Mechanisms of Glutamate Release from Astrocytes

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Presently, glutamate removal from the extracellular space:
- ~80% astrocytes
- ~20% neurons
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Depolarization +
Increased [K+]o

Ischemia

A

B

C

Extracellular space

Xo
(cysteine)

K+

3 Na+ 1 H+

Cystol

3 Na+ 1 H+

Glu

2 Cys (cysteine)

GSH (glutathione)

Out

In

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BK: bradykinin

Astrocytes pre-incubated for 40-60 minutes in low extracellular Ca²⁺ (0 Ca)
α-LTX: α-latrotoxin

LP-1: (neuronal) synaptosomal plasma membrane enriched preparation
AsP: non-nuclear membrane extract from cultured astrocytes
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Dynamics can be followed by:
- Capacitance measurements (R. Zorec)
- Imaging using fluorescent proteins

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• Release of glutamate from cultured astrocytes during hypo-osmotically induced swelling was first reported using radiolabeled glutamate.

• In addition to glutamate release, there is characteristic co-release of another endogenous signaling molecule, the sulfonic acid taurine.

The release of glutamate occurred through an anion channel since it could be blocked by various anion channel inhibitors:

- Furosemide
- SITS - (4-acetamido-4'-isothiocyanato-stilbene-2,2'-disulfonate)
- L-644,711 - (R(+)) [(5,6-dichloro 9a-propyl 2,3,9,9a-tetrahydro 3-oxo-1H-furoen-7-yl)oxy] acetic acid

Neither bumetanide (K⁺/Na⁺/2Cl⁻ cotransport system blocker), nor TBHA (threo beta-hydroxyaspartate), had effect on swelling-induced glutamate efflux from astrocytes.

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The first evidence that P2X7 channels could mediate the release of glutamate from astrocytes was obtained using cultured astrocytes. Confluent astrocytes were bathed in Lucifer Yellow (LY), known to enter cells through large channels. Activation of the P2X7 receptor with its agonist BzATP enabled astrocytic uptake of Lucifer Yellow in standard extracellular solution containing divalent cations. This dye uptake was increased when astrocytes were bathed in divalent cation-free external solution. In both conditions, the dye uptake was inhibited by the specific P2X7 antagonist, oxidized ATP (Ox-ATP). These data indicated that astrocytes in culture possess functional P2X7 receptors.


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• Release of radiolabeled glutamate from cultured astrocytes was induced by application of ATP, and more potently by BzATP, both in standard and divalent cation free external solutions
• Release was blocked by Ox-ATP
• Release was increased in Divalent Cation Free Solution (DCFS)
• These results suggest that astrocytes can release glutamate through plasma membrane P2X7 channels

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- Extracellular divalent cation reduction induced release of glutamate from astrocytes*
- Aspartate was also released*
- The release of both amino acids was blocked in a concentration-dependent manner by extracellular calcium*
- Conditions favored during injury and ischemia and could possibly occur during times of high frequency synaptic transmission*
- The observed release of glutamate was reduced by application of the gap junction blockers*:
  - Septanol, and octanol blocked DCF5-induced glutamate release from astrocytes
  - Shorter-chain alcohols such as hexanol (Hex) had less effect
  - Butanol (But) and cyclohexanol (cHex) had no effect
- Gx-ATP ineffective*
- When compared to astrocytes originating from control wild type animals, Cx43-lacking astrocytes, exposed to low extracellular divalent cations, show minimal LY dye uptake and glutamate release**

** Spray DC, Ye ZC, Ransom BR (2006) Functional connexin "hemichannels": a critical appraisal; Glia 54: 758-773

Possible future mechanisms: a “watch-out” list

- Pannexon:
  - Non-junctional pannexin channels
  - Not sensitive to extracellular Ca2+*
  - Intracellular Ca2+ dependency
  - Voltage-dependency: within astrocytic range
  - ATP release from astrocytes*

- Bestrophin-1:
  - Intracellular Ca2+ activated anion channel (CAAC)
  - GABA release from astrocytes**

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• Employment: How often and to what extent?
• (Patho) physiological conditions?

THANK YOU

• Parpura Laboratory (chronologically):
  • Steven E. Rosenwald (2000-01)
  • Xue Hu (2001-03)
  • Yingzhun Ni (2001-06)
  • Vaidreva Montara (2002-07)
  • Vic Suñer (2002)
  • Eric G. Malayev (2003-05)
  • Ramo C. Reyes (2005-06)
  • William Lee (2005-present)
  • Randy F. Stout, Jr. (2005-present)
  • Kyle D. Osborne (2005-07)
  • ChangMan-Ho (2005-07)
  • Oliver C. Leomin (2006-07)
  • Kirk A. Fischer (2006-07)
  • Vladimir Gudkoff (2005, 06, 08-present)
  • Wei Liu (2007-10)
  • Roberto Gomez (2008-10)
  • Joshua J. Samuels (2009-10)
  • J. Robert Grammer (2009-11)
  • Brandi J. Baker (2009-10)
  • Manoj K. Gottipati (2010-present)

• Collaborators:
  • Philip C. Haydon
  • Edward S. Young
  • Umar Mahdavian
  • Edwin R. Chapman
  • Robert C. Haddon
  • Glenn L. Hatton
  • Elena Scammel
  • David C. Spray
  • Andrew J. Irving
  • Mathieu Lesort
  • Candice L. Floyd
  • Dale J. Bennett
  • Michelle Gray
  • Robert Zorec
  • Alexei Verkhratsky

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