Original Article

Access this article online

Quick Response Code:

Website: http://www.braincirculation.org

DOI: 10.4103/bc.bc_27_24

The cellular distribution of P2X7, P2Y6, and P2Y12 during or after pilocarpine-induced status epilepticus and literature review

Yue Li[1,2], Fengru Tang[3#], Yumin Luo[4#]

Abstract:

BACKGROUND: When a seizure occurs, the distribution of purine receptors in different cell types at various time points remains poorly understood. Our literature review revealed that P2X7, P2Y6, and P2Y12 are expressed in different cells during epilepsy pathogenesis. Therefore, we studied the protein expression patterns of the purinergic receptors P2X7, P2Y6, and P2Y12 in the normal mice hippocampus, as well as during or after pilocarpine-induced status epilepticus (DPISE or APISE).

MATERIALS AND METHODS: Immunohistochemical staining and double-labeling immunofluorescence staining were used to study the cellular distribution of various purinergic receptors across several groups: control, 2-hour DPISE, 1-day APISE, 2-day APISE, 3-day APISE, and 1-week APISE.

RESULTS: In the normal mouse brain, P2X7, P2Y6, and P2Y12 were predominantly expressed in the neurons. Microglia and astrocytes were found to express these receptors at the onset of seizures. Immunofluorescence analysis showed that P2X7 and P2Y12 are expressed in microglia, whereas P2Y6 is mainly expressed in astrocytes.

CONCLUSION: Different purinergic receptors are expressed in neurons, microglia, and astrocytes, mediate their interactions, and are involved in epileptogenesis.

Keywords:

Astrocytes, microglia, neuron, P2X7, P2Y12, P2Y6, purinergic receptors, status epilepticus

Departments of [1]Education and [4]Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China, [2]Department of Anatomy, National University of Singapore, [3]Department of Radiation Physiology Lab, Singapore Nuclear Research and Safety Initiative, National University of Singapore, Singapore

[#]These authors contributed equally to this work

Address for correspondence:

Dr. Fengru Tang, MD, PhD, Radiation Physiology Lab, Singapore Nuclear Research and Safety Initiative, National University of Singapore, Singapore 138602, Singapore. E-mail: tangfr@gmail.com; Yumin Luo, Xuanwu Hospital, Capital Medical University, No 45, Changchun Street, Xicheng District, Beijing, 100053, E-mail: yumin111@ccmu.edu.cn

Submission: 03-04-2024
Revised: 30-06-2024
Accepted: 02-07-2024
Published: 28-12-2024

Introduction

Background

Epilepsy is a spontaneous, unprovoked seizure affecting approximately 70 million people worldwide, with an incidence of 1%.[1] Pilocarpine-induced status epilepticus (SE) is a common epilepsy animal model. Pilocarpine induces SE by acting on the M1 muscarinic receptor subtype. Pilocarpine-induced SE can be divided into four phases: during pilocarpine-induced SE (DPISE), characterized by spontaneous remitting tonic-clonic seizures for several hours; the acute stage, characterized by the loss of neurons (days 1 to 3 post-SE); the latent stage, characterized by a seizure-free period with more prominent neuron loss (days 7 to 14 post-SE); and the chronic stage, characterized by uncontrolled spontaneous seizures and sclerosis (days 60 post-SE), these three stages were called after pilocarpine-induced SE (APISE).[2,3]

Increased blood–brain barrier (BBB) permeability, altered synaptic structure and plasticity, degeneration, neurogenesis, and inflammation are the key pathological process of epilepsy.[4] A study on both experimental models and epileptic patients

How to cite this article: Li Y, Tang F, Luo Y. The cellular distribution of P2X7, P2Y6, and P2Y12 during or after pilocarpine-induced status epilepticus and literature review. Brain Circ 2024;10:343-53.

demonstrates that astrocytes and microglia contribute to increased brain hyperexcitability. Astrocytes have been proven to participate in gliotransmission, the balance of extracellular water and ions, BBB maintenance, arteriolar blood flow regulation, energy supply, metabolism, oxidative stress, and inflammation.[5] Astrocyte activation can cause epileptic seizures that do not involve other pathologies.[6] Microglia can be classified into homeostatic microglia and disease-associated microglia and they are involved DPISE and APISE in rodent epileptic models.[7]

Inflammation contributes to both seizure generation and epilepsy development.[8] The discovery of novel soluble inflammatory mediators is in exponential growth. Besides glutamatergic and GABAergic neurotransmission, purinergic signaling has been proven to be the third neuronal transmitter involved in seizure generation and epilepsy development.[9,10] During epilepsy, adenosine triphosphate (ATP) acts as a neurotransmission and gliotransmission factor and it has been proven to cause seizures when microinjected into the rodent peripheral form cortex.[11] Targeting purine receptors has been shown to be one of the promising treatments for epilepsy.

Purinergic receptors can be divided into P1 receptors (adenosine sensitive), which can be further subdivided into A1, A2A, A2B, and A3 receptors, and P2 receptors (nucleotide sensitive), which can be further subdivided into inotropic P2X and metabotropic P2Y receptors. P2X receptors respond to ATP and have seven mammalian subtypes, whereas P2Y receptors respond to ATP, adenosine diphosphate (ADP), uridine triphosphate (UTP), uridine diphosphate (UDP), or UDP-glucose and have eight subtypes. The detailed function and classification of purinergic receptors are shown in Figure 1. Evidence confirms that the nucleoside adenosine has an anticonvulsant effect via P1 receptors,[10] and this effect has also been observed for P2 receptors.[9]

Neurons and glia can release ATP via exocytosis, transporters, membrane channels, and P2X7 (P2X7R) channels.[12] ATP can be converted to ADP and adenosine monophosphate (AMP), and finally to adenosine by ectoenzymes, such as NTPDases, NPPases, and alkaline phosphatases. ATP is classified as a damage-associated molecular pattern and is an endogenous dangerous signal.[13] ATP (ADP and UDP) activated by purines induces intracellular Ca^{2+} increases in astrocytes, from extracellular P2XRs and intracellular P2YRs.[14] The P2 receptor is involved in diverse signaling cascades, such as proinflammatory cytokines release and processing, cytoskeletal rearrangements, and transcription factors activation.[15-17]

Objectives

Astrocytes and other cell types also express purinergic receptors.[18] The distribution and function of the P2 receptors have generated much interest as their expression patterns will change after the onset of several neurodegenerative diseases and play roles in pathological processes. We determined the distribution of the P2 receptors DPISE or APISE by choosing ionotropic P2X7 and metabotropic P2Y6 and P2Y12 to study their expression patterns, as they have received great attention recently. In the hippocampus, P2X7 receptors are upregulated by 80% when rats enter the pilocarpine-induced chronic epileptic stage.[19] P2X7 expression is thought to alter APISE.[20] The P2Y12 receptor is thought to be expressed in microglia cells during epilepsy.[21]

Materials and Methods

Pilocarpine treatment

The National Neuroscience Institutional Animal Care and Use Committee of Tan Tock Seng Hospital, Singapore, approved this study. The animal used was 8–10 weeks male Swiss Albino mice weighing 25 g–30 g without spontaneous seizures. Methylscopolamine nitrate (1 mg/kg) was injected subcutaneously into the mice. Thirty minutes later, saline was injected subcutaneously into the control group and pilocarpine (300 mg/kg) was injected subcutaneously into the experimental group.

Immunohistochemical Study of the Expression of P2X7, P2Y6 and P2Y12

The experimental animals were divided into six groups, and each group included six mice. The groups were as follows: control group, 2 h DPISE group, 1 day APISE group, 2 days APISE group, 3 days APISE group, and 1 week APISE group. After anesthetization with phenobarbital (40–50 mg/kg), 100ml saline was transcardially perfused into the mice, and then 100 ml 0.1M phosphate buffered saline (PBS, pH 7.4) was dissolved in 4% paraformaldehyde. After perfusion, the brains were removed, fixed in 4% paraformaldehyde for 4 h, and soaked overnight in PBS dissolved in 30% sucrose. Freezing microtome (HM505E, Microm; Zeiss, Oberkochen, Germany) was used to cut the brain into 40-μm thickness coronal sections. The sections were put into 24 well-tissue culture dishes according to the order. They were washed in PBS and then in 0.1M Tris-buffered saline (TBS), and their peroxidase activity was quenched with 3% H_2O_2 for 15 minutes. The sections were then washed in TBS for three times, and treated with 2% BSA or 4% normal goat serum (this step was adapted to these primary antibodies, which were made in goats). These sections were incubated in 0.1% Triton-X100 diluted with 0.1M TBS (TBS-TX, pH 7.6) for 2 h. Next, the sections were incubated overnight with primary antibodies diluted in TBX-TX against P2X7, P2Y12 (1:1000, 1:300,

Sigma, St Louis, MO, USA), and P2Y6 (1:350, Abcam, Boston, MA, USA). The next day, sections were washed once with TBS-TX and incubated with biotinylated IgG secondary antibodies for 2 h. After washing thrice with TBS-TX, the sections were incubated in an avidin-biotin complex (ABC) reagent diluted with TBS-TX for 2 h. Washed by 0.1M Tris buffer (TB, pH 7.6), reacted with 0.012% H_2O_2 and 0.05% 3, 3′-diaminobenzidine hydrochloride TB solution (Sigma, St Louis, MO, USA) for 3-5 minutes and then washed by TB again. Finally, the sections were mounted on slides, dehydrated, and covered with coverslips.

Double-labeling immunofluorescence staining

Double-labeling immunofluorescence staining was carried out to colocalize P2X7 (1:500), P2Y6 (1:100), and P2Y12 (1:100) with different cell markers, i.e. NeuN (1:1000, Chemicon International Inc., CA, USA) for neurons, CD11b (1:1000, AbD Serotec, Oxford, UK) for microglia, and GFAP (1:1000, Chemicon International Inc., CA, USA) for astrocytes. The sections were washed with 0.1 M TBS and incubated with 3% H_2O_2 for 15 minutes. Next, they were incubated with 2% BSA for 2 h, and with primary antibodies overnight. The next day, sections were incubated with Cy3-or FITC-conjugated secondary antibodies (Chemicon International Inc., CA, USA) for 4 h. They were then mounted on slides, dried, and coverslipped using FluorSaveTM Reagent (Calbiochem, San Diego, CA, USA). Images were captured using an Olympus FluoView FV500 confocal laser scanning biological microscope (Olympus Corporation, Tokyo, Japan).

Results

Behavior monitoring

After treatment with pilocarpine, the mice developed hyperactivity, recurrent myoclonic convulsions, and SE, those symptoms lasting for more than 4 h (Classification of seizures referred to Racine's 1972, only those with Racine scale score ≥3 were chosen for the experimental group). Mice that did not experience continuous seizures for 4 h were considered as non-SE mice and were excluded from the study. Mice who were convulsed and died were also excluded. Approximately 14 days after the SE, the mice began to experience spontaneous recurrent seizures.

Immunohistochemistry results of P2X7, P2Y6, and P2Y12 in the hippocampus of the control and pilocarpine-induced status epilepticus mice

P2X7

In control mice, P2X7 was found to be expressed in neurons in different layers of the cornu ammonis (CA) area of the hippocampus [in the border between the stratum oriens and alveus-O/A border, the stratum oriens-SO, the stratum pyramidale-SP, the stratum radiatum-SR, and stratum lacunosum moleculare-SLM, Figure 2a]. P2X7 immunopositive neurons were also observed in the stratum granular cells (GC) and hilus of the dentate gyrus (DG) [Figure 3a]. The same situations were observed at 2 h DPISE [Figures 2b and 3b]. At 1 day APISE, P2X7 was found to be expressed in activated glial cells in the CA and DG are as [Figures 2c and 3c]. At 2 days APISE, more P2X7 positive neurons were stained in the SP of the CA area and the GC and hilus of the DG area. Activated glial cells were observed [Figures 2d and 3d]. Three days APISE, most of the P2X7 immunopositive cells were glial cells [Figures 2e and 3e]. At higher magnification (×100), P2X7 immunopositive glial cells were observed, they changed from the hyper ramified to amoeboid and finally to the phagocytic glial cells from 1 to 3 days APISE [Figure 2p-r].

P2Y6

In control mice, P2Y6 immunopositive neurons were scattered in different layers of the CA area and the GC and hilus of the DG area [Figures 2f and 3f]. Except for the SP layer, the number of P2Y6 immunopositive neurons decreased at 2 h DPISE and 1 day APISE. At 3 days APISE, except for P2Y6 immunopositive neurons in the SP layer, P2Y6 immunopositive glial cells were observed in the CA and DG areas [Figures 2i and 3i]. At 1 week APISE, P2Y6 immunopositive glial cells increased and they were activated based on their morphology [Figures 2j and 3j].

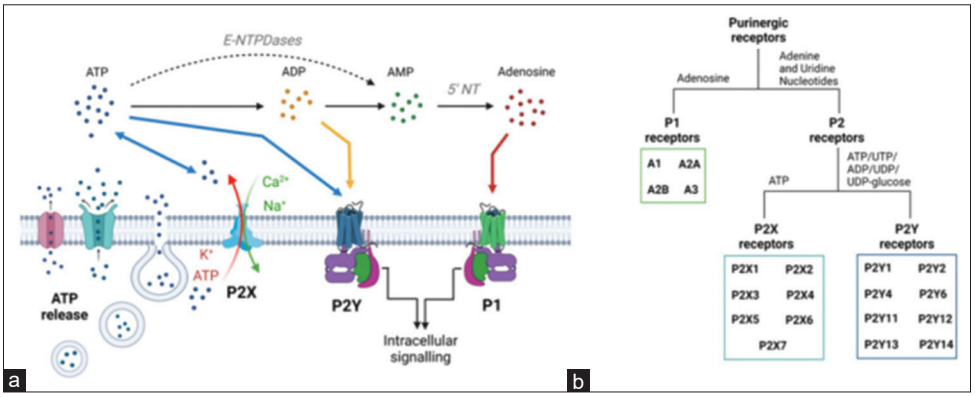

Figure 1: Purinergic signaling overview. (a) Detailed function of purinergic receptors, (b) Classification of purinergic receptors[4]

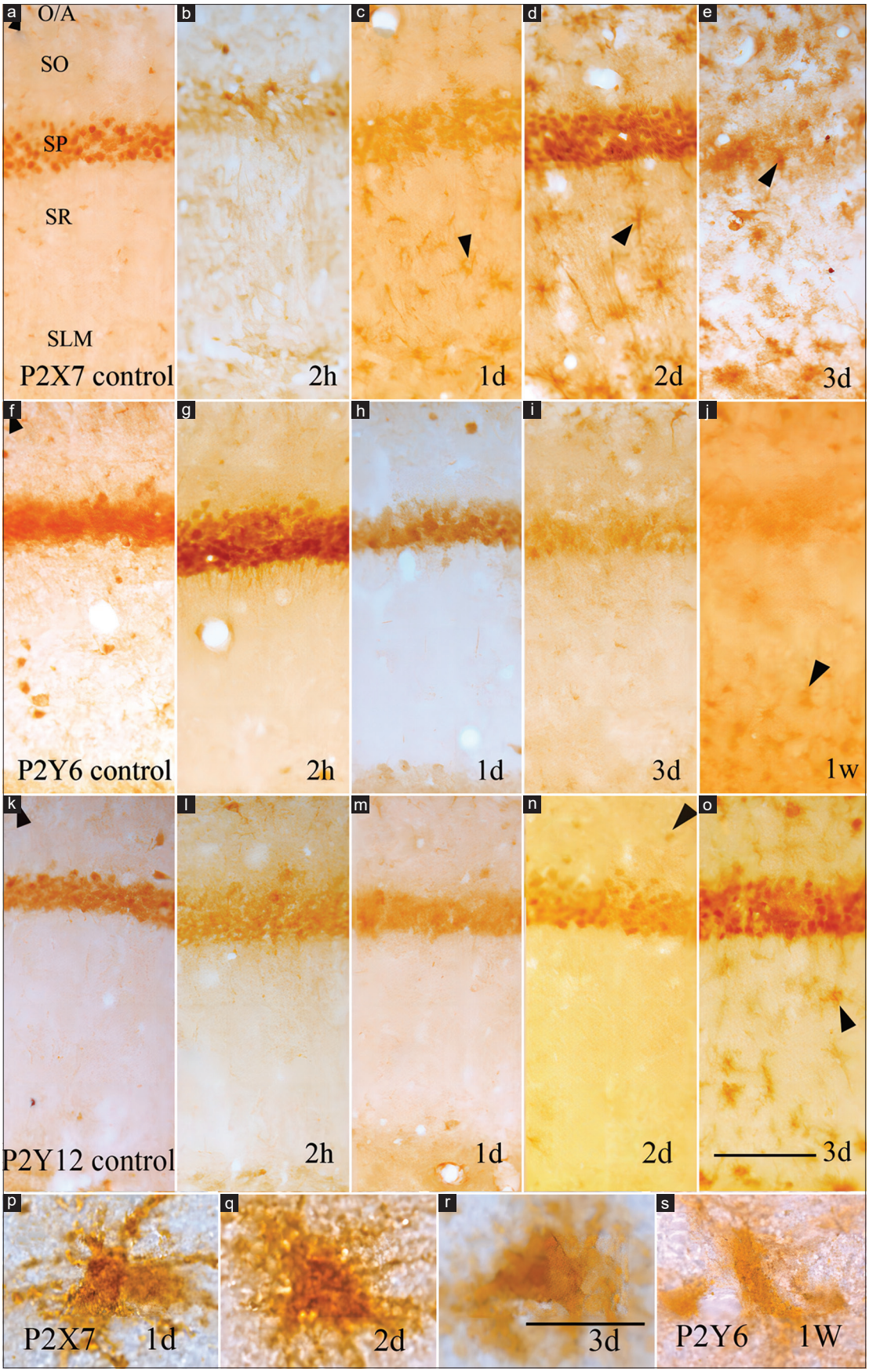

Figure 2: The distribution of P2X7, P2Y6, and P2Y12 receptors in the cornu ammons (CA) area of the hippocampus in control and pilocarpine-induced status epilepticus mice. The detailed descriptions of Figure 2 (a-s) were under Figure 3.

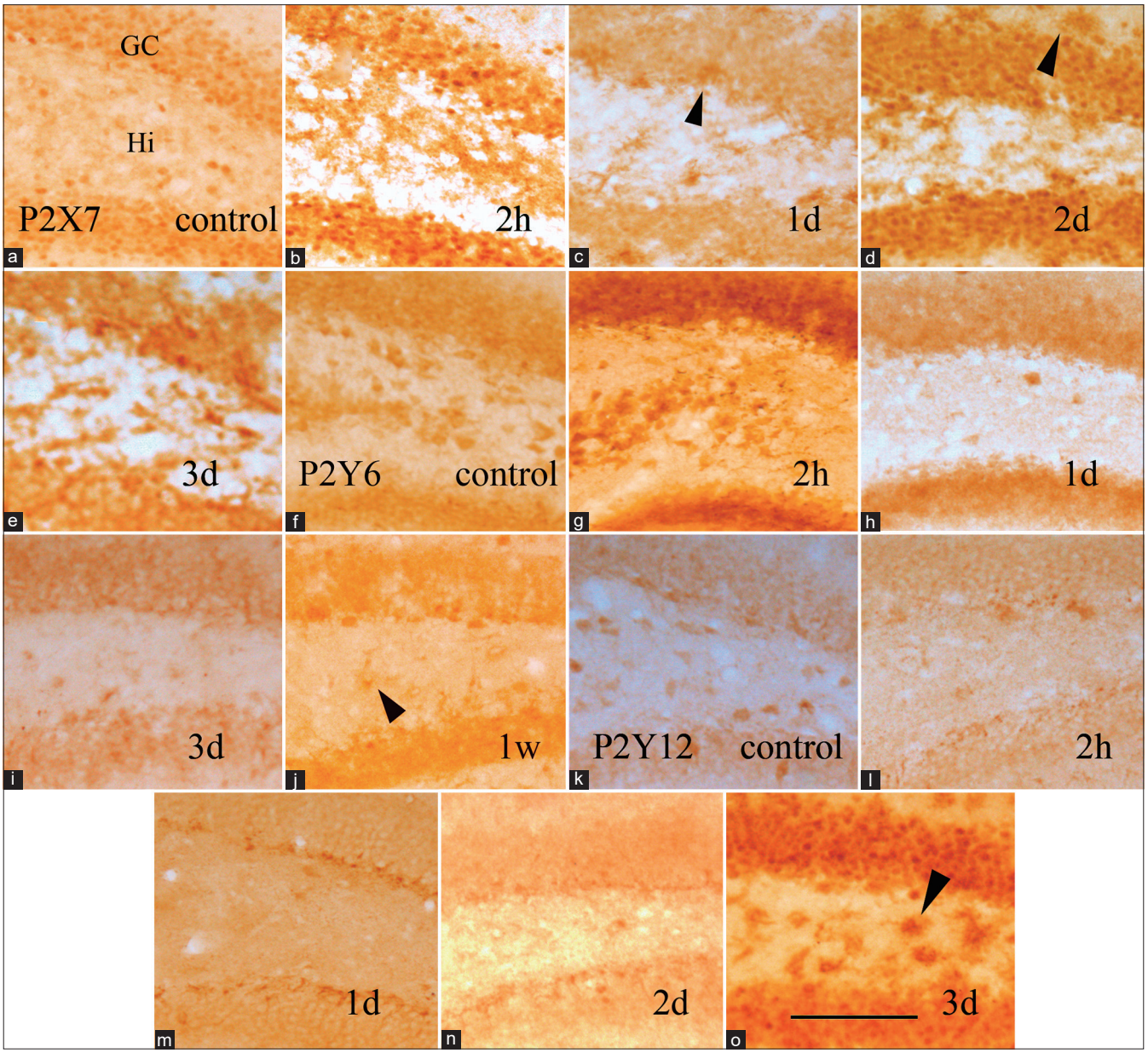

Figure 3: The distribution of P2X7, P2Y6, and P2Y12 receptors in the denta te gyrus (DG) area of the hippocampus in control and pilocarpine-induced status epilepticus mice. In the control and 2h DPISE mice, P2X7 immunopositive neurons were scattered across different layers of the CA and DG areas of the hippocampus.The P2X7 immunopositive neurons were mainly located in the SP layer of the CA area and GC and hilus of the DG area [Figures 2, 3a, 3b]. At 1–2 days APISE, P2X7 staining was observed in glial cells scattered in different layers of the CA and DG areas except for the SP layer and GC [Figures 2, 3c, 3d]. The P2X7 positive glial cells were found in each layer of the hippocampus at 3 days APISE [Figures 2, 3e]. In the control and2 h DPISE to 1 d APISE, P2Y6 immunopositive neurons were mainly in the SP, GC and hilus of the hippocampus [Figures 2, 3f-h]. At 3 days to 1 week APISE, P2Y6 positive glial cells were observed [Figures 2, 3i, 3j]. From the control to 2 days APISE, P2Y12 positive neurons were mainly observed in the SP layer of the CA area and GC and hilus of the DG area [Figures 2, 3k-n]. At 3 days APISE, except for P2Y12 positive neurons in the SP layer of the CA area, P2Y12 positive glial cells were also observed in other layers of the CA area and the hilus of the DG area [Figures 2, 3o]. P2X7 and P2Y6 immunopositive glial cells were identified at ×100 [Figure 2p-s]. The scale bars indicate 100 μm in a-o in Figures 2 and 3, and 20 μm in p-s in Figure 2.

At high magnification (×100), the morphology of the P2Y6 immunopositive glial cells could be observed more clearly [Figure 2s].

P2Y12

From the control to 2 days APISE, P2Y12 immunopositive neurons were mainly observed in the SP layer [Figure 2k-n] and in the GC and hilus of the DG [Figure 3k-n]. At 3 days APISE, except for P2Y12 immunopositive neurons in the SP layer of the CA area and GC of the DG area, P2Y12 immunopositive glial cells could be seen in different layers of the CA area without SP, and hilus of DG [Figures 2o and 3o].

Double immunofluorescence staining of P2X7, P2Y6, and P2Y12 with CD11b or GFAP or NeuN in the hippocampus of the control and SE mice

Colocalization of P2X7 with CD11b confirmed

the microglial expression of P2X7 at 1-3 days APISE [Figure 4a-c]. The colocalization of P2X7 with NeuN confirmed the neuronal expression of P2X7 at 2 days APISE [Figure 4e-h]. No colocalization of P2X7 and GFAP at 3 days APISE confirmed that P2X7 was not expressed in astrocytes [Figure 4j-l], which was also confirmed by three-dimensional scanning [Figure 4i]. P2X7 immunopositive products were also found in the stratum lucidum of the CA3 area and the hilus of the DG at 1 day APISE [Figure 4d, arrow].

At 1 week APISE, the P2Y6 receptor localized to the neuronal membrane [Figure 5a] in the hippocampus. It was also colocalized with GFAP immunopositive astrocytes [Figure 5a-f].

In the hippocampus of the 3-day SE mice, immunopositive P2Y12 neurons and microglia cells are shown in red, and the CD11b immunopositive microglia cells are shown in green. As they are colocalized, the microglial cells in Figure 6a-i appear yellow. A diagram of our purine receptor immunohistochemical staining results are shown in Figure 7. P2X7 and P2Y12 are expressed in neurons and microglia, whereas P2Y6 is expressed in neurons and astrocytes DPISE and APISE.

Discussion

Key results

The authors studied the cellular distribution of P2X7, P2Y6, and P2Y12 DPISE and APISE. In the hippocampus of the control mice, the P2X7, P2Y6, and P2Y12 immunopositive cells were neurons, in principal cells (pyramidal neurons and granule cells) and nonprincipal neurons. From 1 day to 3 days APISE, P2X7 was found to be expressed in active microglia, and at 1 day APISE, P2X7 was expressed by mossy fibers and/or terminals in the hilus of the DG, that is, it can also be expressed in axonal or presynaptic locations.[22] At approximately 3 days APISE, P2Y12 was expressed in reactive microglia. At approximately 3 days to 1 week APISE, P2Y6 was expressed in reactive astrocytes.

Using Western blotting, Huang *et al*. found that P2X7 receptors increased at 1 day, reached their peak at 2 days, and returned to baseline at 2 weeks in the hippocampus in a rat SE model induced by Coriaria lactone.[23]

There is a range of P2Y receptors expressed in microglia, with P2Y1 receptors inducing microglial migration,[24]

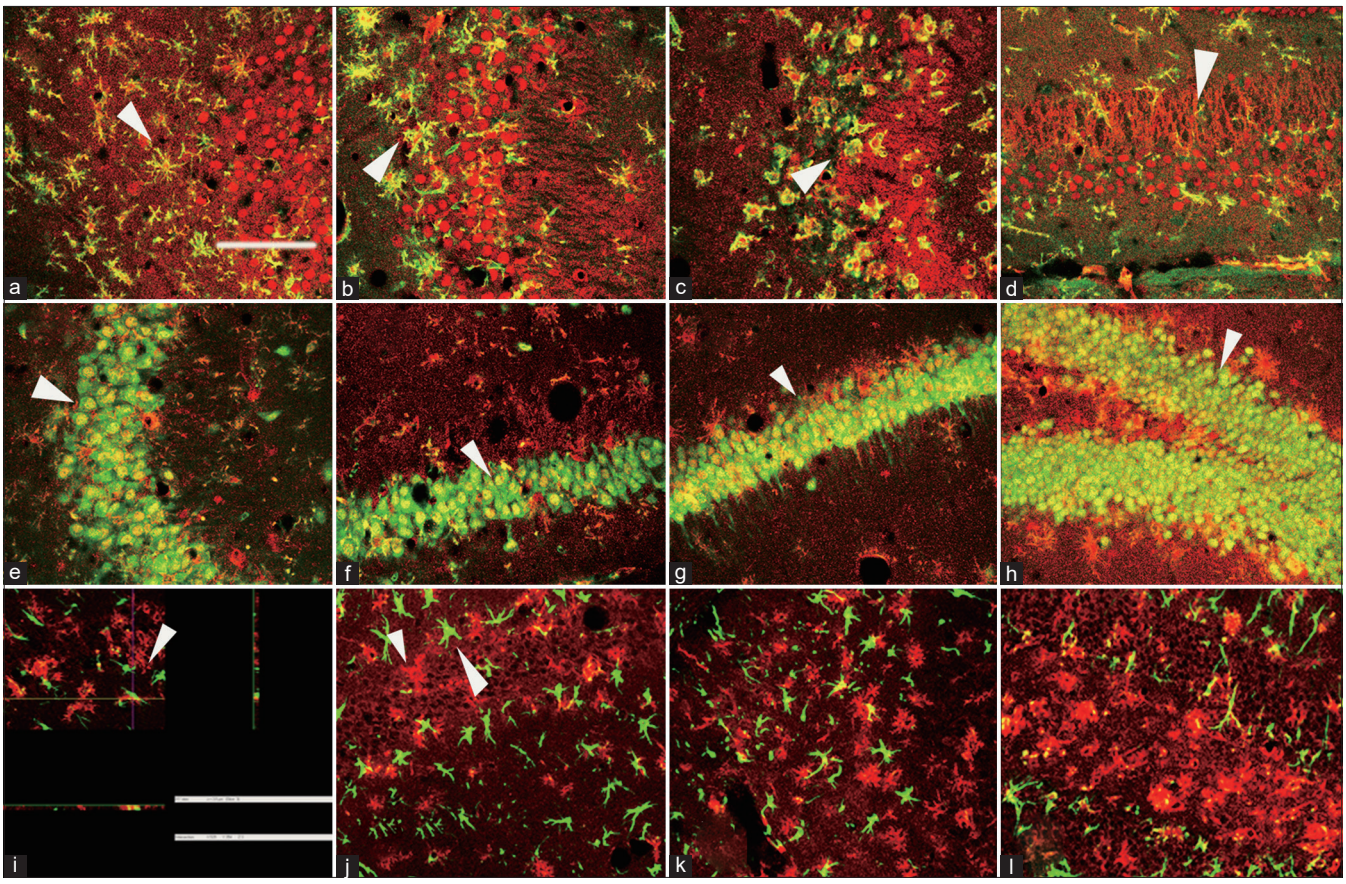

Figure 4: Double immunofluorescence labeling of P2X7 with CD11b, NeuN, and GFAP in the hippocampus of the experimental mice. Double-labeling immunofluorescence staining shows colocalization (yellow, arrows) of P2X7 (red) with CD11b (green). (a and d): 1-day after pilocarpine-induced status epilepticus (APISE), (b):2-day APISE, (c): 3-day APISE, and colocalization of P2X7(red) with NeuN (green). (e-h): 2-day APISE. It also shows no colocalization of P2X7 (red) with GFAP (green). (i-l): 3-day APISE. Scale bar = 50 μm in a-l.

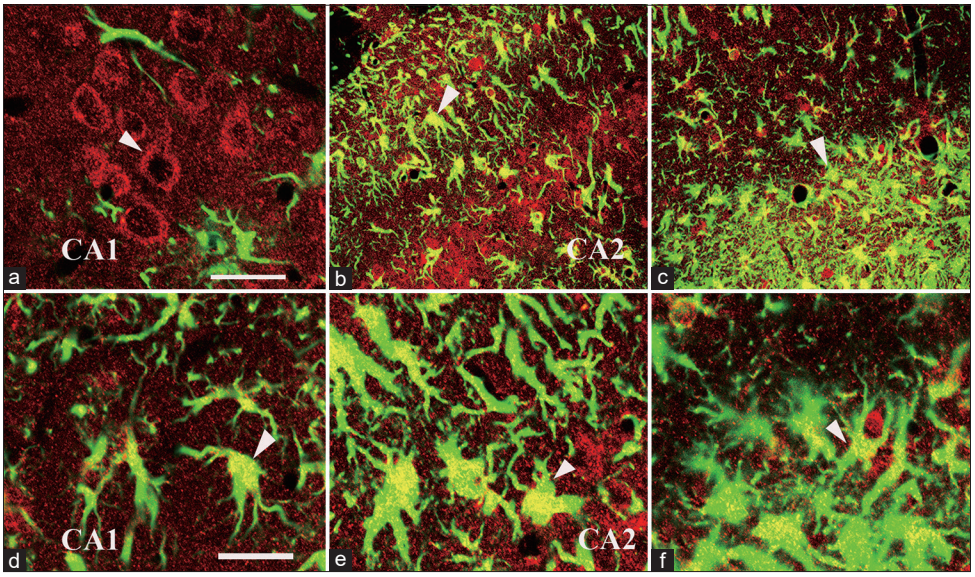

Figure 5: Double immunofluorescence labeling of P2Y6 with GFAP in the hippocampus of experimental 1-week APISE mice. Double-labeling immunofluorescence staining shows colocalization (yellow, arrows) of P2Y6 (red) with GFAP (green, a-f) at 1 week APISE. Scale bar = 50 μm in b-c, and 12 μm in a,d-f

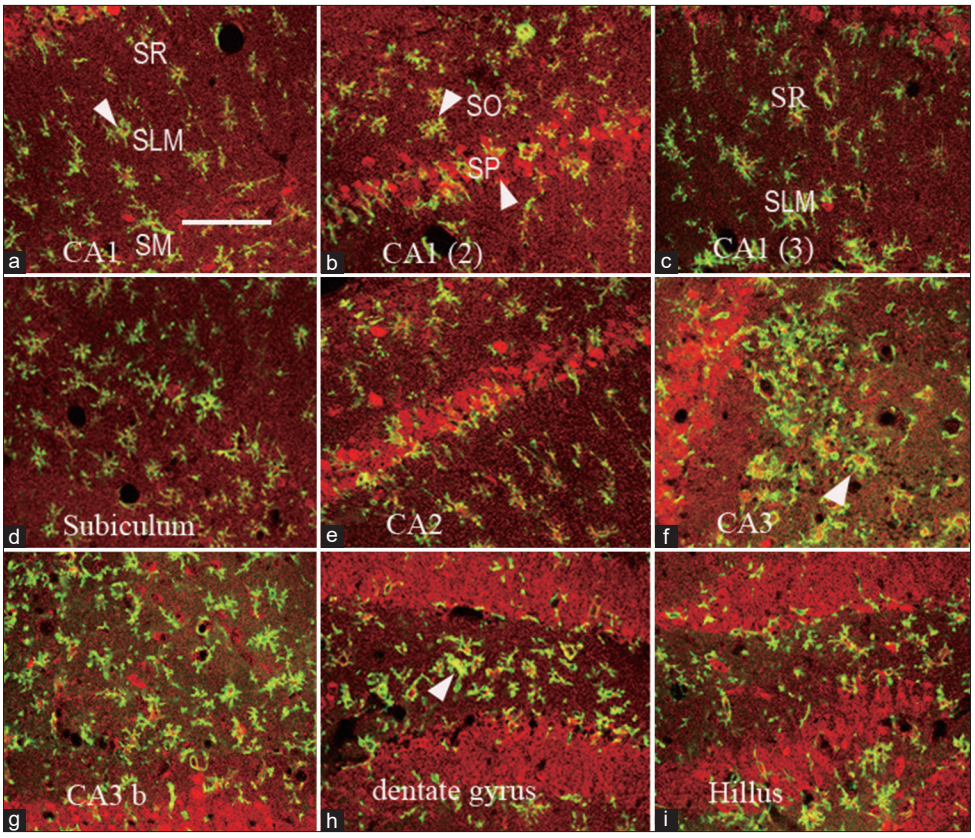

Figure 6: Double immunofluorescence labeling of P2Y12 with CD11b in the hippocampus of the experimental 3-day APISE mice. Double-labeling immunofluorescence microscopy shows colocalization (yellow, arrows) of P2Y12 (red) with CD11b (green, a-i) at 3 days APISE. Scale bar = 50 μm in a-i

P2Y6 receptors inducing microglial phagocytosis,[25] and P2Y12 receptors inducing chemoattraction of microglia to ATP accumulation sites.[26] The significance of the induced P2Y6 expression in astrocytes but not in microglia in the present immunofluorescence study needs to be investigated further.

The interactions between neurons or neurons and glia in the hippocampus are complex. As different layers of the hippocampus receive input from different brain or hippocampal regions, we described the detailed cellular distribution of these three purinergic receptors and hope that this information can provide clues for future

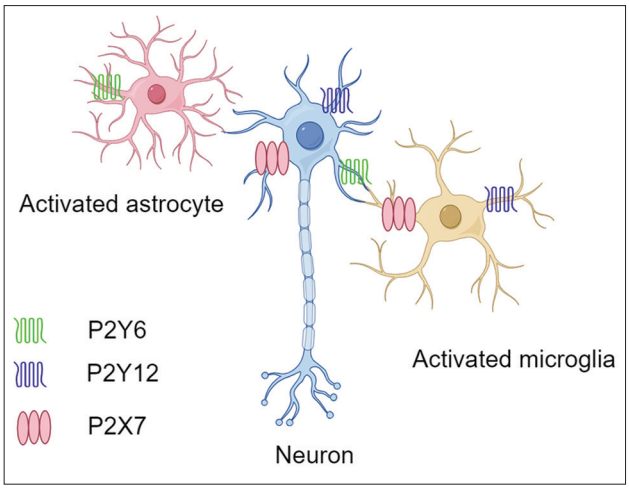

Figure 7: The cellular distribution of P2X7, P2Y6, and P2Y12 during or after pilocarpine-induced status epilepticus which is created by Figdraw. P2X7 and P2Y12 are expressed in neurons and microglia, whereas P2Y6 is expressed in neurons and astrocytes DPISE and APISE.

functional studies. ATP modulates the neuron–neuron and neuron–glia interactions and produces physiological effects via the P2X and P2Y receptors, which can be modulated using pharmacological methods.

Significance and the progressive changes of the expression of P2X7, P2Y6, and P2Y12 in hippocampal neurons and glial cells during or after pilocarpine-induced status epilepticus

P2 receptors are mainly expressed in neurons and glial cells and can be involved in neurotransmission, neurogenesis, and neuron–glia interactions.[27,28] Among them, P2X4 and P2X7 function in tandem and are involved in neuroinflammation.[29] P2X4 is located on chromosome 12, close to P2X7, and P2X4 and P2X7 have different affinities for ATP, milimolar ATP activates the former, while the latter is activated by micromolar concentrations.[30] P2X4 works with pannexin 1 to release ATP and trigger the reaction, and P2X7 amplifies this signal and elevates the information in the brain.[31] P2X7 can promote the release of proinflammatory cytokines such as IL1α, IL1β,[32] IL6,[33] and IL18,[34] and act as gate keeper of inflammation. Spontaneous seizures are the manifestation of epilepsy, and epileptogenesis is the underlying mechanism.[35,36] It was found that P2X7Rs are preferentially expressed on resident macrophages-microglia cells in the central nervous system(CNS),[37] which is consistent with our findings.

As stated in the introduction section, ATP can mediate signaling among astrocytes and the signal between astrocytes and neurons or microglia. Astrocytes can release ATP in different ways under physiological or pathological conditions.[12] When epilepsy occurs, astrocytes release ATP, and through P2X7 receptors, mediate communication between microglia and astrocytes. Activated P2X7 receptors on the microglia trigger inflammatory signals and promote astrogliosis.[38] The interaction of astrocytes with neurons occurs mainly through metabotropic P2Y1 receptors, while the interaction of astrocytes with microglias occurs mainly through ionotropic P2X7 receptors.[39] Their interactions are characterized by immune cells entering the CNS system and the activation of astrocytes and microglia.[40] Neuroinflammation has two functions: one is to promote astrogliosis, and the other is to alter the excitability of neuronal networks.[41] In conclusion, purinergic signaling is vital for astrocytic cell-to-cell communication.[42]

P2X7 expression is elevated in the hippocampus and cortex in both epilepsy models and in patients with temporal lobe epilepsy.[20,43-45] It has been found to be expressed on microglia, mossy fibers, and nerve terminals in epilepsy rat models induced by pilocarpine, which is consistent with our results. We also demonstrate the cellular distribution of it in different time points of SE.[19,20] Activation of P2Y12 on microglia can elicit microglial process outgrowth, and stimulate migration to the inflammation sites via PI3K/PLC signaling. It guides the inflammatory factors to the injury site and mediates neuronal damage.[46,47]

Figure 8 summarizes the different functions of P2X and P2Y receptors in the progression of epilepsy. A shows that (1) when the brain has an acquired insult, ATP is released through different pathways: ATP and its breakdown products, such as ADP, activate P2 receptors and induce cell motility, proliferation, and activation of microglia, and astrocytes. (2) The activation of microglial P2X receptors leads to up-regulation of inflammatory transcription factors nuclear factor-κ-gene binding (NF-κB) and nuclear factor of activated T cells (NFAT); this is also applied for P2Y1. ATP and ADP act on the P2Y12 receptors of microglia, initiating proinflammatory cascades, and releasing ATP. (3) P2Y1 activation in the endoplasmic reticulum of astrocytes releases Ca^{2+}, inducing cytokine and ATP release. ATP further potentiates these inflammatory cascades via paracrine-signaling. (4) Inflammation and ATP increase brain excitability and epilepsy progression. Chronic inflammation and excitotoxicity during seizures induce further release of ATP and exacerbate the development of epilepsy. B shows seizure suppression effects when some of the P2 receptors are blocked at different stages of acquired epilepsy.[8] Apoptosis/necrosis and neuronal progenitor cells (NPCs) differentiation are associated with P2X7R activation.[48] P2Y1 receptors are involved in NPC proliferation and migration.[49] Therefore, the function of P2Y6 in epilepsy warrants further investigation.

Limitation

As stated in the Introduction, there are seven types of P2X receptors and eight types of P2Y receptors; however, we

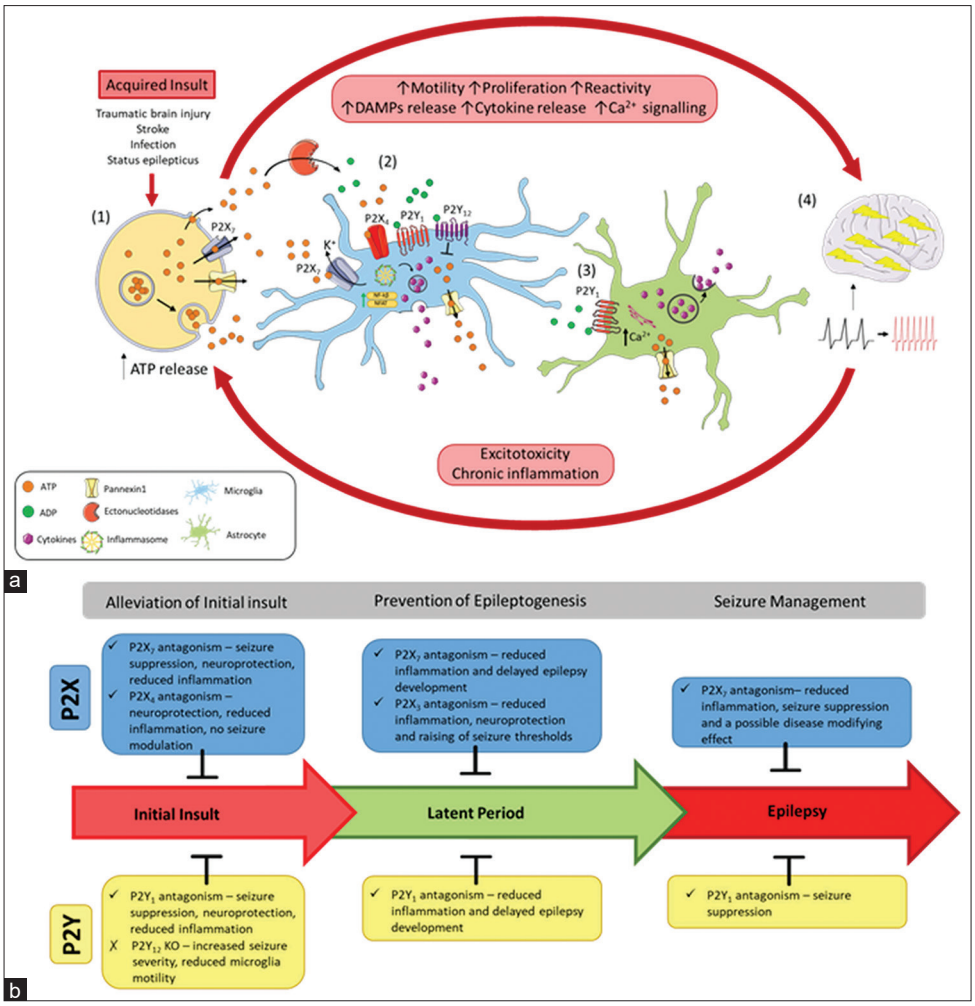

Figure 8: The different function of P2X and P2Y in the progression of epilepsy[8]. (a) After an insult, ATP is released along with its breakdown products. These products activate P2 receptors in microglia and astrocytes, which in turn initiates proinflammatory cascades and further ATP release. This process increases brain excitability and epilepsy progression. (b)Regarding what happens when P2 receptors are blocked at different stages of acquired epilepsy: At the initial insult stage, blocking P2X7 or P2Y1 receptors will cause seizure suppression. However, blocking P2Y12 receptors will lead to increased seizure severity. During the latent stage, blocking P2X7, P2X3, or P2Y1 receptors will delay the development of epilepsy. In the later stage, blocking P2X7 or P2Y1 receptors will again cause seizure suppression.

studied only three of them. The roles of other purinergic receptors in epileptogenesis remain unknown. We failed to obtain positive results for P2X4 expression.

Interpretation

Our study supports the notion that the expression of purinergic receptors changes during epilepsy progression. P2X7, P2Y6, and P2Y12 triggered the interaction of neurons, astrocytes, and microglia with neuroinflammation, and play a role in neuronal death and astrogliosis. Microglial dysregulation may reinforce inflammation and cause cell death. P2X7 receptor antagonists, such as Brilliant Blue G, can pass through the blood–brain barrier and ameliorate the neuropathology in several animal models of neurodegeneration. Klaft *et al*.[50] demonstrated that P2X7 receptor antagonists exert antiepileptic effects in the medial entorhinal cortex of pilocarpine-induced rat SE models. MicroRNA-22 (miR-22), which targets P2X7 and negatively regulates its expression, and its inhibitor (antagomir-22)-treated epileptic mice show more severe epileptic symptoms than scramble-treated epileptic mice.[51] In general, purinergic receptors may be targets for the treatment of epilepsy.[52] Clinical trials have demonstrated that brain-penetrant P2X7 receptor antagonists are safe.[53] Even the purine levels in the blood have been proven to be relevant to seizure severity. They are elevated in patients with epilepsy, which can be developed as a biomarker for epilepsy patients.[54] Anti-inflammatory drugs such as purinergic receptors antagonists have reached the clinical trial stage, which could pave the way for their future clinical use.[55]

Conclusion

Purinergic receptors are differently expressed during the specific phases of the epilepsy progression. For P2X7, it is predominantly expressed in neurons, and after seizures occur, it is expressed in neurons and microglial cells as

well as mossy fibers indicating cell over-excitation. P2Y6 is expressed in astrocytes at 3-7 days APISE, while P2Y12 is expressed in microglial cells at 3 days APISE.

The purinergic pathway plays a crucial role during the acute to latent stages of epileptogenesis. At these stages, nucleotides act as mediators to induce the interaction between microglial cells and neurons, and astrocytes with different pathways leading to the convergence of microglial cells at the site of damage and consequential engulfing of the dead neurons, thereby leading to astrogliosis. Purinergic agents can stimulate P2 receptors on neurons or trigger the release of proinflammatory cytokines from astrocytes or microglia, thereby contributing to neurodegenerative diseases. These observations agree with those of Abbracchio and Frankes'.[56,57] The selected P2X or P2Y antagonists could be used as prospective treatment strategies for epilepsy. Purinergic receptors in blood can be used as biomarkers for the early diagnosis of epilepsy.

Author contributions

YL1 performed the experiment, interpreted the results, and drafted the manuscript. FT designed and supervised the study, revised the manuscript, and obtained the fund. Moreover, YL2 revised the manuscript, did some administration work and gave financial support.

The manuscript has been read and approved by all the authors, the requirements for authorship, as stated earlier in this document, have been met, and each author believes that the manuscript represents honest work.

Ethical policy and institutional review board statement

The study was approved by the National Neuroscience Institutional Animal Care and Use Committee of Tan Tock Seng Hospital, Singapore (No.: B27/09&098/09, dated on 22-Sep-2010).

Data availability statement

Data sharing is not applicable to this article as no datasets were generated and/or analyzed during the current study.

Financial support and sponsorship

This study was supported by the A*STAR Biomedical Research Council. Grant 08/1/33/19/570 from FT when he was in the National Neuroscience Institute, Singapore and Foundation of Human Brain Banking in Beijing Geriatric Medical Research about Pathological Mechanism Study of Neurological Diseases (grant No. 11000024T000002829630) from YL2.

Conflicts of interest

Yumin Luo is an Editorial Board member of Brain Circulation. The article was subject to the journal's standard procedures, with peer review handled independently of this member and their research groups.

Acknowledgments

This work is done when Yue Li is supported by the Scholarship from the Chinese Scholarship Council. Part of the work was done in Dr. Fengru Tang's former Epilepsy Research Laboratory, National Neuroscience Institute of Singapore, before he left there. We would like to thank Dr. Dongliang Ma and Mr. Yongcheng Tang for their technical support.

Conflicts of interest

There are no conflicts of interest.

References

1. Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. Lancet 2019;393:689-701.
2. Curia G, Longo D, Biagini G, Jones RS, Avoli M. The pilocarpine model of temporal lobe epilepsy. J Neurosci Methods 2008;172:143-57.
3. Kim H, Choi Y, Joung HY, Choi YS, Kim HJ, Joo Y, *et al.* Structural and functional alterations at pre-epileptic stage are closely associated with epileptogenesis in pilocarpine-induced epilepsy model. Exp Neurobiol 2017;26:287-94.
4. Gil B, Smith J, Tang Y, Illes P, Engel T. Beyond seizure control: Treating comorbidities in epilepsy via targeting of the P2X7 receptor. Int J Mol Sci 2022;23:2380.
5. Verhoog QP, Holtman L, Aronica E, van Vliet EA. Astrocytes as guardians of neuronal excitability: Mechanisms underlying epileptogenesis. Front Neurol 2020;11:591690.
6. Robel S, Buckingham SC, Boni JL, Campbell SL, Danbolt NC, Riedemann T, *et al*. Reactive astrogliosis causes the development of spontaneous seizures. J Neurosci 2015;35:3330-45.
7. Paolicelli RC, Sierra A, Stevens B, Tremblay ME, Aguzzi A, Ajami B, *et al.* Microglia states and nomenclature: A field at its crossroads. Neuron 2022;110:3458-83.
8. Engel T, Smith J, Alves M. Targeting neuroinflammation via purinergic P2 receptors for disease modification in drug-refractory epilepsy. J Inflamm Res 2021;14:3367-92.
9. Engel T, Alves M, Sheedy C, Henshall DC. ATPergic signalling during seizures and epilepsy. Neuropharmacology 2016;104:140-53.
10. Boison D. Adenosinergic signaling in epilepsy. Neuropharmacology 2016;104:131-9.
11. Knutsen LJ, Murray TF. Adenosine and ATP in epilepsy. In: Adenosine and ATP in epilepsyNew York: Wiley-Liss; 1997.
12. Bijelić DD, Milićević KD, Lazarević MN, Miljković DM, Bogdanović Pristov JJ, Savić DZ, *et al*. Central nervous system-infiltrated immune cells induce calcium increase in astrocytes via astroglial purinergic signaling. J Neurosci Res 2020;98:2317-32.
13. Chen GY, Nuñez G. Sterile inflammation: Sensing and reacting to damage. Nat Rev Immunol 2010;10:826-37.
14. Burnstock G. Short- and long-term (trophic) purinergic signalling. Philos Trans R Soc Lond B Biol Sci 2016;371:20150422.
15. Guerra AN, Fisette PL, Pfeiffer ZA, Quinchia-Rios BH, Prabhu U, Aga M, *et al.* Purinergic receptor regulation of LPS-induced signaling and pathophysiology. J Endotoxin Res 2003;9:256-63.
16. Rothwell N. Interleukin-1 and neuronal injury: Mechanisms, modification, and therapeutic potential. Brain Behav Immun 2003;17:152-7.
17. Verhoef PA, Estacion M, Schilling W, Dubyak GR. P2X7 receptor-dependent blebbing and the activation of Rho-effector kinases, caspases, and IL-1 beta release. J Immunol 2003;170:5728-38.

18. Burnstock G. Introduction to purinergic signaling. Methods Mol Biol 2020;2041:1-15.
19. Vianna EP, Ferreira AT, Naffah-Mazzacoratti MG, Sanabria ER, Funke M, Cavalheiro EA, *et al.* Evidence that ATP participates in the pathophysiology of pilocarpine-induced temporal lobe epilepsy: Fluorimetric, immunohistochemical, and Western blot studies. Epilepsia 2002;43 Suppl 5:227-9.
20. Doná F, Ulrich H, Persike DS, Conceição IM, Blini JP, Cavalheiro EA, *et al*. Alteration of purinergic P2X4 and P2X7 receptor expression in rats with temporal-lobe epilepsy induced by pilocarpine. Epilepsy Res 2009;83:157-67.
21. Avignone E, Ulmann L, Levavasseur F, Rassendren F, Audinat E. Status epilepticus induces a particular microglial activation state characterized by enhanced purinergic signaling. J Neurosci 2008;28:9133-44.
22. Morimoto K, Fahnestock M, Racine RJ. Kindling and status epilepticus models of epilepsy: Rewiring the brain. Prog Neurobiol 2004;73:1-60.
23. Huang C, Chi XS, Li R, Hu X, Xu HX, Li JM, *et al.* Inhibition of P2X7 receptor ameliorates nuclear factor-kappa B mediated neuroinflammation induced by status epilepticus in rat hippocampus. J Mol Neurosci 2017;63:173-84.
24. De Simone R, Niturad CE, De Nuccio C, Ajmone-Cat MA, Visentin S, Minghetti L. TGF-β and LPS modulate ADP-induced migration of microglial cells through P2Y1 and P2Y12 receptor expression. J Neurochem 2010;115:450-9.
25. Koizumi S, Shigemoto-Mogami Y, Nasu-Tada K, Shinozaki Y, Ohsawa K, Tsuda M, *et al.* UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 2007;446:1091-5.
26. Ohsawa K, Irino Y, Sanagi T, Nakamura Y, Suzuki E, Inoue K, *et al.* P2Y12 receptor-mediated integrin-beta1 activation regulates microglial process extension induced by ATP. Glia 2010;58:790-801.
27. Beamer E, Fischer W, Engel T. The ATP-gated P2X7 receptor as a target for the treatment of drug-resistant epilepsy. Front Neurosci 2017;11:21.
28. Guzman SJ, Gerevich Z. P2Y receptors in synaptic transmission and plasticity: Therapeutic potential in cognitive dysfunction. Neural Plast 2016;2016:1207393.
29. Illes P, Verkhratsky A, Tang Y. Pathological ATPergic signaling in major depression and bipolar disorder. Front Mol Neurosci 2019;12:331.
30. Kaczmarek-Hájek K, Lörinczi E, Hausmann R, Nicke A. Molecular and functional properties of P2X receptors – Recent progress and persisting challenges. Purinergic Signal 2012;8:375-417.
31. Burnstock G. P2X ion channel receptors and inflammation. Purinergic Signal 2016;12:59-67.
32. He Y, Taylor N, Fourgeaud L, Bhattacharya A. The role of microglial P2X7: Modulation of cell death and cytokine release. J Neuroinflammation 2017;14:135.
33. Lu W, Albalawi F, Beckel JM, Lim JC, Laties AM, Mitchell CH. The P2X7 receptor links mechanical strain to cytokine IL-6 up-regulation and release in neurons and astrocytes. J Neurochem 2017;141:436-48.
34. Mehta VB, Hart J, Wewers MD. ATP-stimulated release of interleukin (IL)-1beta and IL-18 requires priming by lipopolysaccharide and is independent of caspase-1 cleavage. J Biol Chem 2001;276:3820-6.
35. Dudek FE, Staley KJ. The time course of acquired epilepsy: Implications for therapeutic intervention to suppress epileptogenesis. Neurosci Lett 2011;497:240-6.
36. Clossen BL, Reddy DS. Novel therapeutic approaches for disease-modification of epileptogenesis for curing epilepsy. Biochim Biophys Acta Mol Basis Dis 2017;1863:1519-38.
37. Bhattacharya A, Jones DN. Emerging role of the P2X7-NLRP3-IL1β pathway in mood disorders. Psychoneuroendocrinology 2018;98:95-100.
38. Campagno KE, Mitchell CH. The P2X (7) receptor in microglial cells modulates the endolysosomal axis, autophagy, and phagocytosis. Front Cell Neurosci 2021;15:645244.
39. Nobili P, Shen W, Milicevic K, Bogdanovic Pristov J, Audinat E, Nikolic L. Therapeutic potential of astrocyte purinergic signalling in epilepsy and multiple sclerosis. Front Pharmacol 2022;13:900337.
40. Yang QQ, Zhou JW. Neuroinflammation in the central nervous system: Symphony of glial cells. Glia 2019;67:1017-35.
41. Sanz P, Garcia-Gimeno MA. Reactive glia inflammatory signaling pathways and epilepsy. Int J Mol Sci 2020;21:4096.
42. Franke H, Verkhratsky A, Burnstock G, Illes P. Pathophysiology of astroglial purinergic signalling. Purinergic Signal 2012;8:629-57.
43. Barros-Barbosa AR, Fonseca AL, Guerra-Gomes S, Ferreirinha F, Santos A, Rangel R, *et al.* Up-regulation of P2X7 receptor-mediated inhibition of GABA uptake by nerve terminals of the human epileptic neocortex. Epilepsia 2016;57:99-110.
44. Jimenez-Pacheco A, Mesuret G, Sanz-Rodriguez A, Tanaka K, Mooney C, Conroy R, *et al*. Increased neocortical expression of the P2X7 receptor after status epilepticus and anticonvulsant effect of P2X7 receptor antagonist A-438079. Epilepsia 2013;54:1551-61.
45. Jimenez-Pacheco A, Diaz-Hernandez M, Arribas-Blázquez M, Sanz-Rodriguez A, Olivos-Oré LA, Artalejo AR, *et al.* Transient P2X7 receptor antagonism produces lasting reductions in spontaneous seizures and gliosis in experimental temporal lobe epilepsy. J Neurosci 2016;36:5920-32.
46. Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, *et al.* The P2Y12 receptor regulates microglial activation by extracellular nucleotides. Nat Neurosci 2006;9:1512-9.
47. Ohsawa K, Irino Y, Nakamura Y, Akazawa C, Inoue K, Kohsaka S. Involvement of P2×4 and P2Y12 receptors in ATP-induced microglial chemotaxis. Glia 2007;55:604-16.
48. Tsao HK, Chiu PH, Sun SH. PKC-dependent ERK phosphorylation is essential for P2X7 receptor-mediated neuronal differentiation of neural progenitor cells. Cell Death Dis 2013;4:e751.
49. Cao X, Li LP, Qin XH, Li SJ, Zhang M, Wang Q, *et al.* Astrocytic adenosine 5′-triphosphate release regulates the proliferation of neural stem cells in the adult hippocampus. Stem Cells 2013;31:1633-43.
50. Klaft ZJ, Schulz SB, Maslarova A, Gabriel S, Heinemann U, Gerevich Z. Extracellular ATP differentially affects epileptiform activity via purinergic P2X7 and adenosine A1 receptors in naive and chronic epileptic rats. Epilepsia 2012;53:1978-86.
51. Jimenez-Mateos EM, Arribas-Blazquez M, Sanz-Rodriguez A, Concannon C, Olivos-Ore LA, Reschke CR, *et al.* microRNA targeting of the P2X7 purinoceptor opposes a contralateral epileptogenic focus in the hippocampus. Sci Rep 2015;5:17486.
52. Takenouchi T, Sekiyama K, Sekigawa A, Fujita M, Waragai M, Sugama S, *et al.* P2X7 receptor signaling pathway as a therapeutic target for neurodegenerative diseases. Arch Immunol Ther Exp (Warsz) 2010;58:91-6.
53. Timmers M, Ravenstijn P, Xi L, Triana-Baltzer G, Furey M, Van Hemelryck S, *et al.* Clinical pharmacokinetics, pharmacodynamics, safety, and tolerability of JNJ-54175446, a brain permeable P2X7 antagonist, in a randomised single-ascending dose study in healthy participants. J Psychopharmacol 2018;32:1341-50.
54. Beamer E, Lacey A, Alves M, Conte G, Tian F, de Diego-Garcia L, *et al.* Elevated blood purine levels as a biomarker of seizures and epilepsy. Epilepsia 2021;62:817-28.
55. French JA, Koepp M, Naegelin Y, Vigevano F, Auvin S, Rho JM, *et al.* Clinical studies and anti-inflammatory mechanisms of treatments. Epilepsia 2017;58 Suppl 3:69-82.
56. Abbracchio MP, Ceruti S. Roles of P2 receptors in glial cells: Focus on astrocytes. Purinergic Signal 2006;2:595-604.
57. Franke H, Krügel U, Illes P. P2 receptors and neuronal injury. Pflugers Arch 2006;452:622-44.