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$P2Y_2$ Nucleotide Receptor-Mediated Responses in Brain Cells

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Abstract Acute inflammation is important for tissue repair; however, chronic inflammation contributes to neurodegeneration in Alzheimer's disease (AD) and occurs when glial cells undergo prolonged activation. In the brain, stress or damage causes the release of nucleotides and activation of the G_q protein-coupled $P2Y_2$ nucleotide receptor subtype ($P2Y_2R$) leading to pro-inflammatory responses that can protect neurons from injury, including the stimulation and recruitment of glial cells. $P2Y_2R$ activation induces the phosphorylation of the epidermal growth factor receptor (EGFR), a response dependent upon the presence of a SH3 binding domain in the intracellular C terminus of the $P2Y_2R$ that promotes Src binding and transactivation of EGFR, a pathway that regulates the proliferation of cortical astrocytes. Other studies indicate that $P2Y_2R$ activation increases astrocyte migration. $P2Y_2R$ activation by UTP increases the expression in astrocytes of $\alpha_V\beta_{3/5}$ integrins that bind directly to the $P2Y_2R$ via an Arg-Gly-Asp (RGD) motif in the first extracellular loop of the $P2Y_2R$, an interaction required for G_o and G_{12} protein-dependent astrocyte migration. In rat primary cortical neurons (rPCNs) $P2Y_2R$ expression is increased by stimulation with interleukin-1β (IL-1β), a pro-inflammatory cytokine whose levels are elevated in AD, in part due to nucleotide-stimulated release from glial cells. Other results indicate that oligomeric β-amyloid peptide ($A\beta_{1\text{-}42}$), a contributor to AD, increases nucleotide release from astrocytes, which would serve to activate upregulated $P2Y_2Rs$ in neurons. Data with rPCNs suggest that $P2Y_2R$ upregulation by IL-1β and subsequent activation by UTP are neuroprotective, since this increases the non-amyloidogenic cleavage of amyloid precursor protein. Furthermore, activation of IL-1β-upregulated $P2Y_2Rs$ in rPCNs increases the phosphorylation of cofilin, a cytoskeletal protein that stabilizes neurite outgrowths. Thus, activation of pro-inflammatory $P2Y_2Rs$ in glial cells can promote neuroprotective responses, suggesting that $P2Y_2Rs$ represent a novel pharmacological target in neurodegenerative and other pro-inflammatory diseases.

Keywords Neurons · Neurodegeneration · Astrocytes · Growth factor receptors · Inflammation · $P2Y_2$ receptors · Cofilin · Nucleotides · Proliferation · RGD motif · SH3 binding domain · Integrins

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Introduction

Chronic neuroinflammation, associated with the pathogenesis and progression of Alzheimer's disease (AD), occurs when glial cells (*i.e.*, astrocytes and microglia) undergo prolonged activation in response to oxidative stress. Oxidative stress is postulated to be an early event in the development of AD that is due to increased production of reactive oxygen species

from mitochondria and NADPH oxidase which can modify lipids, nucleic acids, and proteins [1–9]. Production of neurotoxic β-amyloid (Aβ) peptides, such as $A\beta_{1\text{-}42}$, also is a widely accepted contributor to neurodegeneration in AD [10, 11], and oxidative stress can enhance Aβ production [12] leading to mitochondrial dysfunction and neuronal apoptosis [13]. Chronic inflammation occurs around β-amyloid plaques [14, 15], and has been associated with the activation by cytokines of receptors in glial cells that promote neuronal cell death [16–18]. Studies have shown that inflammation begins as a neuroprotective mechanism, but becomes neurodegenerative when sustained [19–22]. Chronic neuroinflammation occurs in brain pathologies including AD, trauma, and stroke and is characterized by increased glial cell migration and proliferation, and morphological changes, including extensive cellular hypertrophy, fiber extension and increased expression of glial fibrillary acidic protein (GFAP) [23, 24]. In the initial stages, neuroinflammation limits brain damage by promoting the clearance of neurotoxic soluble β-amyloid peptide [25, 26]. Activated glial cells migrate to the edge of an injured area and secrete cytokines, chemokines, and growth factors, and also upregulate antigens and cell adhesion molecules [27, 28]. Glial cell activation in the central nervous system under physiological conditions facilitates axonal growth during development [29]. In adult brain, glial cell activation is critical for structural plasticity and repair of damaged brain cells [24]. In the chronic stages, neuroinflammation may exacerbate neurotoxic effects induced by the formation of glial-derived amyloid plaques [24–30] that contribute to neurodegeneration and loss of brain function in AD. Anti-inflammatory drugs have been shown to alter Aβ deposition in an animal model of AD [31]. Among the agents that can contribute to glial cell activation in AD, nucleotides released from the cytoplasm of oxidatively stressed cells have garnered little attention despite the fact that multiple nucleotide receptor subtypes are expressed in glial cells and neurons. Studies have shown that ATP release due to stretch-induced injury increases GFAP expression and proliferation in astrocytes [32], and nucleotides cause responses indicative of astrogliosis in vivo [33] and in primary rat cortical astrocyte cultures [34]. Release of nucleotides has been proposed to occur by exocytosis of ATP/UTP-containing vesicles, facilitated diffusion by putative ABC transporters, cytoplasmic leakage, and electrodiffusional movements through ATP/nucleotide channels [35].

Our studies have shown that the G_q protein-coupled $P2Y_2$ receptor subtype is an important mediator of neuroinflammatory responses mediated by astrocytes. Nucleotides are present at millimolar concentrations in the cytoplasm and when released activate a variety of P2 nucleotide receptors in the brain that have nanomolar to micromolar affinities for nucleotides [36]. Therefore, a small amount of nucleotide released from damaged or oxidatively-stressed cells can activate P2 receptors [37]. It has been demonstrated that ATP released from the leading edge of the cell surface amplifies chemotactic signals and directs neutrophil orientation by feedback through $P2Y_2$ nucleotide receptors ($P2Y_2Rs$) [38]. Our previous results indicate that the pro-inflammatory cytokine IL-1β upregulates $P2Y_2R$ expression in neurons [39], which can be activated by released nucleotides (unpublished data). Thus, the release of nucleotides in the brain is hypothesized to stimulate the generation of extracellular pro-inflammatory cytokines by astrocytes and microglial cells that promote the upregulation of neuronal $P2Y_2Rs$. This review will discuss our findings relating to the mechanisms underlying the pro-inflammatory and neuroprotective effects mediated by $P2Y_2Rs$ in astrocytes and neurons and their potential relationship to the pathophysiology of AD.

The P2 Receptor Family

In the early 1970 s, it was reported that ATP was released into the extracellular space by stimulation of nonadrenergic, noncholinergic nerves to activate responses postulated to be mediated by P2 purinergic receptors for nucleotides [40, 41]. Over the next few decades, it was recognized that activation of P2 receptors can modulate a variety of responses in cells of the mammalian central nervous system (CNS), including neurotransmission, cell growth, and apoptosis [42–44]. It is now accepted that nucleotides are released from excitatory neurons, injured cells, cells undergoing mechanical or oxidative stress, aggregating platelets, degranulating macrophages, and astrocytes by exocytosis from ATP/UTP-containing vesicles, facilitated diffusion, or cytoplasmic leakage [35–38, 45–50]. Extracellular nucleotides activate cell surface P2 receptors belonging to two structurally distinct families: the G protein-coupled P2Y receptors (P2YRs) and P2X receptors (P2XRs) that are ligand-gated ion channels. Eight P2Y receptor subtypes have been cloned and characterized to date, including the G_q-coupled $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors, and the G_i-coupled $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ receptors [51]. Seven P2X receptors have been cloned and characterized as ligand-gated ion channels, including $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_6$, and $P2X_7$ receptors [52]. Activation of P2 receptors in neurons and glia under normal and pathological conditions regulates pro-inflammatory responses, ion transport, neurotransmission and cell apoptosis, proliferation, and migration [42–44, 52–54]. Therefore, P2 receptors in the CNS represent potential targets for pharmaceutical approaches to treat neurological disorders. Among these P2 receptor subtypes, our research has focused on the $P2Y_2R$ and its signaling

pathways in the regulation of pro-inflammatory responses in astrocytes associated with reactive astrogliosis, and neuroprotective responses associated with neurite growth and stability and the non-amyloidogenic processing of amyloid precursor protein (APP).

The $P2Y_2$ Nucleotide Receptor

Activation of the G_q-coupled $P2Y_2R$ stimulates phospholipase C (PLC) and leads to the production of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [54, 55], second messengers for calcium release from intracellular storage sites and protein kinase C (PKC) activation, respectively. Interestingly, we have found that the $P2Y_2R$ by virtue of a Arg-Gly-Asp (RGD) motif in its first extracellular loop (Fig. 1) can bind to $\alpha_V\beta_{3/5}$ integrins and enable UTP to stimulate G_o and G_{12} proteins leading to the activation the small GTPases Rac and Rho, respectively (Fig. 2) [56, 57]. Mutation of the RGD sequence to Arg-Gly-Glu (RGE), prevents both integrin binding and UTP-induced activation of G_o, G_{12}, Rac and Rho by the mutant $P2Y_2R$ expressed in human 1321N1 astrocytoma cells that lack endogenous P2Y receptors. In 1321N1 astrocytoma cells, activation of the wild-type $P2Y_2R$, but not the RGE-mutant $P2Y_2R$, leads to cytoskeletal rearrangements and increases in cell migration, suggesting that association with $\alpha_V\beta_{3/5}$ is required for these $P2Y_2R$-mediated responses. The $P2Y_2R$ also contains 2 PXXP motifs in the intracellular C-terminal domain that represent consensus Src-homology-3 (SH3) binding sequences (Fig. 1). Activation of the wild type $P2Y_2R$ expressed in 1321N1 astrocytoma cells induces the phosphorylation of Src and EGFR, responses that are attenuated for a mutant $P2Y_2R$ in which the SH3

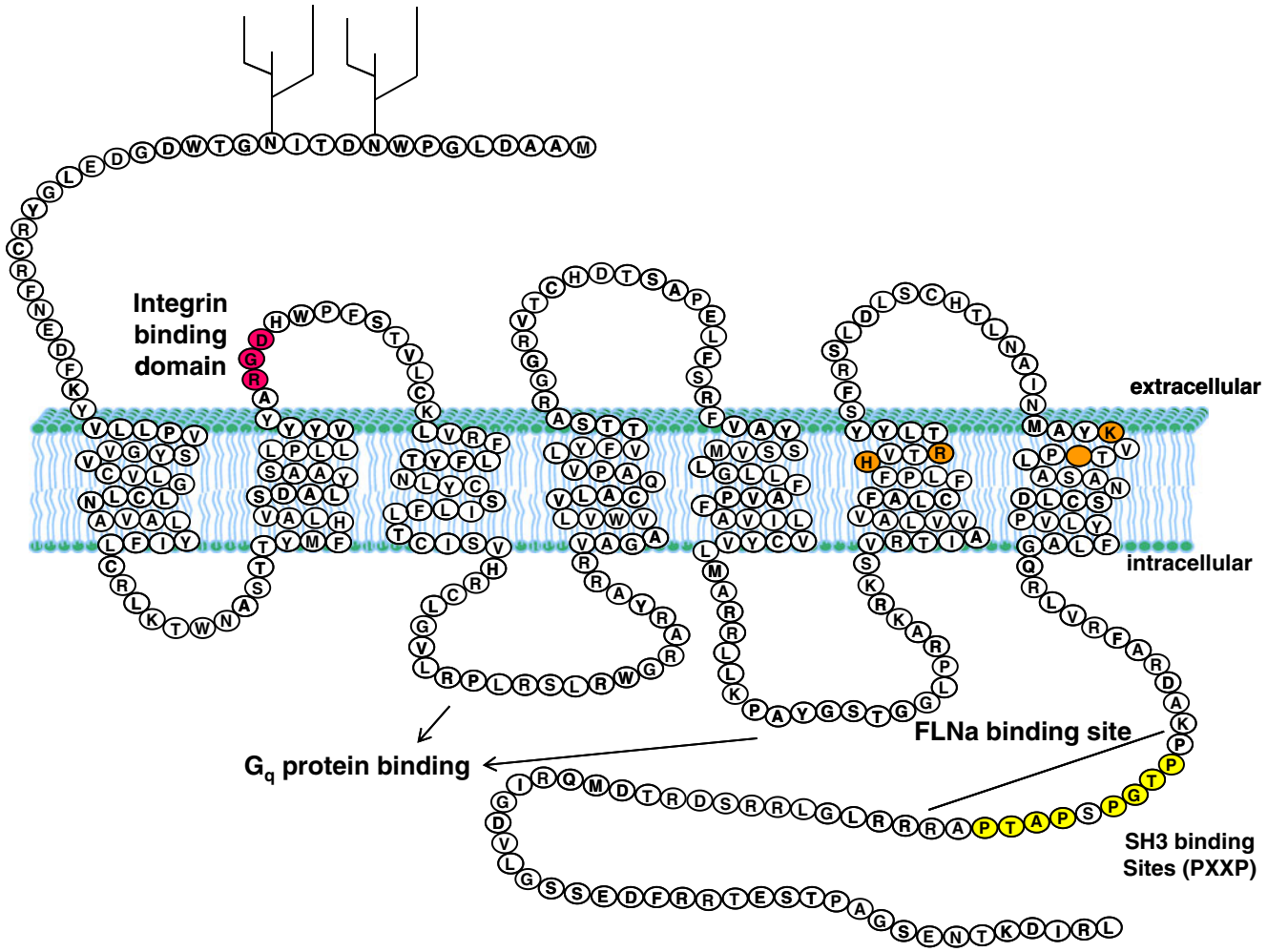

Fig. 1 $P2Y_2R$ structure and domains: the $P2Y_2R$ is a seven pass transmembrane G protein-coupled extracellular nucleotide receptor. It is activated equipotently by ATP and UTP and has been shown to be upregulated in response to stress or injury in various cell types. Highlighted features include the consensus RGD integrin-binding domain (in *pink*), positively-charged amino acid residues known to be involved in ATP/UTP binding (in *orange*), two consensus PXXP SH3 domain binding sites (in *yellow*), the FLNa binding site, the intracellular loops that regulate Gq protein binding, and two glycosylation sites

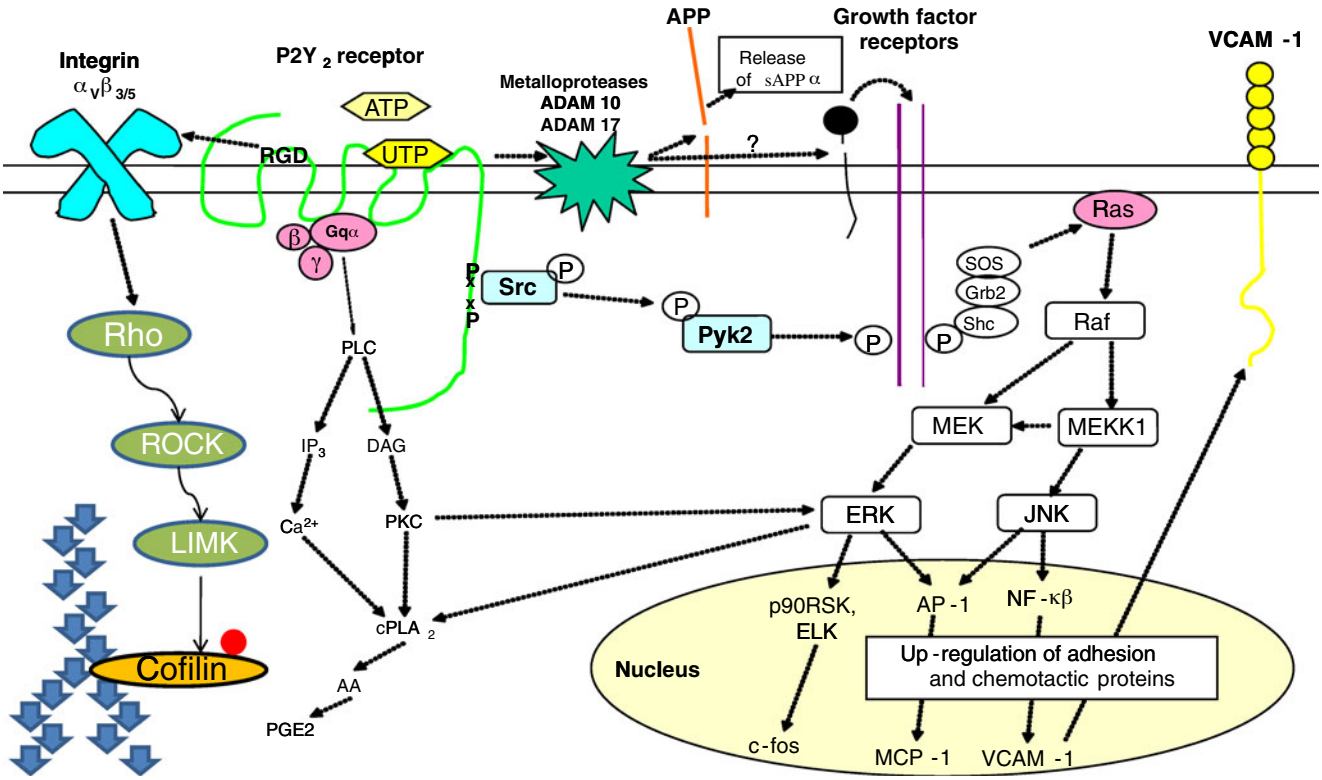

Fig. 2 $P2Y_2$ receptor-mediated signal transduction: activation of the $P2Y_2$ receptor (*$P2Y_2R$*) is coupled to several intracellular signal transduction pathways including: **a** Gqα-dependent activation of phospholipase C (*PLC*) that generates inositol 1,4,5 trisphosphate (*IP3*) and diacylglycerol (*DAG*), second messengers for intracellular calcium mobilization and protein kinase C activation, respectively; **b** Src-mediated transactivation of growth factor receptor phosphorylation that stimulates mitogen-activated protein kinase cascades to regulate gene transcription; **c** association with and activation of αvβ3/5 integrins that stimulates Rho kinase leading to cofilin phosphorylation; and **d** activation of metalloproteases (*i.e.*, ADAM10/17) to stimulate the non-amyloidogenic processing of amyloid precursor protein (*APP*). Other abbreviations: *AA* arachidonic acid, *PGE2* prostaglandin E2, *VCAM-1* vascular cell adhesion molecule-1

binding domains for Src in the intracellular C-terminus of the $P2Y_2R$ have been deleted [58]. Since the activated $P2Y_2R$ co-localizes with EGFR in the plasma membrane [58], these findings suggest that the previously reported ability of the $P2Y_2R$ to regulate EGFR phosphorylation [59, 60] is due to Src-dependent recruitment of the $P2Y_2R$ to a signaling complex containing EGFR, thereby inducing EGFR phosphorylation in response to $P2Y_2R$ ligands. These studies used kinase inhibitors to demonstrate that $P2Y_2R$-mediated activation of the mitogen-activated protein kinases ERK1/2, is dependent on the kinase activities of Src [58] and EGFR [59]. Whereas the activities of ERK1/2 are important for $P2Y_2R$-mediated cell/astrocyte proliferation [61], the activity of another MAP kinase, p38, is important for $P2Y_2R$-mediated upregulation of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), which is involved in tight binding of monocytes to endothelial cells [62] and lymphocytes to epithelial cells [63]. In astrocytic cells, the p38 signaling pathway is also required for the $P2Y_2R$ to inhibit trauma-induced cell death [64]. Other studies indicate that EGFR signaling regulates neuronal survival by promoting cortical but not midbrain astrocyte apoptosis [65], which suggests an endpoint for $P2Y_2R$ activation in the CNS. Additionally, it has been shown that the $P2Y_2R$ interacts directly with filamin A (FLNa) [66], a crosslinking cytoskeletal maintenance protein [66].

The ability of the $P2Y_2R$ to regulate signal transduction via activation of integrins and growth factor receptors, in addition to PLC, suggests that $P2Y_2R$ activation could have significant physiological and pathophysiological consequences in a variety of cell types that express the $P2Y_2R$. $P2Y_2Rs$ are expressed in epithelial cells, smooth muscle cells, endothelial cells, monocytes, macrophages, neutrophils, and cardiomyocytes and in brain, heart, kidney, liver, spleen, placenta, and skeletal muscle tissue [35, 36, 55, 67–70]. In cells derived from the peripheral and central nervous systems, $P2Y_2Rs$ also are expressed in immortalized astrocytes, NG108-15 neuroblastoma × glioma hybrid cells, Schwann cells, dorsal horn and cortical astrocytes, astrocytoma cells, rat cortical neurons, microglia and oligodendrocytes [37, 54, 71–74]. The $P2Y_2R$ subtype is upregulated in activated thymocytes, in response to pro-inflammatory cytokines including IL-1β, interferon-γ, and

tumor necrosis factor-α, and in animal models of injury or disease of the salivary gland epithelium or the vasculature [63, 67, 75–77] and nucleotides have been reported to activate pro-monocytic cells [78]. For example, placement of a silicone collar around a rabbit carotid artery upregulates $P2Y_2R$ expression in smooth muscle and endothelium and upon activation of the $P2Y_2R$ in vivo promotes intimal thickening and monocyte infiltration due to increased smooth muscle cell proliferation and VEGF receptor-2-dependent upregulation of VCAM-1, respectively [62, 67]. $P2Y_2R$-mediated VCAM-1 expression also promotes lymphocyte adherence to salivary epithelial cell monolayers, a potential consequence of $P2Y_2R$ upregulation detected in a mouse model of Sjögren's syndrome, an autoimmune exocrinopathy that leads to salivary gland dysfunction [63, 77]. The $P2Y_2R$ agonists ATP and UTP have been shown to stimulate the adherence of monocytes and neutrophils to endothelial cell monolayers [62, 79]. $P2Y_2R$ activation also regulates the synthesis of superoxide, prostaglandins, nitric oxide, and cytokines in response to the elicitors IFN-γ and LPS [34, 37, 38, 55, 80, 81].

Very few studies have investigated the consequences of $P2Y_2R$ expression in the brain. We utilized in situ hybridization and reverse transcriptase–polymerase chain reaction to identify $P2Y_2R$ messenger RNA (mRNA) expression in normal rodent (*i.e.*, rat, mouse, and gerbil) brain slices, where expression levels were highest in the hippocampus (*i.e.*, dentate gyrus) and cerebellum [34]. $P2Y_2R$ mRNA expression was also detected in rat primary astrocytes and microglial cells, although rat primary neurons express very low levels of $P2Y_2R$ mRNA [37, 39]. Under non-inflammatory conditions, $P2Y_2R$ expression in neurons and oligodendrocytes is low, therefore, these cells are unresponsive to UTP [82], unless the presence of the pro-inflammatory cytokine IL-1β increases functional expression of the $P2Y_2R$ in neurons [39].

$P2Y_2$ Receptors Regulate Neuroinflammatory Responses

It is well accepted that nucleotides can be released into the extracellular milieu from aggregating platelets, degranulating macrophages, excitatory neurons, and injured cells [35, 49, 50]. Under pathophysiological conditions in the brain and other tissues, extracellular nucleotides can be released in response to oxidative stress, ischemia, hypoxia or mechanical stretch [45–50], consistent with the ability of released ATP and UTP to induce migration [67, 68, 83, 84] and chemotaxis of microglial cells [85] and primary rat cortical astrocytes [86]. We have also determined that the amyloidogenic peptide, oligomeric $A\beta_{42}$, whose levels are elevated in Alzheimer's brain, induces the release of ATP from mouse primary cortical astrocytes (Fig. 3). Primary rat cortical astrocytes were isolated from postnatal 2- to 3-day old rat pups. Briefly, cerebral cortices were cut into very small pieces and incubated with trypsin-EDTA at 37°C for 7 min. The suspension was filtered through 85 μm nylon mesh and centrifuged at ~250 g for 5 min. The cell pellet was resuspended in DMEM with 10% FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin and 7.5 μg/ml fungizone, and transferred to T75 culture flasks. Cells were maintained in an incubator with 5% CO_2 at 37°C and the medium was changed every two days. When cells reach ~80-90% confluence, flasks were shaken at 225 rpm for 6 h at room temperature to remove microglial cells. Then, 10^6 cells were seeded into 12-well plates and cultured for 2 days when ATP release assays were performed. Our results showed that oligomeric $A\beta_{42}$ induces the release of endogenous ATP from rat primary cortical astrocytes (Fig. 3). The basal release of ATP, determined after incubation of cells in HEPES buffer supplemented with 200 μM AOPCP, an inhibitor of 5'-nucleotidase, was 7.9, 7.8, 4.2 and 5.9 nmoles/well for 1, 2, 4 and 10 min, respectively. After stimulation of the cells with oligomeric Aβ, the endogenous ATP release was14.2, 28.7, 21 and 29.2 nmoles/well, for 1, 2, 4 and 10 min, respectively, and results compared with controls were significantly different at 4 and 10 min ($p<0.01$). Thus, pro-inflammatory conditions in AD that include oxidative stress and the increased production of $A\beta_{42}$ [4–14], are likely to induce the release of $P2Y_2R$ agonists. Once released, these

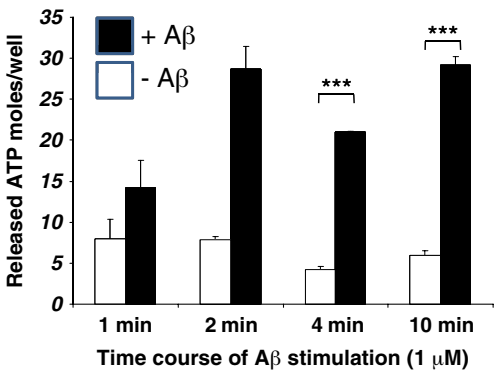

Fig. 3 Effect of oligomeric Aβ42 on ATP release from primary cultured rat cortical astrocytes: the cells were incubated for 15 min at 37°C with HEPES buffer supplemented with 200 μM AOPCP, an inhibitor of 5′-nucleotidases, to retard ATP breakdown. Cells were washed 3 times using the same buffer and then incubated for different time periods at 37°C with or without oligomeric Aβ. Supernatants were collected and released ATP was measured with the ATP Bioluminescence Assay kit HSII (Roche). The ATP levels were calculated based on an ATP standard curve. The results are expressed as nmoles of ATP released per well of 12-well plate and are presented as means±S.E.M.; $n=3$. *White bars* are basal levels at each time point (without oligomeric Aβ) and *black bars* are stimulated ATP release (with oligomeric Aβ). $^{**}p<0.01$

agonists will activate $P2Y_2Rs$ expressed in astrocytes and microglial cells to induce integrin-dependent activation of Rho and Rac to promote glial cell migration, and transactivation of growth factor receptors to increase glial cell proliferation, responses associated with neuroinflammation, [34, 37, 43, 56–58] (Fig. 4), although nucleotides have been suggested to exert anti-inflammatory effects in LPS-treated microglial cells [74].

P2 receptor activation in vascular smooth muscle and glial cells also has been shown to increase the release of pro-inflammatory cytokines, including IL-1β and IFNγ [76, 87, 88]. Since cytokine release is dependent on metalloprotease activation, we postulate that IL-1β release from astrocytes is dependent upon $P2Y_2R$-mediated metalloprotease activation (see Fig. 2). Consistent with this hypothesis, $P2Y_2R$ activation has been shown to activate the metalloproteases ADAM10 and ADAM17 in astrocytoma cells, primary neurons and salivary epithelial cells [39, 89].

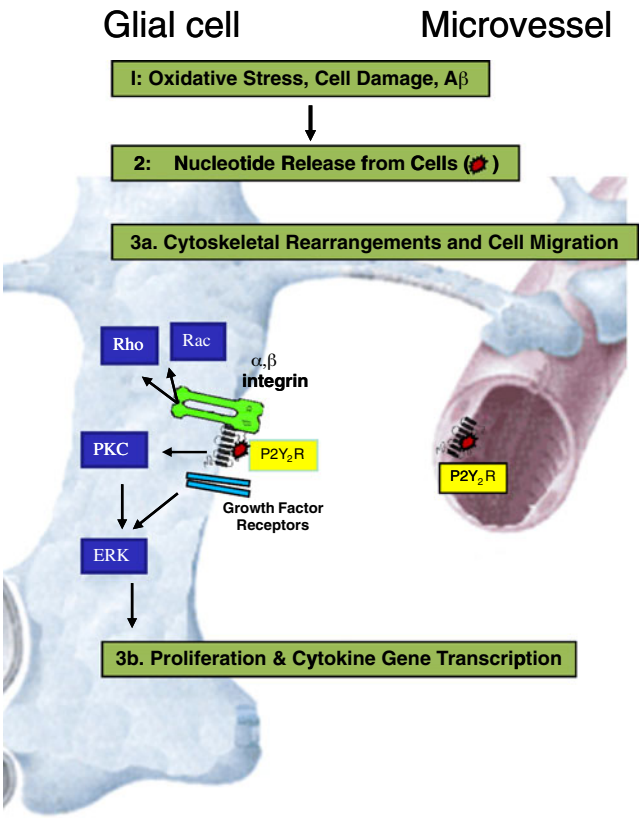

Fig. 4 $P2Y_2Rs$ in astrocytes and microvessels: chronic inflammation caused by oxidative stress in brain is mediated by $P2Y_2Rs$ via cytokine-like actions of nucleotides in astrocytes and microvessels through transactivation of integrins and growth factor receptors. Nucleotides are released from oxidatively-stressed brain cells activating $P2Y_2Rs$ in astrocytes and microvessels. Activation of endogenously expressed $P2Y_2Rs$ in glial cells leads to integrin-mediated cell migration (via the $P2Y_2R$ RGD domain), which has been shown to be necessary for cell migration. Nucleotideinduced and integrin-dependent activation of Rho and Rac promotes glial cell migration, and $P2Y_2R$-induced transactivation of growth factor receptors increases cell proliferation and pro-inflammatory gene expression

$P2Y_2Rs$ Mediate Neuroprotective APP Processing

The inflammatory cytokine IL-1β whose levels are elevated in AD [90] has been shown to upregulate functional expression of the $P2Y_2R$ in rat primary cortical neurons [39]. IL-1β release from astrocytes and microglia has been shown to be induced by exogenous ATP acting through the $P2X_7$ receptor, however, the contribution of other P2 purinergic receptors was not excluded [91]. In primary rat and mouse neuronal cultures, the $P2Y_2R$ is expressed at very low levels (39, unpublished data). However, Il-1β induces an increase in $P2Y_2R$ expression by activating the NF-κB signaling pathway, since Bay-11-7085, an irreversible inhibitor of IκB-α phosphorylation and thus NF-κB activation, decreases IL-1β-induced $P2Y_2R$ expression levels in rat primary cortical neurons [39]. These results are consistent with the finding that the *$P2Y_2R$* promoter contains an NF-κB binding site that regulates *$P2Y_2R$* transcription in intestinal epithelial cells [92]. Since the pro-inflammatory cytokine IL-1β upregulates $P2Y_2R$ expression in neurons, it was somewhat surprising to find that the $P2Y_2R$ serves a potential neuroprotective role by stimulating the non-amyloidogenic processing of APP [89] and the activation of cofilin [56], a cytoskeletal actin-binding protein that is known to promote dendritic spine growth and stabilization [26, 93–95] (Fig. 5).

Our findings indicate that $P2Y_2R$ activation stimulates the α- and γ-secretase-dependent proteolytic processing of APP to generate the non-amyloidogenic peptide soluble amyloid precursor-α (sAPPα) in both astrocytoma cells expressing the wild type $P2Y_2R$ [89] and in primary rat cortical neurons treated overnight with IL-1β [39]. Production of sAPPα from APP would be anticipated to decrease the production of amyloidogenic Aβ peptide, the main component of senile plaques in the AD brain [96, 97]. APP is either proteolytically processed by β- and γ-secretases to release Aβ, or by α- and γ-secretases to produce sAPPα. APP is a transmembrane glycoprotein that is present in a variety of tissues, but predominantly in the brain [98]. APP contains an extracellular N terminus and a short C-terminal region that lies in the cytoplasm. Within APP, a single membrane-spanning region of 39-42 amino acids represents Aβ [99, 100]. Proteolytic cleavage of APP *in vivo* can occur at the amino terminus of the Aβ domain (by β-secretase), within the Aβ domain (by α-secretase), and at the C-terminus of the Aβ domain (by γ-secretase) [101]. Thus, the ability of the $P2Y_2R$ to activate α-secretase and generate sAPPα, the soluble, non-amyloidogenic N-terminal fragment (~100–140 kD) of APP,

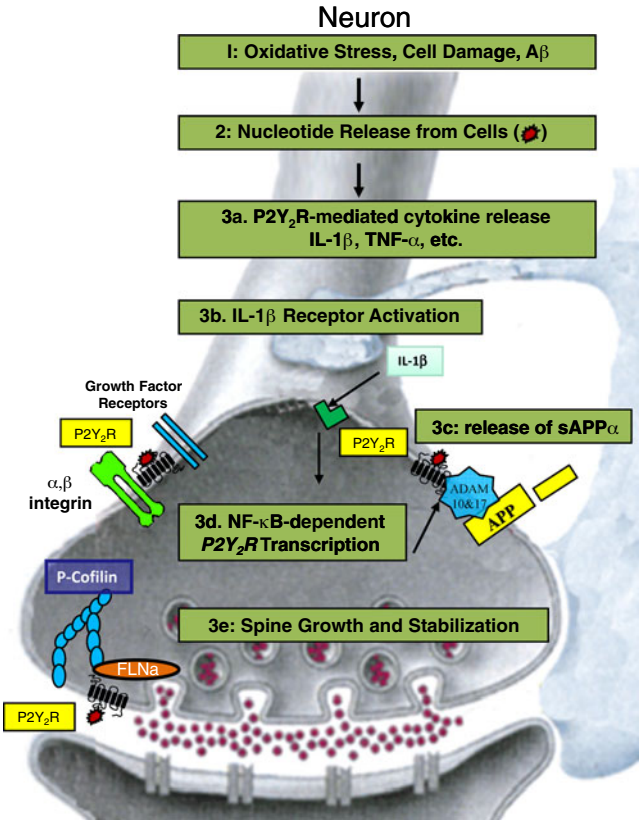

Fig. 5 $P2Y_2Rs$ in neurons: nucleotides released from oxidatively stressed brain cells activate $P2Y_2Rs$ on neurons. $P2Y_2R$ activation induces release of cytokines, which upregulate the expression of the $P2Y_2R$. Additionally, extracellular nucleotides activate matrix metalloproteases to increase production of the non-amyloidogenic APP fragment, sAPP-α. Activation of the $P2Y_2R$ also promotes binding of FLNa to the C-terminal domain of the receptor and phosphorylation of cofilin

precludes the potential release of amyloidogenic $A\beta_{1\text{-}42}$ from the same APP molecule. Although not determined in our studies, it has been reported that the membrane-retained fragment resulting from sAPPα release undergoes further cleavage and endocytotic processing [102–104]. The released sAPPα fragment has been shown to have both neurotrophic [105] and neuroprotective [106–109] activities, suggesting that the pro-inflammatory upregulation of $P2Y_2Rs$ in neurons may be beneficial.

PKC-dependent and -independent pathways stimulated by several G protein-coupled receptors (GPCRs) have been reported to induce sAPPα release [110–112]. Overexpression of the human M1 and M3 muscarinic receptors in HEK293 cells stimulates sAPPα secretion [113]. Subsequently, thrombin, bradykinin, glutamate, and serotonin (5-HT) receptors have been shown to regulate sAPPα release [114–118]. Other studies indicate that reduction in $A\beta_{42}$ is associated with receptor-mediated activation of sAPPα release [119–121]. We have found that $P2Y_2R$ activation stimulated α-secretase by the furin-dependent activation of two members of the ADAM (for a disintegrin and metalloprotease) family [39, 89], ADAM10, the Kuz enzyme [122] and ADAM17/TACE (tumor necrosis factor-α converting enzyme), the protease responsible for releasing TNF-α from the plasma membrane [123]. The cleavage of pro-IL-1β into mature IL-1β is achieved by a cysteine protease belonging to the caspase family, the IL-1β-converting enzyme (ICE), known to be activated by ATP [124].

$P2Y_2R$-mediated Cytoskeletal Signaling in Primary Rat Neurons

It has been demonstrated that ATP released from the leading edge of the neutrophil surface amplifies chemotactic signals and directs cell orientation by activation of the $P2Y_2R$ [38]. Our previous studies indicate that $P2Y_2R$ activation in astrocytoma cells promotes the formation of actin stress fibers and induces cell migration [56, 57], although little is known about the effect of $P2Y_2R$ activation on cytoskeletal functions in neurons. We found that treatment of primary cortical neurons from mice and rats with IL-1β induced $P2Y_2R$ upregulation (39, unpublished data). Subsequent $P2Y_2R$ activation with UTP induces Rho and LIM kinase activation that increases the phosphorylation of the actin-depolymerization factor cofilin [56], a response known to promote localized F-actin expansion and the stabilization of dendritic spines [56, 94, 95, 125, 126]. Since we have found that $P2Y_2R$ interaction with $\alpha_v\beta_{3/5}$ integrins mediates cytoskeletal rearrangements and cell migration in astrocytoma cells via activation of Rho kinase, we postulate that a similar pathway regulates cofilin phosphorylation in neurons (Fig. 5). Previous studies have shown that inhibition of cofilin activation by expressing a phosphomimetic mutant of cofilin (cof-S3D) prevented Aβ-induced spine loss [26]. Activation of the $P2Y_2R$ causes dynamic reorganization of the actin cytoskeleton in migratory cell types, and our results indicate that the $P2Y_2R$ directly binds FLNa, activates focal adhesion molecules, and induces the phosphorylation of cofilin, suggesting that $P2Y_2Rs$ utilize these signaling pathways to regulate actin cytoskeletal rearrangements that promote dendritic spine growth and stabilization in neurons.

Conclusion

The neuroprotective mechanisms underlying acute inflammatory responses in the brain become neurodegenerative when sustained [19–21], as occurs in brain pathologies including AD, trauma, and stroke [22]. The ATP and UTP-activated G_q protein-coupled $P2Y_2R$ is expressed in glial

cells and regulates a variety of intracellular signal transduction pathways via activation of integrins, growth factor receptors, and PLC to promote cytoskeletal rearrangements, cell migration and proliferation, associated with reactive astrogliosis in the AD brain. In neurons, upregulation of $P2Y_2Rs$ by IL-1β promotes the nucleotide-induced non-amyloidogenic processing of APP and the phosphorylation of cofilin, responses that are neuroprotective. Thus, the $P2Y_2R$ may represent a novel target for the prevention of neuronal damage in AD and related neuroinflammatory diseases.

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