Preconditioning the Brain for Stroke Prevention

The Peritz Scheinberg Cerebral Vascular Disease Research Laboratories; Department of Neurology

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The main goal in this field is to define the pathophysiological mechanisms of cerebral ischemia-induced cell death with the goal of finding therapies to ameliorate the consequences of the insult.
Strategies for Neuroprotection

- Cocktail of drugs that target all these pathological mechanisms

- Or, the use of a drug that have pleiotropic properties that can protect against most of these pathological phases
Strategies for Neuroprotection with Pleiotropic Properties

• Ischemic Conditioning
Ischemic Preconditioning

‘That which does not kill us makes us stronger’ Friedrich Nietzsche, Ecce Homo – (1908)

Ischemic preconditioning refers to the ability of a brief (“sublethal”) ischemic episode, or mild stress insult followed by a period of reperfusion, to increase an organ’s resistance to injury (ischemic tolerance)
Dave et al. J Neurosci Res. 2005, 82, 665-673
Other models of conditioning

Remote organ ischemic preconditioning protect brain from ischemic damage following asphyxial cardiac arrest

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IPC

Pre-synaptic

DPCPX → A1 Receptor → Phospholipase-C → DAG → OAG → PKCε Translocation to its RACKs → εV1-2 → ERK activation → PD-98059 → NEUROPROTECTION

Post-synaptic

MK-801 → BAPTA-AM → U73122 → Calcium → BAPTA
Protein Kinase C epsilon (PKCε) activation is necessary for IPC neuroprotection

Raval et al., 2003, J Neurosci. 23(2):384-91.
IPC

- Pre-synaptic
  - DPCPX
  - U73122
  - A1 Receptor

- Post-synaptic
  - MK-801
  - NMDA
  - Calcium
  - BAPTA-AM
  - BAPTA

Phospholipase-C

- DAG
  - OAG

PKCε Translocation to its RACKs

- εRACK
- εV1-2

ERK activation

- PD-98059

HIPPOCAMPAL CA1
NEUROPROTECTION
Preconditioning enhances GABA release

Glutamate

GABA

Dave et al. J Neurosci Res. 2005, 82, 665-673

Baseline   Ischemia   Reperfusion
Whole Cell Recording
Preconditioning alters GABA mPSC

Defazio et al. JCBFM. 2009, 29, 375
Preconditioning alters GABA mPSC

Defazio et al. JCBFM. 2009, 29, 375
PKCε pre-conditioning alters action potential properties in CA1 hippocampal neurons

Neumann et al. JCBFM. 2015 Jan;35(1):121-30
PKCε pre-conditioning induces a delay in membrane depolarization during OGD

Neumann et al. JCBFM. 2015 Jan;35(1):121-30

n=9
*p<0.05
Ischemic preconditioning

Presynaptic

εPKC

NF-κB

ERK1/2

CREB

BDNF

Glutamate

GABAergic

Glial cells

cAMP

TrkB

NMDA receptor

Ca++

CaMKII

Postsynaptic

p75

p75

TrkB

TrkB

Glial cells

TrkB
TrkB activation is necessary for ψεRACK mediated protection
SUMMARY

• ψεRACK increases BDNF expression and TrkB phosphorylation
• BDNF expression and TrkB phosphorylation alter action potential properties
• ψεRACK-mediated arc expression triggers a decrease in AMPAR mEPSCs
• ψεRACK delays the onset of anoxic depolarization
• TrkB activation and arc expression are necessary for ψεRACK-mediated neuroprotection
MITOCHONDRIAL DYSFUNCTION

PKCε Enhances Maximal NADH Fluorescence

**Major Biosynthetic Pathway for NAD⁺ Production**

- **Nampt** = nicotinamide phosphoriboslytransferase
- **NAM** = nicotinamide
- **NMN** = Nicotinamide mononucleotide
- **Nmnat** = NMN adenyl transferase

The pathway involves the following reactions:

1. **NAM** → **NAD⁺** via **Nampt**
2. **NMN** → **NAD⁺** via **NMNAT**
3. **NAD⁺** → **NAM**
4. **NMN** → **NAD⁺** via **NMNAT**

The diagram illustrates these processes with arrows indicating the direction of the reactions.
PKCε Enhances Nampt Fluorescence in the Rat Cortex

**Nampt** = nicotinamide phosphoriboslytransferase

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**Nmnat** = NMN adenyl transferase

¡Nampt= nicotinamide phosphoriboslytransferase

NAM= nicotinamide

NMN=Nicotinamide mononucleotide

Nmnat=NMN adenyl transferase
Global gene repression
Histone deacetylation

Inflammatory response
Inhibition of NF-κB transcriptional activity

Mitochondrial activity
Promotes mitochondrial biogenesis and activity by activation of PGC-1α

Oxidative stress
Shift in FOXO regulated gene expression from cell death to survival

Apoptosis
Inactivation of p53-dependent transcription of pro-apoptotic proteins
Resveratrol → SIRT1 → Caloric Restriction

- Decreased Reactive Oxygen Species
- Preserved Mitochondrial Function
- Insulin Growth Factor Signaling Pathway
- Enhanced Lifespan
Resveratrol preconditioning (RPC) decreases CA1 cell death in organotypic slices after OGD.
Resveratrol preconditioning (RPC) reduces infarct after MCAo in mice

RPC decreases CA1 cell death after cardiac arrest in rats

CAN YOU DRINK YOUR WAY TO PRECONDITIONING???

Resveratrol – 1 bottle of red wine

<table>
<thead>
<tr>
<th>Wine Type</th>
<th>Country</th>
<th>Resveratrol (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinot Noir</td>
<td>California</td>
<td>5.01</td>
</tr>
<tr>
<td>Beaujolais</td>
<td>France</td>
<td>3.55</td>
</tr>
<tr>
<td>Zinfandel</td>
<td>California</td>
<td>1.38</td>
</tr>
<tr>
<td>Cabernet Sauvignon</td>
<td>California</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Resveratrol in one liter of red wine (milligrams)

Images courtesy of Google Images
A SINGLE APPLICATION OF RPC INDUCES ISCHEMIC TOLERANCE AGAINST FOCAL ISCHEMIA FOR TWO WEEKS

Koronowski et al. 2015. Stroke.

↓ RPC (10 mg/kg) or Veh (DMSO-Saline)
Sirt1

β-Actin

80 kDa

45 kDa

AB

**

**

*

UCP2

VDAC

70 kDa

32 kDa

B

Sirt1-Promoter

Binding

C

BDNF

UCP2

pg / mg protein

D

BDNF

Fold Change
Transcriptomic changes induced by RPC within the long-term window of IPC

A. IP injection
   Vehicle/ Resveratrol (30mg/kg)
   C57Bl/6 mice
   Day 0 → 14

   Obtain Cortex → Isolate RNA → RNA-seq
   Illumina Hiseq 2500

B. 116/36 downregulated (85%)
   20/138 upregulated (15%)

C. [Biological Processes(GO)] p-value
   Genes
   Transcription from RNA polymerase II promoter 2.18E-08
   Autos1, Npas4, Ep300, Fos, Junb, Nfia, Six3, Sall1, Lef1, Foxo1, Kmt2a, Foxi1, Zmiz1, Nrf1a, Tead1, Pitrk2, Notch1, Aga2, Brd4, Nos1, H1vپ3, Hipk2, Gli3, Sox9, Kmt2c, Zfhx3, Epas1, Rnf165, Arid1a, Atn1

   Regulation of signaling 2.26E-08
   Unc13c, Npas4, Ep300, Kcnj10, Six3, Sall1, Lef1, Foxo1, Kmt2a, Zmiz1, Doc2b, Fshr1, Ki, Peg10, Shank1, Tacr1, Vamp2, Shank2, Ca2, Calm1, Notch1, Ksr2, Shisa7, Sipa1, Ubqn2, Nos1, Irnin59, Hipk2, Gli3, Sox9, Kmt1b, Ca2, Dusp1, Kcnj2, Arc, Fli1, Rnf165, Acdy5, Arcrg1, Wasf

   Synaptic signaling 2.26E-05
   Gpr88, Unc13c, Nos1, Kcnj10, Sirc5a7, Kmt2a, Doc2b, Shank1, Tacr1, Vamp2, Shisa6, Shisa7, Nos1, Arc

   Regulation of ion transmembrane transport 5.17E-05
   Kcnj10, Atp1b2, Scd1a, Shank3, Vamp2, Nos1, Ca2, Kcnj2, Arc, Atrf1

   Positive regulation of neurotransmitter levels 3.77E-01
   Unc13c, Kcnj10, Sirc5a7, Doc2b, Vamp2,Nos1

   Histone H3 acetylation 3.03E-04
   Ep300, Lef1, Kmt2a, Brd4

   Histone H4 acetylation 2.04E-05
   Autos2, Ep300, Lef1, Kmt2a, Brd4

   Histone acetylation 8.05E-05
   Autos2, Ep300, Lef1, Kmt2a, Brd4, Nos1

Nathalie Khoury et al. Mol Neurobiol. 2018 Oct 20
1. Signaling pathways activated by Ischemic Preconditioning exhibit pleiotropic properties that lead to neuroprotection.

2. PKCε and Resveratrol are two pharmacological agents that emulate IPC and have pleiotropic properties.

3. Pharmacological agents that emulate IPC could be used in a prophylactic manner to enhance ischemic tolerance in patients prone to cerebral ischemia.
Clinical Scenarios for IPC Implementation

a) Carotid endarterectomy and coronary artery bypass graft (CABG) which both result in high incidence of strokes and cognitive deficits
b) Transient ischemic attacks (TIA) patients, from which 10% will have a stroke within a month
c) Sub-arachnoid hemorrhage (SAH) patients from which 20-30% undergo delayed cerebral ischemia (DCI)
d) Neurosurgical procedures
e) All stroke patients have a higher incidence of subsequent strokes
"Precondition-able" population

Stroke Survivors
~ 660,000
Carotid Endarterectomy
~ 132,000
Coronary Artery Bypass Graft
~ 500,000
TIA
~ 240,000
Sub-arachnoid Hemorrhage
~ 55,650
Neurosurgical Procedures
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Stradecki H.
About the Image

The role of mitochondrial dysfunction in age-related neurodegenerative diseases is significant.

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