The Modulation of Synaptic Transmission by the Glial Purinergic System

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Abstract: Accumulating evidence indicates that bioactive substances produced by glia play an important role in the modulation of synaptic transmission. Astrocytes and microglia express many types of P2 purinoceptors and the stimulation of these receptors causes the release of bioactive substances, termed "gliotransmitters", such as ATP, glutamate and cytokines. Gliotransmitters are able to modulate synaptic transmission. In this article, the $P2X_4R$ and $P2Y_{12}R$ systems of microglia, which modulate the synaptic transmission between dorsal root ganglion neurons and dorsal horn neurons, are described. In addition, the role of the astrocyte purinergic system in synaptic transmission is discussed. The modulation of synaptic transmission by glial purinergic systems is a novel perspective on the regulation of brain and nerve function and is a new target for the development of medicines.

Keywords: ATP receptors, microglia, astrocyte, synaptic transmission.

1. INTRODUCTION

In 1972, Burnstock proposed new a role for nucleotides; that of neurotransmission [1]. Recently, numerous subtypes of ATP and adenosine receptor have been cloned, which has led to the acceptance of the "purinergic nervous system". Now purinergic receptors are divided into two big families, P1 (receptors for adenosine and AMP) and P2 (receptors for nucleotides). Four subtypes of P1 receptors have been cloned, namely, A_1, A_{2A}, A_{2B}, and A_3. P2 purinoceptors are divided into two families, ionotropic receptors (P2X) and metabotropic receptors (P2Y) [2]. P2X receptors (7 types; $P2X_1$ - $P2X_7$) contain intrinsic pores that allow the flow of ions. P2X receptors switch conformation from closed to open on binding ATP. P2Y receptors (8 types; $P2Y_{1, 2, 4, 6, 11, 12, 13 \text{ and } 14}$) are activated by purine or pyrimidine nucleotides or by sugar-nucleotides (which is subtype-dependent) and couple to intracellular second-messenger systems through heterotrimeric G-proteins [3-5].

Nucleotides are released or leaked from non-excitable cells, as well as from neurons and play a role in cell-to-cell communication in physiological and pathophysiological conditions[2, 6]. One of the most interesting non-excitable cells is the glial cell, which makes up over 70% of the total cell population in the central nervous system (CNS) and is classified into astrocytes, oligodendrocytes and microglia. Astrocytes express several types of P2 purinoceptors and release bioactive substances, including ATP in response to various stimuli or even spontaneously, and communicate with neurons and microglia. Microglia also express some types of P2 purinoceptors and are known as resident macrophages in the CNS, accounting for 5-10% of the total population of glia [7, 8]. Recent evidence has suggested that glial cells activated by the result of neuron-glia and glia-glia interactions through purinergic receptors release gliotransmitters (ATP, glutamate, cytokines, etc.), thereby affecting synaptic transmission. In this article, we describe the modulation of synaptic transmission by the glial purinergic system.

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2. MICROGLIA

2.1. Microglial Activation

Microglia express functionally active P2 receptors [9]. In the adult, microglia are ubiquitously distributed throughout the CNS and represent a morphologically unique type of cell which, under normal conditions, have a small soma bearing thin and branched processes. Such microglia were considered as 'resting', but recent studies investigating a transgenic mouse line that expressed green fluorescent protein in microglia using two-photon microscopy to image the behavior of these cells have revealed that microglial processes are highly dynamic in the brain [10, 11]. The processes of microglia rapidly move toward the site of injury, an effect that is mimicked by local injection of ATP and can be inhibited either by the ATP-hydrolyzing enzyme apyrase or by blockers of P2YRs [10]. Furthermore, Haynes *et al*. (2006) have shown that microglia in $P2Y_{12}R$-deficient mice exhibit normal basal motility but diminished directional branch extension toward nucleotides or sites of cortical damage *in vivo* [12]. Thus, microglia appear to act as sensors using the nucleotide/$P2Y_{12}R$ system.

When neurons are injured or over-stimulated, microglia undergo a stereotypical program of changes in morphology, gene expression, function and number to become activated microglia [13, 14]. Activated microglia migrate to the site of injury, release gliotransmitters, and engulf and phagocytose damaged cells or debris. These actions of microglia will af-

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fect synaptic transmission. For the activation of microglial motility, extracellular nucleotides have a central role. Extracellular ATP functions as a chemoattractant. Microglial chemotaxis towards ATP, *via* $P2Y_{12}$ receptors, was originally observed by Honda *et al*. (2001) [15] and has recently been confirmed *in vivo* in $P2Y_{12}$ receptor knock-out animals [12].

2.2. Microglial $P2X_4R$ Modulation of Synaptic Transmission in Primary Afferent Nerves and Dorsal Horn Neurons

There is a type of chronic pain that does not abate even though tissue damage has healed. This type pain is called 'neuropathic pain' and it typically develops when peripheral nerves are damaged, such as during surgery, bone compression in cancer, diabetes or infection. In addition to spontaneous pain and hyperalgesia (the increased pain perception of noxious stimuli), a troublesome symptom is known as tactile allodynia, a phenomenon of pain sensation evoked by innocuous stimuli. In normal conditions, innocuous stimuli cause touch sensation, mediated through the dorsal root ganglia (DRG) neuron Aβ, which does not conduct pain sensation. Therefore, tactile allodynia is thought to be a shift in the modality of sensation. The mechanism of neuropathic pain is still not fully understood. A variety of animal models for studying neuropathic pain have been developed, and in all models examined, activation of microglia in the dorsal horn is observed after nerve injury [13, 16-25]. Peripheral nerve damage leads to a dramatic change in the microglia within the spinal dorsal horn; these cells become activated through a step-by-step process [13]. Within the first 24 hrs after peripheral nerve injury, the first signs of microglial activation can already be observed: the small soma become hypertrophic and the long and thin processes withdraw [16]. This is followed by a burst of proliferation, with a peak around 2~3 days after nerve injury [21, 22, 26-28]. Also, activated microglia upregulate the expression of complement receptor 3 leading to enhanced OX-42 labeling [16-19, 29], with peak enhancement observed around 14 days after injury [17, 20].

An important question is how microglia in the spinal cord become activated when the nerve injury may occur at a very great distance from the spinal cord. The signal(s) of injury in sensory or mixed nerves remains obscure but candidate signaling molecules involved in microglial activation are growth factors, cytokines, chemokines and neurotransmitters [13]. A potential candidate is monocyte chemoattractant protein-1 (MCP-1) whose expression is markedly increased in DRG neurons after nerve injury [20, 30, 31]. The time-course of MCP-1 upregulation in DRG neurons is similar to that of microglial activation [20]. Recently, it was found that mice lacking either toll-like receptor 4 (TLR4) or 2 (TLR2), which are type I transmembrane signaling proteins that recognize pathogen-associated molecular patterns, showed impaired microglia activation in the dorsal horn after nerve injury [32, 33]. Thus, TLRs also appear to play an important role in nerve injury-induced microglia activation in the spinal cord. However, the identity of endogenous ligands for these receptors remains to be determined. A recent study has indicated that interferon-γ (IFN-γR) levels are increased in the spinal cord after nerve injury, leading to speculation that it has a role in neuropathic pain [32]. However, there was no direct evidence indicating that IFN-γ signaling contributes to microglial activation in the dorsal horn and to tactile allodynia under neuropathic pain conditions. More recently, Tsuda *et al*. reported that in naive animals, spinal microglia express a receptor for IFN-γR in a cell-type specific manner and that stimulating this receptor converts microglia into activated cells and produces a long-lasting tactile allodynia [34]. Conversely, ablating IFN-γR severely impairs nerve injury-evoked microglia activation and tactile allodynia without affecting microglia in the contralateral dorsal horn or basal pain sensitivity. These results imply that IFN-γR is a key element in the molecular machinery through which 'resting' spinal microglia transform into an activated state and, thereby, drive neuropathic pain.

There is abundant evidence demonstrating activation of spinal microglia in the neuropathic pain state, but until recently it remained an open question as to whether spinal microglia play a causal role in neuropathic pain. Tsuda *et al*. (2003) directly implicated activated microglia in the pathogenesis of neuropathic pain by determining the role of the purinoceptor $P2X_4R$. A clue to identifying $P2X_4Rs$ in the spinal cord as being required for neuropathic pain first came from pharmacological investigation of pain behavior after nerve injury using the P2X receptor antagonists TNP-ATP and PPADS [22]. The marked tactile allodynia after an injury to the spinal nerve was found to be reversed by acute intrathecal administration of TNP-ATP but was unaffected by the administration of PPADS. From the pharmacological profiles of TNP-ATP and PPADS, it was inferred that the tactile allodynia depends upon $P2X_4Rs$ in the spinal cord. Expression of $P2X_4R$ protein progressively increased in the days following nerve injury, the time-course of which parallels that of the development of tactile allodynia. Immunohistochemical analyses showed that many small cells in the dorsal horn of the nerve-injured side were positive for $P2X_4R$ protein, and these cells were identified as microglia. The cells expressing $P2X_4R$ in the nerve-injured side of the dorsal horn showed high levels of OX-42 and morphological hypertrophy, which are characteristic markers of activated microglia. Moreover, it was found that reducing the upregulation of $P2X_4R$ protein in spinal microglia using $P2X_4R$ antisense oligodeoxynucleotides prevented the development of nerve injury-induced tactile allodynia. Finally, Tsuda *et al*. reported that in mice with a disrupted *p2rx4* gene (*p2rx4*$^{-/-}$ mice), tactile allodynia caused by an injury to the spinal nerve was markedly blunted [22, 35, 36]. Together, these observations implied that activation of $P2X_4Rs$ in spinal microglia is necessary for neuropathic pain. $P2X_4R$ activation in microglia was shown to be sufficient for the development of allodynia by intrathecal administration of activated, cultured microglia in which these receptors had been stimulated by ATP *in vitro* [22]. In otherwise naïve animals, allodynia develops progressively over the 3-5 hours following the administration of the $P2X_4R$-stimulated microglia. Moreover, in rats in which tactile allodynia was caused by ATP-stimulated microglia this allodynia was reversed by administering TNP-ATP [22]. Thus, the allodynia caused by ATP-stimulated microglia is pharmacologically similar to that caused by peripheral nerve injury. Together, these findings indicate that $P2X_4R$ stimulation of microglia is not only necessary for tactile allodynia but also is sufficient to cause the allodynia.

Enhanced synaptic transmission and firing discharge of neurons in the dorsal horn pain-processing network following nerve injury is crucial for neuropathic pain syndromes [37, 38]. The first evidence that microglia may participate in the hyperexcitability of dorsal horn neurons was revealed by Coull *et al*. (2005). They used spinal cord slices taken from rats displaying allodynia following intrathecal administration of $P2X_4R$-stimulated microglia and found that ATP-stimulated microglia positively shifted the anion reversal potential (E_{anion}) in spinal lamina I dorsal horn neurons [39]. In normal conditions, a touch stimulation evokes an impulse in DRG Aβ neurons, which partially innervate inhibitory interneurons resulting in the release of the neurotransmitter, GABA. GABA opens chloride channels and normally causes hyperpolarization of dorsal horn neurons to diminish the pain sensation. In this abnormal condition, GABA released by touch stimulation causes depolarization rather than hyperpolarization in DH neurons, thereby evoking pain sensation [39]. Moreover, TNP-ATP which can reverse nerve injury-induced allodynia [22] acutely reverses the depolarizing shift in E_{anion} in lamina I neurons after peripheral nerve injury [39]. Together with the findings that the depolarizing shift in E_{anion} and the excitatory response to GABA are key events in dorsal horn neurons in neuropathic pain after nerve injury [40], these results imply that spinal microglia stimulated by $P2X_4R$ cause neuropathic pain through a rise in intracellular Cl^- in spinal lamina I DH neurons.

The next question is, 'what are the underlying mechanisms for the microglial affect on the anion balance in lamina I DH neurons'? Coull *et al*. (2005) determined the role of brain-derived neurotrophic factor (BDNF) as a signaling factor between microglia and dorsal horn lamina I neurons. It was found that intrathecal application of BDNF mimicked tactile allodynia and the depolarizing shift in E_{anion} in lamina I neurons following peripheral nerve injury or intrathecal administration of $P2X_4R$-stimulated microglia. Furthermore, interfering with signaling between BDNF and its receptor (TrkB), either by a function-blocking TrkB antibody or by a BDNF-sequestering fusion protein (TrkB-Fc), prevented tactile allodynia caused by peripheral nerve injury or by intrathecal administration of P2X4R-stimulated microglia. Moreover, activation of $P2X_4R$ on microglial cells caused the release of BDNF [39, 41], an effect that is dependent on activation of p38 [41], a member of the mitogen-activated protein kinase family that is implicated in neuropathic pain [42, 43]. Interestingly, $P2X_4$-mediated BDNF release was abolished by inhibiting SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor)-mediated exocytosis. Thus, these results indicate that $P2X_4$-stimulated microglial release of BDNF causes a collapse in the transmembrane anion gradient and subsequent neuronal hyperexcitability in lamina I neurons (Fig. **1**). The $GABA_AR$-mediated depolarization might also produce an excitation through voltage-sensitive Ca^{2+} channels and NMDA receptors. There is also evidence that several proinflammatory cytokines which are known to be released from microglia [9, 44] modulate excitatory synaptic transmission. Interleukin-1β was reported to enhance the NMDA receptor-mediated Ca^{2+} response [45], while long-term treatment with interferon-γ produced an increase in neuronal excitability in dorsal horn neurons [46]. Thus, the net enhanced transmission in the dorsal horn pain network by these factors might be responsible for nerve injury-induced neuropathic pain (Fig. **1**).

Upregulation of $P2X_4R$ in microglia is an important process in producing neuropathic pain. Nasu-Tada *et al*. (2006) reported the role of fibronectin, an extracellular matrix protein, as a potential candidate for inducing $P2X_4R$ upregulation in microglia. It was found that microglia cultured on fibronectin-coated dishes showed a marked increase in $P2X_4R$ expression both at the mRNA and protein levels [47]. This upregulation of $P2X_4R$ protein by fibronectin might be functional as the $P2X_4R$ -mediated Ca^{2+} response was enhanced in fibronectin-treated microglia. Tsuda *et al*. found that in fibronectin-stimulated microglia, activation of phosphatidylinositol 3-kinase (PI3K)-Akt and mitogen-activated protein kinase kinase (MAPK kinase, MEK)-extracellular signal-regulated kinase (ERK) signaling cascades occurred divergently downstream of the Src-family kinase (SFK), Lyn, in microglia [48]. Pharmacological interference of PI3K-Akt signaling inhibited fibronectin-induced $P2X_4R$ gene expression. Activation of PI3K-Akt signaling resulted in a decrease in the protein level of the transcription factor p53 *via* mouse double minute 2 (MDM2), an effect that was prevented by MG-132, an inhibitor of the proteasome. In microglia pretreated with MG-132, fibronectin failed to upregulate $P2X_4R$ expression. Conversely, an inhibitor of p53 caused increased expression of $P2X_4R$, implying a negative regulatory role of p53. On the other hand, inhibiting MEK-ERK signaling activated by fibronectin suppressed an increase in $P2X_4R$ protein levels but, interestingly, did not affect the level of $P2X_4R$ mRNA. Fibronectin stimulation resulted in the activation of the translational factor eIF4E *via* MAPK-interacting protein kinase-1 (MNK1) in a MEK-ERK signaling-dependent manner, and an MNK1 inhibitor attenuated the increase in $P2X_4R$ protein. Together, these results suggest that the PI3K-Akt and MEK-ERK signaling cascades have distinct roles in the upregulation of $P2X_4R$ expression in microglia at transcriptional and post-transcriptional levels, respectively [48]. Tsuda *et al*. also found that IFN-γ-stimulated spinal microglia showed upregulation of Lyn (a tyrosine kinase) and the purinergic $P2X_4$ receptor, which are crucial events for the generation of neuropathic pain. Genetic approaches also provide evidence linking these events to IFN-γR-dependent microglial and behavioral alterations [34, 49]. It was also reported that activating both TLRs and NOD2 in cultured microglia increased expression of $P2X_4R$ at the mRNA level [50], thus implying an involvement of these receptors in the regulation of $P2X_4R$. More research is required to reveal the complete mechanism underlying over-expression of $P2X_4R$ in activated microglia after nerve injury.

3. ASTROCYTES

3.1. ATP Receptors in Cultured Astrocytes

Since the first reports of elevations in $[Ca^{2+}]i$ in cultured astrocytes in response to neurotransmitters [51, 52] it has become apparent that many types of neurotransmitter stimulate Ca^{2+} elevations in glial cells by activating specific receptors. These receptors include metabotropic glutamate receptors, dopamine receptors, noradrenaline receptors, serotonin receptors and purinergic receptors [53], whose activation results in elevations in $[Ca^{2+}]i$ in astrocytes. Glutamate can

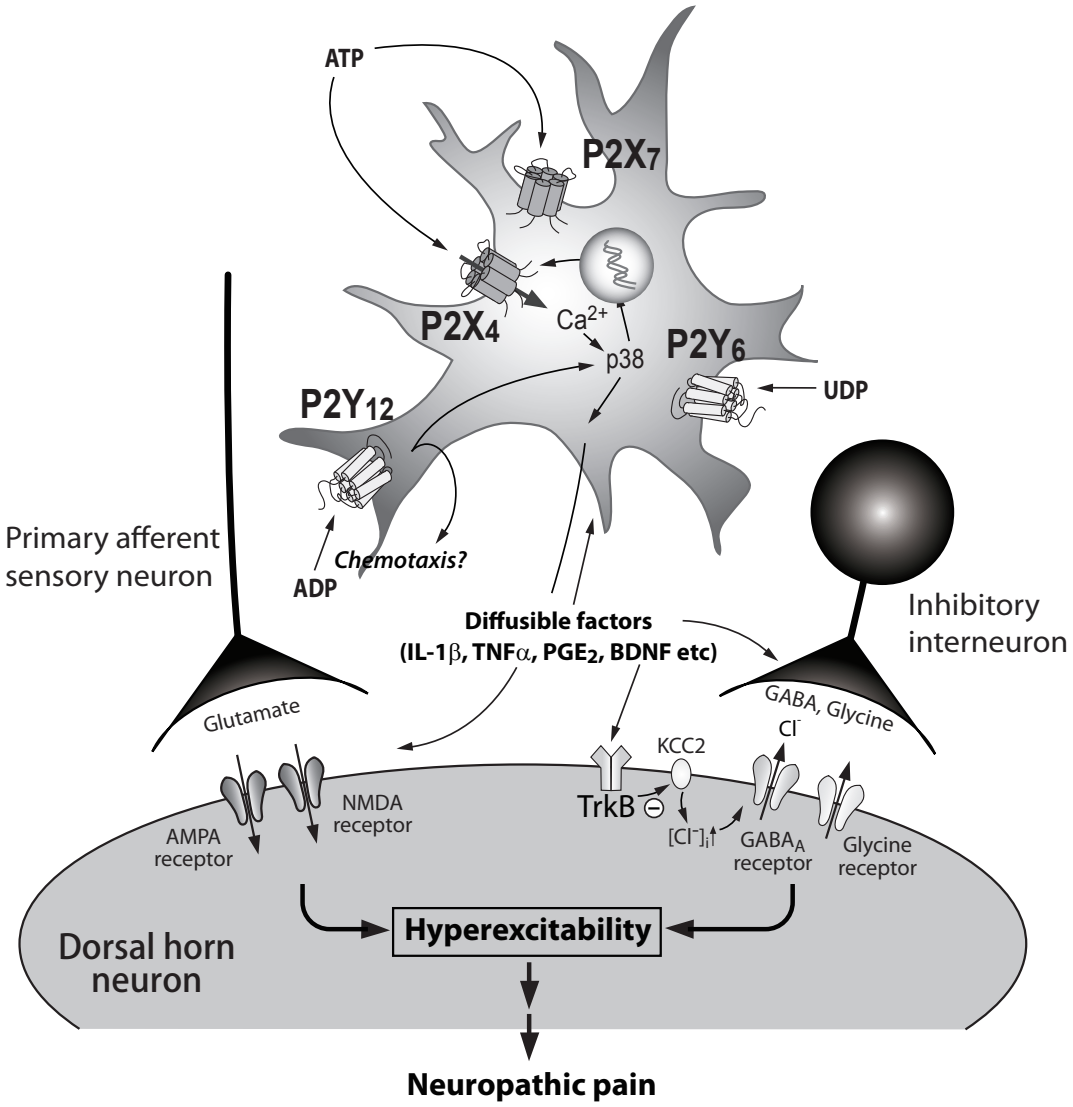

Fig. (1). Schematic illustration of microglial modulation of synaptic transmission in primary afferent nerves and dorsal horn neurons. After peripheral nerve injury, microglia in the spinal cord ipsilateral to the nerve injury become transformed to the activated phenotype (hypertrophy, proliferation, and the expression of cell-surface molecules). Activated microglia after nerve injury increase the expression of $P2X_4R$. The $P2X_4R$ are activated by ATP resulting in the release of bioactive diffusible factors such as BDNF and other proinflammatory factors (cytokines and chemokines). BDNF causes a collapse of the transmembrane anion gradient in dorsal horn lamina I neurons presumably through the downregulation of KCC2, which in turn renders GABA and glycine affects depolarizing, rather than hyperpolarizing, in these neurons. Microglial factors (cytokines, PGE_2, etc) may also interact with excitatory synapses of neighboring dorsal horn neurons and enhance the excitability in dorsal horn neurons. The net hyperexcitability in the dorsal horn network by these factors from activated microglia may be responsible for abnormal pain signaling.

elicit $[Ca^{2+}]i$ elevation not only in individual cells but also in intercellular waves $[Ca^{2+}]i$ that are propagated from single cells to multiple neighboring cells [52]. Neuronal activity can directly initiate such a Ca^{2+} wave in astrocytes [54]. Other stimuli, such as local mechanical or electrical stimulation, were subsequently observed to initiate similar intercellular Ca^{2+} signaling in astrocytes. Ca^{2+} waves were thought to propagate *via* gap junctions [55-57], through which the internal messenger inositol 1,4,5-triphosphate (IP_3) can diffuse to mobilize Ca^{2+} release [57]. Recent experiments in culture showed that Ca^{2+} waves can be propagated between astrocytes, even when the cells do not contact each other directly, and the extent and direction of the Ca^{2+} wave propagation are significantly influenced by movement of the extracellular medium [58]. Recent reports suggest that substances released from astrocytes can activate receptor systems on astrocytes, evoking the release of gliotransmitters. Importantly, it was found that extracellular ATP is the major messenger for this event. ATP is released from astrocytes during Ca^{2+} wave propagation [58], and the propagation can be reduced or abolished by a purinergic antagonist [58] [59-62] or by the ATP degrading enzyme apyrase [58, 62]. In addition, it was demonstrated by a method for the visualization of ATP release that the velocity of ATP release correlates well with that of the Ca^{2+} wave in astrocytes [62]. These findings suggest that the extracellular molecule ATP

could be a primary signal for the Ca^{2+} wave propagation, and highlights the importance of ATP in cross-talk among astrocytes and even with other cell types in the CNS (Fig. **2**).

Astrocytic ATP also mediates presynaptic inhibition in cultured hippocampal neurons [62, 63]. Cultured hippocampal neurons exhibit synchronous spontaneous Ca^{2+} oscillation, which is extracellular Ca^{2+}-dependent, tetrodotoxin-sensitive and inhibited by inhibitors of ionotropic glutamate receptors, suggesting that the neuronal Ca^{2+} oscillation is mediated by glutamatergic synaptic transmission [62, 64, 65]. Endogenous ATP released from astrocytes dynamically down-regulates spontaneous neuronal Ca^{2+} oscillations [62] and EPSCs in the hippocampal culture [63] by inhibiting presynaptic functions of glutamatergic neurons.

ATP differs from glutamate as a signaling molecule between astrocytes and neurons in that it inhibits rather than potentiates synaptic transmission. It is hypothesized that the opposing actions of glutamate and ATP released from astrocytes represent a means by which astrocytes can dynamically modulate neuronal activity by releasing distinct transmitters which can either excite or inhibit synaptic transmission. In addition to mediating inhibitory rather than excitatory effects on synaptic transmission, ATP-mediated astrocyte-to-neuron signaling further differs from glutamate-dependent signaling mechanisms by the fact that it occurs in a tonic fashion [62] [63, 66]. When the ATP-degrading enzyme apyrase was applied, a potentiation of spontaneous neuronal Ca^{2+} oscillations or EPSCs was induced in the absence of any astrocytic stimulation. This means the presence of a constitutive ATP-dependent inhibition of synaptic transmission. Furthermore, spontaneous astrocytic Ca^{2+} responses occur in both purified astrocyte cultures and in mixed cultures of astrocytes and neurons. The spontaneous Ca^{2+} signals in astrocytes were inhibited by apyrase but persisted in the presence of TTX. Therefore, astrocytes constitutively release ATP in the absence of neuronal activity, thereby exerting tonic down-regulation of excitatory synaptic transmission [62, 63]. ATP mediates astrocytic Ca^{2+} waves and can evoke neuronal Ca^{2+} responses in various parts of the CNS, suggesting that ATP may be a ubiquitous mediator of astrocyte-to-neuron signaling in the modulation of synaptic activity. Such a tonic modulation by astrocytic ATP might be a mechanism by which neurons tune their communications in the CNS.

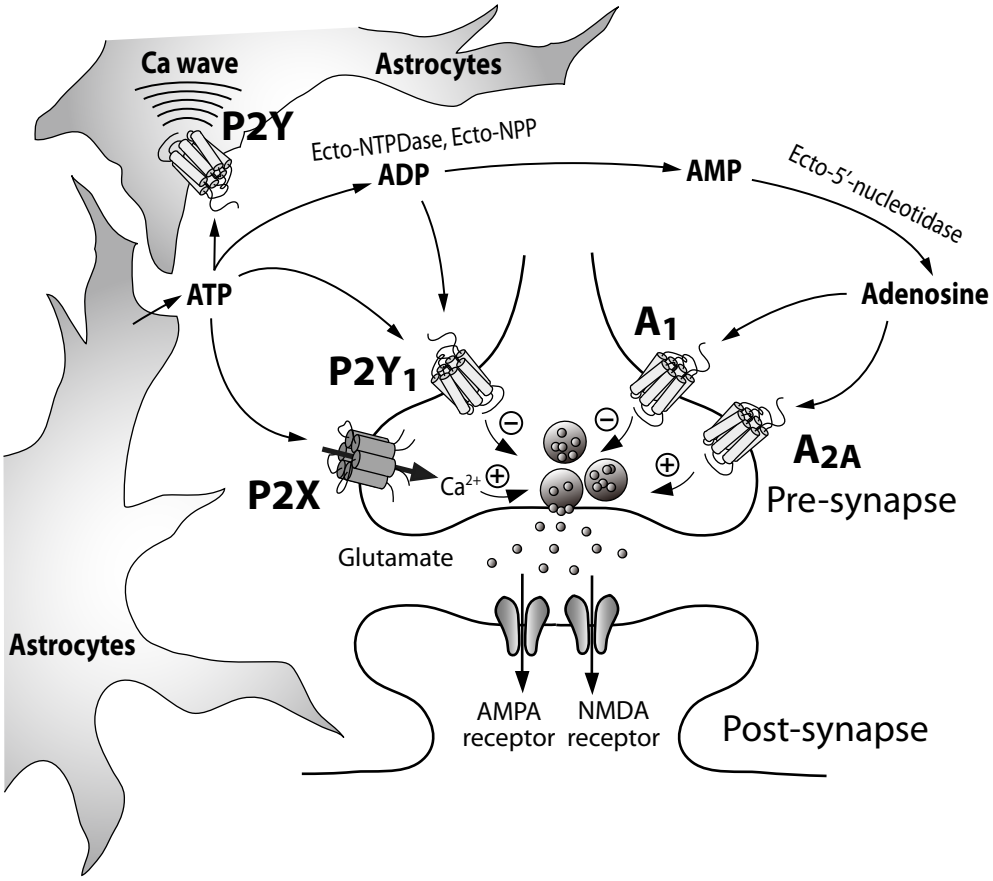

Fig. (2). Schematic illustration of astrocytic modulation of synaptic transmission.

Suppression of glutamatergic transmission by ATP of astrocyte-origin occurs through the activation of presynaptic $P2Y_1$ receptors in cultured hippocampal neuron-glia co-cultures and the activation of presynaptic A_1 receptors in acute slices, due to strong hydrolyzing activity around the synapses in the slices. In contrast, activation of A_{2A} receptors rather potentiates excitatory transmission. Additionally, in a large variety of central structures, the stimulation of presynaptic P2X receptor by α,β-methylene ATP facilitate glutamatergic synaptic transmission *via* direct Ca^{2+} entry through P2X receptors.

3.2. Adenosine Receptors in the Retina

ATP molecules are readily cleaved by enzymatic hydrolysis to yield ADP, AMP and adenosine. Adenosine activates P1 purinergic receptors, for which there are the subtypes A_1, A_{2A}, A_{2B}, and A_3. Newman reported that astrocytic purinergic signaling in the mammalian retina is related to the suppression of neuronal activity [67]. He also showed that light activation of photoreceptors in the retina leads to Ca^{2+} signaling in associated Muller glial cells, evoking the release of ATP which causes outward currents in these neurons [68, 69]. Importantly, these inhibitory actions were not mediated directly by ATP, but instead by its metabolite adenosine. Indeed, stimulation of the adenosine A_1-receptor induces outward currents, and DPCPX, an A_1-receptor antagonist, inhibits the light-induced neuronal suppression that is mediated by glia.

3.3. The Purinergic System in the Synaptic Glia-Neuron Interaction of *In Situ* Brain Tissue

Diverse Effects of Exogenous Purines in Brain Slices

Exogenous application of various types of P2X, P2Y, A_1 and A_{2A} receptor agonist exerts potent and robust effects on synaptic transmission in brain slices through activating these receptors expressed on the pre- and/or postsynapses. Application of P2X agonists results in a robust inward current, presumably activating the postsynaptic receptors, in the medial habenula [70], cortex [71, 72], locus coeruleus [73], and other structures [74-77] [78]. These postsynaptic P2X receptors can also be activated by electrical stimulation of presynaptic afferent fibers in the slice, suggesting ATP/P2X receptor-mediated fast synaptic transmission [79].

However, compared with the wide distribution of P2X receptor subunit expression in the brain and also to the P2X agonist responses reported in isolated or cultured neurons of brain origin, such ATP/P2X receptor-mediated fast transmission has been identified only in limited structures, as listed above. Even in the cases where such transmission occurs, the resulting postsynaptic responses are so small that complete blockade of principal glutamatergic transmission and extremely strong presynaptic stimulation are required to detect them [80, 81]. Rather, in a large variety of central structures, degradation-resistant P2X receptor agonists such as α,β-methylene ATP robustly facilitate release of transmitters such as glutamate and GABA, mostly through direct Ca^{2+} entry through presynaptic P2X receptors [82-88], suggesting that the major site of action for the extracellular ATP in CNS neurons *in situ* is in presynaptic structures.

The effects of exogenously applied ATP to neurons in brain slices are more complicated than those in isolated or cultured cells primarily due to the higher-density of enzymes involved in ATP to adenosine conversion [89]. Local application of ATP to the hippocampal pyramidal neurons, for example, evokes robust outward current in a manner sensitive to A_1 receptor antagonists and inhibitors of ecto-5'-nucleotidase [90], suggesting that ATP undergoes a rapid degradation down to adenosine within a few hundred seconds near the synapse sites (Fig. **2**). In a similar manner, application of both ATP and adenosine suppresses excitatory transmission in many brain synapses, which are also sensitive to A_1 antagonists [85, 91-94]. Involvement of A_1 receptors in this ATP-evoked synaptic suppression in the hippocampus was unequivocally demonstrated by the lack of ATP effect in mice lacking A_1 receptors [91]. Such effects of ATP resulting from activation of A_1 receptors are also found in other structures, where ATP plays dual roles, one as a P2 receptor agonist and another as a P1 receptor agonist [85, 92, 95], resulting in complex bi-directional regulation of synaptic transmission (Fig. **2**). The situation in the brain slice is more complicated because not only the neurons but also astrocytes express P2Y and adenosine receptors (but not P2X receptors [96]), activation of which makes them release many transmitters and mediators including glutamate and ATP, which in turn activates specific neuronal and astrocytic receptors and gives rise to specific responses [97].

Suppression of glutamate release by exogenously applied adenosine A_1 receptor agonists seems to be a ubiquitous mechanism of synaptic regulation [93, 94]. In contrast, activation of A_{2A} receptors with exogenously applied agonists rather potentiates excitatory transmission, a mechanism that might have important implications in corticostriatal regulation[98-100]. Moreover, interactions between presynaptic A_1 and A_{2A} receptors [101] and A_{2A} and dopamine D_2 receptors [102] increase the complexity of such purinergic regulation (Fig. **2**). This field, which is extremely important for the development of therapeutic treatments against epilepsy [103], Parkinson's disease [98], sleep disorder [104] and age-dependent cognitive decline [105, 106], awaits further studies to obtain more complete understanding.

Astrocyte-Neuron Interaction in Synaptic Transmission

Identification of ATP as a gliotransmitter released from astrocytes [107] helped the understanding of how these pre- and postsynaptic purinoceptors are activated by endogenous purines in brain slices and in the brain *in vivo*. Whereas suppression of glutamatergic transmission by ATP of astrocyte-origin occurs through the activation of presynaptic $P2Y_1$ receptors in cultured hippocampal neuron-glia co-cultures, it occurs through activation of presynaptic A_1 receptors in acute slices, due to strong hydrolyzing activity around the synapses in the slices, as evidenced by its inhibition using ecto-5'-nucleotidase inhibitors [63]. Such effects of ATP of astrocyte-origin on A_1 receptors are confirmed by the use of gliotoxins [63], by perfusion of gap junction-linked astrocyte syncytium with a Ca^{2+} chelator, BAPTA, combined with evaluation of heterosynaptic depression in the hippocampus [108], and by using mice carrying astrocyte-specific expression of a dominant negative form of synaptobrevin-2 [66]. This mouse also shows abnormalities in sleep homeostasis and cognitive deficits associated with sleep loss, suggesting adenosine derived from ATP of astrocyte origin plays a role in sleep maintenance [109].

The mechanism of ATP release from astrocytes is a topic of debate. The following four mechanisms have been proposed, each with convincing demonstration: 1) through vesicular exocytosis [66, 110], 2) through hemi-channels, which are connexin or pannexin channels expressed on the membrane surface without their counterpart to form cell-to-cell gap junctions [111, 112], 3) through $P2X_7$ receptor channels that have large conductance pores [113], and 4) through large-conductance anion channels, the molecular identity of which remains unidentified [114, 115]. At this moment, it seems that pathways for ATP release from astro-

cytes may depend on the type of stimulus, region, and subcellular structures.

Nevertheless, direct demonstrations of modulation of synaptic transmission by ATP or adenosine of astrocyte origin in native brain tissue are currently scarce. Yamazaki *et al*. demonstrated, in the hippocampal slice, that experimental depolarization of a single astrocyte suppresses excitatory transmission recorded in adjacent neurons in a manner sensitive to ecto-5'-nucleotidase inhibitor and adenosine A_1 antagonist[116], suggesting activation of astrocytes can indeed modulate synaptic transmission through the release of ATP and subsequent conversion to adenosine. In addition, they also demonstrated a continuous firing of the neuron, which in turn modifies the membrane properties of astrocytes, arguing strongly for mutual astrocyte-neuron interaction. Open questions for understanding the physiological and pathophysiological significance of the purinergic neuron-astrocyte interaction include, in which situations and through which mechanisms do astrocytes release ATP to synapses and how is the quantitative balance between ATP, ADP and adenosine determined by ecto-5'-nucleotidase to determine the resulting synaptic responses?

CONCLUSIONS

Astrocytes and microglia express many types of purinoceptors and the stimulation of these receptors cause the release of gliotransmitters (ATP, glutamate, cytokines etc.) affecting synaptic transmission. In this article, $P2X_4R$ and $P2Y_{12}R$ systems of microglia for modulation of the synaptic transmission between dorsal root ganglion neurons and dorsal horn neurons are mainly described. $P2X_7R$ of microglia is also tightly correlated with the release of cytokines that affect synaptic transmission in several part of nervous system though we did not mentioned the role of this receptors in this paper. Astrocytic purinergic system in synaptic transmission was also mentioned mainly in the case of hippocampus. The modulation of synaptic transmission by glial purinergic system is a novel insight of the regulation of brain and nerve function, especially abnormal and disease of brain functions. Therefore, glial purinergic system might be a new target for developing medicines.

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