused by the enhancement of Ca2+ influx through the NMDA receptor

- **Damage linked to an increase in appetite**
  - Can lead to weight gain/greater intake of fat-rich diets
    - Which negatively affects processes related to energy homeostasis
Experimental Examples

- **Cerebral Cortex**
  - selective lesions alter feeding behavior

- **Cerebellum**
  - leptin-deficient rodents display reduced mobility
  - lesions show signs of reduced food-seeking behavior

- **Hippocampus**
  - damaged in patients $\rightarrow$ inability to inhibit consumption of a second meal because they cannot remember eating beforehand
Hyperleptinemia

- Having more than normal amounts of leptin in the bloodstream
  - Damages hippocampal synaptic transmission
  - Causing factors not well known, but increased food intake/insulin resistance play a part
- Associated with the onset of depressive symptoms
  - Suggests leptin resistance may have a deleterious effect
  - Central infusion of leptin shown to induce antidepressive behavior
    - But may promote an inflammatory profile - correlates with markers of the metabolic syndrome

- Implies an important association between obesity and depression
Neurodegenerative Diseases

- Leptin promotes neurogenesis, axonal growth and synaptogenesis
  - By activating JAK2/STAT3 pathways, there is neurotrophin withdrawal, leading to reduced cell death
    - May help reverse the effects of dopaminergic neuron loss in Parkinson’s Disease (PD)
- High leptin levels may have harmful effects on other diseases, ie. Multiple Sclerosis (MS)
  - With MS, leptin production is only enhanced in serum and cerebrospinal fluid of patients
Neurodegenerative Diseases

- Alzheimer’s Disease: characterized by the aggregation of $\beta$-amyloid proteins and neurofibrillary tangles of hyperphosphorylated tau
- Leptin modulates APOE expression and decreases activity of beta secretase
  - APOE - role of removing $\beta$-amyloid proteins
  - Beta secretase produces $\beta$-amyloids

https://www.jscimedcentral.com/AlzheimersDisease/alzheimersdisease-1-1009.php
Neurodegenerative Diseases

- Large connection between effects on insulin sensitivity and neuropsychiatric diseases, ie. AD
  - Insulin receptor binding regulates cognitive functioning
    - Promote the inhibition of apoptosis
    - Stimulate metabolism and plasticity
      - Mediated by Akt phosphorylation and GSK3β inhibition
      - In an experimental model, activation of PI3K/Akt mediates neuroprotection in AD and PD
  - Lower activation of IRS/PI3K pathways disrupts Aβ processing.
    - Increases AD-related symptoms