Ca\(^{2+}\)-dependent modulation of GABA\(_A\) and NMDA receptors by extracellular ATP: implication for function of tripartite synapse

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Abstract

The importance of communication between neuronal and glial cells for brain function is recognized by a modern concept of ‘tripartite synapse’. Astrocytes encase synapses and can modulate their activity by releasing gliotransmitters such as ATP, glutamate and \(\delta\)-serine. One of the regulatory pathways in the tripartite synapse is mediated by P2X purinoreceptors. Release of ATP from synaptic terminals and astrocytes activates Ca\(^{2+}\) influx via P2X purinoreceptors which co-localize with NMDA (N-methyl-\(\delta\)-aspartate) and GABA (\(\gamma\)-aminobutyric acid) receptors and can modulate their activity via intracellular cascades which involve phosophatase II and PKA (protein kinase A).

Introduction

Communication between neuronal and glial cells is regarded to be very important for brain functions such as memory and cognition and many brain pathologies such as ischaemia, epilepsy and Alzheimer’s disease [1,2]. The importance of astrocytes for brain function is recognized by a modern concept of ‘tripartite synapse’, postulating equal importance of presynaptic, postsynaptic and astroglial membranes [2–4].

Astrocytes encasement neurons and therefore are exposed to various neurotransmitters that spill out of the synaptic cleft. Astrocytes express a variety of neurotransmitter receptors which can trigger intracellular Ca\(^{2+}\) signalling [1–3]. Glial cells possess the SNARE (soluble N-ethylmaleimide-sensitive fusion protein-attachment protein receptor)-like Ca\(^{2+}\)-dependent machinery for vesicular release of ‘gliotransmitters’ such as ATP, glutamate and \(\delta\)-serine. Acting via the release of gliotransmitters, astrocytes can regulate synaptic strength [1–5].

Release of ATP as a glio- and neuro-transmitter

Extracellular ATP acts as a signalling molecule in the brain, triggering glial Ca\(^{2+}\) waves [4–6] and signals to neurons [7–9]. We found that synaptically released ATP mediated a distinct population of mPSCs (miniature spontaneous postsynaptic currents) in the neocortical and hippocampal pyramidal neurons [9,10]. Furthermore, stimulation of single neocortical and hippocampal axons evoked ATP-mediated unitary synaptic currents in the pyramidal neurons. These results are in line with the observation of vesicular release of ATP from GABAergic (where GABA is \(\gamma\)-aminobutyric acid) synaptic terminals in the hypothalamus [11]. Hence, ATP can be released from inhibitory and excitatory nerve terminals as a co-transmitter along with GABA and glutamate.

Other pathways of ATP release to the extracellular space include connexin hemichannels and spill out from damaged cells during brain insults [5,7,12]. Until recently, the main attention of studies of ATP release as a gliotransmitter was on connexins due to their high permeability [6,12–14]. However, the opening of connexins requires a much lower than physiological level of extracellular Ca\(^{2+}\). Vesicular release from astrocytes by SNARE complex-dependent mechanism seems to be more suitable for modulation of synaptic activity under physiological conditions [4,15].

Release of ATP from cortical astrocytes

We studied Ca\(^{2+}\)-dependent release of ATP from cortical astrocytes in situ and astrocytes acutely dissociated from tissue using a non-enzymatic isolation procedure with the aid of a vibrating glass ball. This technique preserves the cell processes and function of membrane proteins [16] and is therefore devoid of artefacts of enzymatic cell isolation and culturing procedures. Release of ATP from dissociated astrocytes were monitored using a ‘sniff-cell’ approach with the aid of HEK (human embryonic...
Figure 1 | Detection of ATP released from astrocytes using a ‘shift-cell’ approach

(A) Cortical astrocytes acutely dissociated from P21 C57BL6 mouse brain slice were resuspended, loaded with the Ca\(^{2+}\) indicator Fluo4-AM (Fluo-4 acetoxymethyl ester) and placed over cultured HEK-293 cells expressing P2X2 receptors. (B) Fluo-4 fluorescent signal monitored in the astrocyte simultaneously with whole-cell recording of transmembrane current in HEK-293 cells, voltage-clamped at −80 mV. Application of the specific astroglial PAR-1 receptor agonist (TFLLR) caused an elevation of cytosolic Ca\(^{2+}\) in the astrocyte followed by the burst of spontaneous ‘synaptic-like’ currents (\(t_{\text{decay}}\) ~5–15 ms) in the HEK-293 cell. Note that spontaneous currents have been eliminated after application of the P2X receptor antagonist (PPADS).

Kidney)-293 cells transfected with P2X2 purinoreceptors (Figure 1). Elevation of intracellular Ca\(^{2+}\) was triggered by TFLLR (Thr-Phe-Leu-Leu-Arg), an agonist of specific astroglial metabotropic PAR-1 (protease-activated receptor 1) [17,18]. We observed spontaneous ‘synaptic-like’ currents activated in the HEK-293-P2X2 cells during astrocytic Ca\(^{2+}\)-transients (Figure 1); these currents were abolished by a specific antagonist of ionotropic ATP receptors [PPADS (pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid), 10 \(\mu M\)]. These observations demonstrate capability of astrocytes to release significant amounts of ATP on a millisecond timescale.

Postsynaptic action of ATP

On the basis of affinity of P2X2 receptors to ATP, one can estimate the concentration of ATP released in the above experiment to be approx. 10 \(\mu M\). Levels of extracellular ATP in brain tissue assessed by various techniques in different physiological and pathological contexts can reach 1–100 \(\mu M\) [6–9,19,20]. Such a concentration is more than enough to activate ionotropic P2X and metabotropic P2Y purinoreceptors abundantly expressed in central neurons and glial cells [7,8,19–21]. They are capable of transmitting robust Ca\(^{2+}\) signals which do not require cell depolarization and are thereby implicated in the synaptic plasticity [19].

One of the important pathways for ATP action as a glio- and neuro-transmitter can be direct Ca\(^{2+}\)-dependent regulation of postsynaptic receptors. Phosphorylation of GABA\(_A\) and NMDA (N-methyl-D-aspartate) receptors provides an endogenous pathway for short- and long-term regulation of synaptic transmission [19,22–24].

We have shown previously that activation of P2X receptors by application of the exogenous ATP to isolated cells and endogenous release of ATP in situ caused the Ca\(^{2+}\)-dependent inactivation of NMDA receptors in the hippocampal [23] and neocortical pyramidal neurons (see Supplementary Figure S1 at http://www.biochemsoctrans.org/bst/037/bst0371407add.htm). Ca\(^{2+}\)-dependent down-regulation is an intrinsic property of NMDA receptors; the most feasible mechanism involves calmodulin and phosphatase II [22,25]. Another important class of neurotransmitter receptors prone to Ca\(^{2+}\)-dependent regulation is GABA\(_A\) receptors [24,26,27]. Interaction between P2X and GABA\(_A\) receptors has been reported recently, but its mechanisms remains controversial; direct coupling of intracellular domains of receptors co-expressed in Xenopus oocytes [28] contradicts the necessity of cytosolic Ca\(^{2+}\) signalling observed in the DRG (dorsal root ganglion) neurons [29].

To study the interaction between P2X and GABA\(_A\) receptors in mouse central neurons, we measured currents activated by fast application of their agonists to acutely isolated cells and mEPSCs (miniature evoked postsynaptic currents) and mPSCs in neocortical slices. Application of P2X receptor agonist significantly inhibited the GABA-activated currents in the isolated pyramidal neurons; this effect was eliminated by intracellular Ca\(^{2+}\)-chelators (Figure 2A).

We have described previously two distinct populations of synaptic currents recorded in the neocortical neurons in situ in the presence of CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) and D-AP5 (D-2-amino-5-phosphonovalerate): fast ATP-mediated current and slow GABA-mediated currents (outwards under our experimental conditions) [9,10]. Application of ATP and its analogues decreased the amplitude of GABA\(_A\) receptor-mediated spontaneous mPSCs (miniature inhibitory postsynaptic currents) without affecting their frequency (Figure 2B); this suggests that the effect was due to interaction of the postsynaptic receptors. Inactivation of GABA\(_A\) receptors in neocortical neurons was dependent on the intracellular Ca\(^{2+}\) and activity of PKA (protein kinase A), but was not altered after inhibition of phosphatase II (Figure 2C); we observed similar behaviour in DRG neurons as well (results not shown). These results strongly support the hypothesis of a Ca\(^{2+}\)-dependent interaction between P2X and GABA\(_A\) receptors. They also agree with previous data of Ca\(^{2+}\)-dependent down-regulation of GABA receptors [25,26].

Activation of cytosolic Ca\(^{2+}\) transient in the neocortical astrocytes in situ via a specific astroglial PAR-1 decreased
the amplitude of evoked and spontaneous GABA-mediated mIPSCs without affecting mIPSC frequency (Figure 3). A simultaneous dramatic increase in the frequency of P2X receptor-mediated spontaneous mEPSCs suggests that inhibition of GABA<sub>A</sub> receptors was caused by ATP released from astrocytes.

**Conclusion**

Our results show a novel pathway of modulation of signalling within tripartite synapse: release of ATP from synaptic terminals and astrocytes activates Ca<sup>2+</sup> influx via P2X purinoreceptors which are co-localized with NMDA and GABA<sub>A</sub> receptors. ATP-triggered modulation
of postsynaptic NMDA and GABA<sub>δ</sub> receptors involves phosphatase II and PKA. The physiological implications of this regulatory pathway are yet to be investigated.

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Figure 3 | Activation of astrocytes inhibits GABA receptor-mediated currents in the neocortex

(A) Time course of amplitude of mIPSCs evoked in the layer 2/3 pyramidal neurons by field stimulation of intracortical pathways in the presence of CNQX and D-AP5; each point represents the mean±S.D. of ten consecutive mIPSCs. (B) Time course of amplitude and frequency of mIPSCs simultaneously recorded in the same neuron; each point presents data averaged over 60 s. P2X receptor-mediated mEPSCs and GABA receptor-mediated mIPSCs were distinguished by their kinetics and reversal potential as described in [10]. Application of agonist of specific astroglial PAR-1 caused a decrease in the mEPSCs and mIPSCs (upper panel) accompanied by increase in frequency of P2X receptor-mediated spontaneous mEPSCs (lower panel); similarly to Figure 2(B), frequency of spontaneous mIPSCs was not altered. Taken together with results presented in Figures 1 and 2, these observations imply that activation of astrocytes induces vesicular release of ATP leading to P2X receptor-mediated down-regulation of postsynaptic GABA<sub>δ</sub> receptors.

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References

17. Shigetomi, E., Bowser, D.N., Sofroniew, M.V. and Khakh, B.S. (2008) Two forms of astrocyte calcium excitability have distinct effects on NMDA receptor-mediated slow inward currents in pyramidal neurons. J. Neurosci. 28, 6659–6663
22. Rycroft, B.K. and Gibb, A.J. (2004) Inhibitory interactions of calcineurin (phosphatase 2B) and calmodulin on rat hippocampal NMDA receptors. Neuropharmacology 47, 505–514


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SUPPLEMENTARY ONLINE DATA

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Supplementary Figure S1 can be found on the following page
Figure S1 | The NMDA receptor-mediated component of cortical mEPSCs is down-regulated by activating P2X receptors, an effect that is inhibited by antagonists of P2X receptors and intracellular Ca^{2+}-chelators

(A) Synaptic currents comprising NMDA and P2X receptor-mediated components were evoked in the layer 2/3 pyramidal neurons by stimulation of intracortical axons in the presence of CNQX (50 μM) and picrotoxin (100 μM) at two membrane potentials, first at +40 mV (mainly NMDA receptor-mediated components) and then at −80 mV (only the P2X receptors are responsible for the inward current measured). Note that a substantial inhibition of the NMDA component after return of the voltage to +40 mV (the mEPSCs measured at the moments indicated by the numbers are demonstrated in the inset, each trace is the average of five mEPSCs). The kinetics of the mEPSCs recorded again at +40 mV become faster because of the decrease in the slow NMDA receptor-mediated fraction. Switching off the stimulation at −80 mV did not produce any reduction in NMDA currents, indicating that effect is not an artefact of holding voltage change. At the same time, application of P2X receptor antagonist PPADS to the same cell diminished the effect. (B) Pooled data (means±S.D. for 20 cells) on inhibition of NMDA receptor-mediated current in neocortical neurons in situ. The effect did not depend on the membrane potential at which NMDA currents were recorded; difference in the inhibitory action of purinergic mEPSCs on NMDA current recorded at −40 and +40 mV was not statistically significant. On the other hand, the inactivation was decreased by the P2X receptor blocker PPADS and was almost abolished with intracellular solution containing the Ca^{2+}-chelator BAPTA [1,2-bis-(o-aminophenoxy)ethane-N,N′,N′,N′-tetra-acetic acid].