Prevention of hypoglycemia-induced neuronal death

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The authors declare no competing financial interests.
Hypoglycemia? 

Brain uniquely depends on glucose for its energy.

Hypoglycemia can be induced by blood glucose reducing agents such as insulin or by insulin producing tumor, insulinoma.

Hypoglycemia-induced brain injury has been a big obstacle to optimal blood glucose regulation in people with type 1 diabetes.

The prevalence of severe hypoglycemia, including hypoglycemic coma could be as high as 40 % in patients with type 1 diabetes alone.

Severe hypoglycemia causes neuronal death and cognitive impairment.
Hypoglycemia correction by glucose?

Hypoglycemic symptom (stupor, unconsciousness, coma and etc) can be recovered by glucose infusion.

However, recurrent episodes of hypoglycemia can cause brain damage, and glucose infusion can not prevent neuron death.

Recently, our lab hypothesized that may be the re-infusion of glucose after hypoglycemia triggers neuronal death cascade.

Glucose reperfusion injury
Hypoglycemia-Induced Neuronal Death

- Severe hypoglycemia causes selective neuronal damage in rat brain (Auer et al., 1984).

- Hypoglycemia-induced neuronal death is associated with severe energy failure and massive release of glutamate (Engelsen et al. 1986).

- Poly(ADP-ribose) polymerase (PARP) is a nuclear enzyme that is activated by DNA damage. Excessive activation of PARP deplete ATP and NAD. PARP inhibitors prevent hypoglycemia-induced neuronal death (Suh et al., 2003).

- Zinc translocation into neurons can induce activation of the PARP. Zinc chelation prevented PARP activation and subsequent hypoglycemia-induced neuronal death (Suh et al., 2004).

- Pyruvate administered after severe hypoglycemia reduced neuronal death and cognitive impairment (Suh et al., 2005).
1. Neuronal depolarization

2. Glut/Zn$^{2+}$ release  
   ? impaired Glut uptake

3. Postsynaptic Zn$^{2+}$ accumulation
   ROS production
   DNA damage
   PARP activation
   Cell death
Zinc is co-localized with glutamate in the neuron terminals.

Zinc has been identified as both an inducer of neuronal NADPH oxidase activation and a contributor to hypoglycemic neuronal death.
Zinc is an essential mineral for our body function.

2\textsuperscript{nd} most transitional metal in our brain.
Essential for transcription factors (zinc binding proteins),
enzyme function, cell growth, growth factors, neurogenesis,
immune function, sperm motility, hair growth, blood coagulation,
and etc\ldots.

So, zinc deficiency caused many clinical symptoms.
**Vesicular Zinc**

- Zinc is localized in the presynaptic vesicle (Haug F.M. 1967)
- < 5 % of the total brain zinc
- Around 1 mM of zinc concentrated in the vesicle
- Co-localized and co-released with glutamate
- Concentration is regulated by zinc transporter
  - **Take in**- Zinc Transporter 3 (ZnT3)
  - **Take out**- Zinc Transporter 1 (ZnT1) (Palmiter et al., 1996)
Vesicular Zinc Distribution in The Brain and Histological Detection

Timm-Danscher

TSQ

EM
Zinc Containing Neuron is a subset of Glutamatergic Neurons
Vesicular zinc release after hypoglycemia
Possible Vesicular Zinc Movement After Release

ZnT3

Glia?

Zinc binding proteins?

Cellular Function & Genetic Expression
Zinc Toxicity

• too much zinc accumulation in the neuron is toxic!!!

1) seizure (Frederickson et al., 1987; Suh et al., 2001)

2) global ischemia (Tonder et al., 1990; Suh et al., 1995; Koh, Suh et al., 1996)

3) head trauma (Suh et al., 2000)

4) exogenous zinc toxicity on neuron, in vitro
   (Yokohama et al., 1986; Choi and Koh, 1998)

5) neurofibrillary tangle in Alzheimer’s disease
   (Suh et al., 2000)
Hypoglycemia-induced zinc translocation & accumulation

A. Zn$^{2+}$ release

- HG/GR HG alone Sham

30 min after HG/GR

- Presynaptic terminal
- Postsynaptic neuron

B. Zn$^{2+}$ translocation

- Sham
- HG/GR

3 hour after HG/GR

- Postsynaptic neuron

C. Zn$^{2+}$ accumulation

- Sham
- HG/GR

24 hour after HG/GR

- Postsynaptic neuron
Hypoglycemia induced vesicular zinc release is potentiated by glucose reperfusion.

A

Sham Hypoglycemia

60 min HG only

30 min HG + 30 min GR

B

Intensity of TSQ

Rat hippocampal hilus, TSQ staining
Glucose reperfusion potentiates superoxide production after hypoglycemia/glucose reperfusion

Hippocampal CA1, dHEt stained section
Hypoglycemia-induced zinc translocation or superoxide production is prevented by zinc chelation after hypoglycemia.
Hypoglycemia → Neuronal depolarization → Glutamate release

Oxidative stress ? ↔ Zinc release ? ↔ NO production ?

PARP1 activation → Glycolytic inhibition ? → MPT → Neuronal death
MATERIALS AND METHODS

• In vivo insulin induced hypoglycemia model.

• Glucose deprivation/reperfusion model using cultured cortical neurons.

• Superoxide production was detected by dihydroethidium (dHEt).
  \[ \text{dHEt} + \text{superoxide} \rightarrow \text{Ethidium} \]

• Zinc ion stained by TSQ.

• \( p47^{\text{phox}} \) KO mice or ZnT3 KO mice were used.
In vivo hypoglycemia

Glucose Reperfusion

insulin → glucose

-- 2.75 hr → 45 min → 45 min → 3 hr → 7 day

--isoEEG--

TSQ  dHEt  FJB
Zinc-induced superoxide production

In vitro, zinc application

Zn 100 uM

1 hr

imaging

dHEt 5 uM + Apocynin or CaEDTA
EEG and BP change before and after hypoglycemia

“Isoelectric EEG”
Hypoglycemia-induced vesicular zinc release is not prevented by SOD over expression but prevented by NOS inhibitor, 7NI.
Hypoglycemia-induced zinc translocation is not prevented by SOD over expression but prevented by NOS inhibitor, 7NI.

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<tr>
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<th>TSQ</th>
<th>Fluoro-Jade B</th>
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<tbody>
<tr>
<td>Sham</td>
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<tr>
<td>Wt</td>
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<td>SOD Tg</td>
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<td>7-NI</td>
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Table showing TSQ and Fluoro-Jade B images for different conditions: Sham, 3 hour, 24 hour, and 24 hour.
Hypoglycemia-induced neuronal death in mice

A  EEG change
  a. 
  b. 
  c. 
  d. 
  e. 
  f. 
  g. 
  h. 

B  Blood glucose

C  Neuronal death
  CA1
  DG
  Subiculum
  Cortex
ZnT3 KO eliminates vesicular zinc
Hypoglycemia-induced superoxide production and neuronal death is prevented by ZnT3^-/-

Superoxide production

![Images showing superoxide production in Wt and ZnT3^-/- conditions](Image)

- Wt
- ZnT3^-/-

Neuronal death

![Images showing neuronal death in WT and ZnT3^-/- conditions](Image)

- WT
- ZnT3^-/-

Hypoglycemia-induced superoxide production and neuronal death is prevented by ZnT3^-/-

Superoxide production

- Comparison of Et intensity between Wt and ZnT3^-/-
- Statistical significance indicated by (*)

Neuronal death

- Comparison of degenerating neurons between Wt and ZnT3^-/-
- Statistical significance indicated by (*)
Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase has been especially well characterized in immune cells and leukocytes for their involvement of ROS production for host defense purpose.

Recently NADPH oxidase subunits have been found in neuronal cell bodies and their process in the brain.

Activation of NADPH oxidase has been considered an important source for elevated levels of superoxide that contribute to cytotoxicity in several brain disease including ischemia.
NADPH oxidase is an enzyme that catalyzes the production of superoxide from oxygen and NADPH.

It is a complex enzyme consisting of two membrane-bound components (gp91\textsuperscript{phox}, p22\textsuperscript{phox}) and three components in the cytosol (p40\textsuperscript{phox}, p47\textsuperscript{phox}, and p67\textsuperscript{phox}), plus rac 1 or rac 2.

Activation of the NADPH oxidase needs the translocation of the cytosolic components to plasma membrane.
Glucose deprivation/reperfusion induced NADPH oxidase subunit translocation is prevented by CaEDTA

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<th>MAP2</th>
<th>p47</th>
<th>merge</th>
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<td><img src="Cont_p47.png" alt="Image" /></td>
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NADPH oxidase assembly inhibitor, apocynin, prevents hypoglycemia-induced neuronal death.
NADPH oxidase subunit, p47<sup>phox</sup>, gene deletion prevents hypoglycemia-induced superoxide production and neuronal death in mice.

**dHET staining**

**Fluoro-Jade B staining**
NADPH oxidase subunit, p47$^{\text{phox}}$, gene deletion mice showed similar level of vesicular zinc in the hippocampus and same zinc release after hypoglycemia. But, no further intracellular zinc increase at 24 hr after hypoglycemia.
NADPH oxidase inhibition or subunit p47<sup>phox</sup> deletion prevents zinc-induced superoxide production.
Hypothermia prevents hypoglycemia-induced neuron death

**CA1**
- Sham
- Normo
- Hyper
- Hypo

**Subiculum**
- Sham
- Normo
- Hyper
- Hypo

**B**
- Degenerating neurons
  - Lt Normo
  - Lt Hyper
  - Lt Hypo

**D**
- Degenerating neurons
  - Lt Normo
  - Lt Hyper
  - Lt Hypo

* indicates statistical significance.
Hypothermia prevents hypoglycemia-induced zinc release and accumulation
SUMMARY

• Glucose reperfusion potentiates superoxide production after hypoglycemia.
• Glucose reperfusion potentiates vesicular zinc release after hypoglycemia.
• nNOS inhibitor, 7-NI, completely prevents vesicular zinc release and translocation after hypoglycemia.
• SOD overexpression inhibits PARP activation and subsequent neuronal death after hypoglycemia.
• Zinc chelation prevents glucose reperfusion-induced superoxide production after hypoglycemia.
• Zinc-induced superoxide production in the cultured neuron is prevented by NADPH oxidase inhibitor or p47 gene deletion.
• Hypothermia prevents hypoglycemia-induced neuron death.
CONCLUSIONS

Hypoglycemia-induced vesicular zinc release is dependent on nitric oxide synthase activity, and then subsequent zinc translocation into hippocampal neurons produced superoxide through an NADPH oxidase mediated pathway.
Thank you~~~