Neuronal-glial chemical transmission mediated by glutamate and ATP

Prof. Alexei Verkhratsky

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Astrocytes form communication “channels” with nearby neurones

Neurotransmission by glutamate: glutamate sensors

Neuronal-glial chemical transmission mediated through glutamate

• Ionotropic glutamate receptors
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Identification of neurotransmitter receptors in cultured astrocytes

Kainate induces massive calcium influx mediated by AMPA receptors into Bergmann glial cells in cerebellar slice preparations

NMDA receptors in astroglial cells

- The NMDA receptor was, for a long time, believed to have exclusively neuronal localisation
- The NMDA receptors possess an important property of Mg²⁺ block that precludes their opening at hyperpolarised membrane potentials
- The astroglial resting potential is set at -80 to -90 mV and therefore it was assumed that NMDA receptors cannot function in glial cells
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- Glutamate induces a complex current response sensitive to inhibitors of AMPA and NMDA receptors and glutamate/Na+ transporters
  Journal of Neuroscience, 26: 2673-2683

NMDA induces glycine-sensitive current response in isolated astrocytes
  Journal of Neuroscience, 26: 2673-2683

Astroglial NMDA receptors are magnesium insensitive
  Journal of Neuroscience, 26: 2673-2683
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Neuronal-glial chemical transmission mediated through glutamate

- Ionotropic receptors
- Metabotropic receptors

Parallel fibers stimulation triggers calcium signals in Bergmann glial cell microdomains


Neuronal-glial chemical transmission mediated through glutamate

- Ionotropic receptors
- Metabotropic receptors
- Glutamate transporters

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Astrocytes form the main glutamate-uptake system in the CNS

Voltage-dependence of GluT- and AMPAR-mediated currents

Application of glutamate triggers inward current and sodium influx
Different sensitivities of glial glutamate sensors allow precise determination of extracellular glutamate concentration

\[ K_D = 2 \text{ mM} \quad K_D = 50 \text{ mM} \quad K_D = 3 - 100 \text{ mM} \quad K_D = 130 \text{ mM} \]

Verkhratsky & Kirchhoff (2007)/Neuroscientist, 13: 1-10

ATP – principal transmitter in neuronal-glial networks

ATP-mediated transmission: the beginning

- ATP was discovered in 1929
- Extracellular signaling role of purines was suggested in 1929
- Geoffrey Burnstock was born on May 10, 1929


- "I came up with this concept in Pharmacological Reviews in 1972, and nobody believed it. At the first Purines Meeting in Canada way back in the late 70s, probably 95% of the science concentrated on adenosine. And my stuff (on signaling through ATP) was very much frowned on. There were certain very strong figures—very influential figures, and gifted—and one of them was Robert Berne, who had early on claimed that adenosine was the physiological regulator of blood flow during hypoxia and ischemia in the heart and other organs. He was charming and he was clever, and although he collected adenosine from the hypoxic heart, he was totally opposed to the fact that it might be originating as ATP. At the opening party of the second Purines Meeting a while later, I was suddenly trapped in the corner by several scientists who were also very opposed to the idea of ATP as an extracellular regulator, and being an ex-boxer, I got very worried because I had the sense that it was going to become physical. People were very passionately against it!"


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ATP: the primordial signalling molecule

- The purines were a part of very early signalling evolution
- Indeed, the purines were in the form of adenine and guanine phosphates occurring in the prebiotic period (as a result of purely thermal synthetic processes) and rapidly acquired high importance
- Purines and pyrimidines were essential for construction of both RNA and DNA, and hence the intracellular concentration was high
- Furthermore, ATP was selected very early as the main source of biological energy, and thus became an indispensable feature of life
- The prominent roles of adenine-phosphates in energy metabolism in the most primitive life forms and ancient origin of adenine-binding sites are widely recognised
- This was a critical evolutionary choice as it immediately resulted in both the universal intracellular signalling system based on calcium ions, as keeping cytosolic Ca²⁺ extremely low became vitally important, since otherwise insoluble Ca²⁺-phosphates would preclude the cell energetics
- Thus even the most primitive ancient cell had high cytosolic concentrations of ATP (or GTP) and upon cell disintegration gradients of both would be present in the surrounding water
- This may have been the initial form of chemical transmission, which remained throughout evolution
- Indeed, every known cell or single cell organism displays some form of sensitivity to ATP, and moreover, the ATP transmission is unique in a sense that it is not confined to a particular tissue or organ of higher organisms — it exists everywhere. Indeed, it is difficult to find a cell type, which does not show sensitivity to ATP


Mechanisms of ATP release in the central nervous system

ATP release and degradation in the CNS

Exocytotic in presynaptic terminals

Non-exocytotic release in astrocytes

ATP

Adenosine

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**Exocytotic ATP release from synaptic terminals**

- Dissection of GLUR/P2X-mediated mEPSCs in cortical neurones P2X antagonists selectively inhibit small amplitude mEPSCs


**Quantal release of ATP from acutely isolated cortical astrocytes**

- HEK293-P2X cell
  - Isolated astrocyte


**Purinoceptors: an overview**

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ATP as a universal transmitter in neuronal-glial circuits


ATP as a universal glial transmitter: oligodendrocytes


Iono- and metabotropic ATP receptors expressed in astroglia trigger calcium signals in the optic nerve


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ATP-induced currents in cortical astrocytes

Concentration-dependence of ATP-induced currents

Pharmacology of ATP-induced currents

* TNP-ATP: 2',3'-O-(2,4,6-trinitrophenyl)adenosine 5'-triphosphate

Journal of Neuroscience, 28: 5473-5480

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Subunit composition of the astroglial P2X receptor

<table>
<thead>
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<th>P2X4 receptor expressed in artificial systems</th>
<th>Astroglial P2X receptor</th>
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<tbody>
<tr>
<td>Sensitivity to ATP</td>
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<td>Ca2+-dependence</td>
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<td>Inhibition by PPADS</td>
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Cortical astrocytes express P2X1 and P2X5 subunits

Synaptic stimulation triggers fast astroglial currents mediated by ionotropic receptors and glutamate transporters

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Glial synaptic currents (GSC) in the neocortex
- Stimulus/TTX dependence of GSCs and EPSCs
- Stimulus-frequency dependence of GSCs


Ionotropic receptors and glutamate transporter mediate GSC in the neocortex


Spontaneous glial synaptic currents

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Calcium permeability of astroglial NMDA and P2X<sub>1/5</sub> receptors

Calcium permeability of NMDA and P2X<sub>1/5</sub> receptors in cortical astrocytes


Stimulation of NMDA and P2X<sub>1/5</sub> receptors triggers Ca<sup>2+</sup> signals in isolated cortical astrocytes

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Synaptic stimulation-induced Ca^{2+} signals in cortical astrocytes *in situ* are sensitive to ionotropic receptors antagonists

**References**


Age-dependent remodelling of ionotropic receptors expression in cortical astroglia

**References**


**In situ** recordings from ageing cortical astroglia

**References**

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Ageing changes the relative weight of GSCs components
Lalo, Palygin, North, Verkhratsky, Pankratov, (2011)
Aging Cell 10: 392-402

Ageing modifies expression of glutamate and purinergic receptors in astroglia
Lalo, Palygin, North, Verkhratsky, Pankratov, (2011)
Aging Cell 10: 392-402

Ageing affects ionotropic Ca\(^{2+}\) signalling in astroglia in situ
Lalo, Palygin, North, Verkhratsky, Pankratov, (2011)
Aging Cell 10: 392-402

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Ionotropic receptors in neuronal-astroglial signalling:
What is the role of “excitable” molecules in non-excitable cells?
Fast local signalling in astroglial perisynaptic compartments?

- Astroglial cells express several classes of fast ionotropic receptors
- These receptors are activated by ongoing synaptic transmission and mediate local cytoplasmic signalling mediated through [Ca²⁺] and [Na⁺] transients
- Rapid activation of astroglial ionotropic receptors can be instrumental for highly localised neuronal-glial signalling at the synaptic level
- Age-dependent decrease in synaptic communication in neuronal-glial circuitry may signify the beginning of age-dependent decline in brain functions
- Alternatively however, the bell-shaped changes in glial synaptic transmission may reflect maturation of the CNS, pruning of functionally irrelevant synaptic contacts and manifest the “attainment of wisdom” which generally accompanies advanced age

Conclusions

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