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REVIEW

Neuroprotective roles of the $P2Y_2$ receptor

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Abstract Purinergic signaling plays a unique role in the brain by integrating neuronal and glial cellular circuits. The metabotropic P1 adenosine receptors and P2Y nucleotide receptors and ionotropic P2X receptors control numerous physiological functions of neuronal and glial cells and have been implicated in a wide variety of neuropathologies. Emerging research suggests that purinergic receptor interactions between cells of the central nervous system (CNS) have relevance in the prevention and attenuation of neurodegenerative diseases resulting from chronic inflammation. CNS responses to chronic inflammation are largely dependent on interactions between different cell types (i.e., neurons and glia) and activation of signaling molecules including P2X and P2Y receptors. Whereas numerous P2 receptors contribute to functions of the CNS, the $P2Y_2$ receptor is believed to play an important role in neuroprotection under inflammatory conditions. While acute inflammation is necessary for tissue repair due to injury, chronic inflammation contributes to neurodegeneration in Alzheimer's disease and occurs when glial cells undergo prolonged activation resulting in extended release of proinflammatory cytokines and nucleotides. This review describes cell-specific and tissue-integrated functions of P2 receptors in the CNS with an emphasis on $P2Y_2$ receptor signaling pathways in neurons, glia, and endothelium and their role in neuroprotection.

Keywords $P2Y_2$ receptor · CNS · P2X and P2Y receptors

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Introduction

It has become apparent that P2 receptors for extracellular nucleotides are ubiquitously expressed in a wide variety of tissues, and the complexity of responses to nucleotides is due in large part to the presence of multiple subtypes of P2X receptor ligand-gated ion channels and G protein-coupled P2Y receptors [1–7]. This functional complexity is well manifested in the central nervous system (CNS) where 7 P2X and 8 P2Y receptor subtypes are expressed under a range of conditions in several different interacting cell types [6, 8–12]. Accordingly, studies on P2 receptor functions in the brain must consider the combined contributions of P2X and P2Y receptors expressed in neurons, microglial cells, astrocytes, and endothelium [10, 13–15]. In addition, the major ligand that activates many of these P2 receptor subtypes is ATP, released in the course of neurotransmission or under proinflammatory or cell apoptotic conditions [1, 16–18]. Since ATP or its degradative products activate most of the P2 nucleotide and P1 adenosine receptor subtypes identified in the CNS [1], unraveling the effects of ATP in vivo is difficult. This analysis can be simplified using animal models with selective knockout of specific P2 receptor subtypes or subtype-selective agonists/antagonists when

available. Another approach is to analyze the effects of uridine nucleotides (i.e., UTP/UDP) that only activate G_q-coupled $P2Y_2$, $P2Y_4$, and $P2Y_6$ receptors among the known P2 receptor subtypes [2, 4, 19, 20]. Among these three receptors, the $P2Y_2$ receptor ($P2Y_2R$) has unique motifs that promote interactions with integrins and growth factor receptors, thereby enabling activation of signaling pathways beyond G_q-dependent phospholipase C (PLC) [21–24]. Furthermore, the contribution of the $P2Y_2R$ subtype to CNS functions is becoming better understood [14, 15, 25] and appears to be most important under pathophysiological conditions, such as inflammation and bacterial infection [15, 26]. In addition, the availability of the $P2Y_2R$ knockout mouse has provided a valuable tool to dissect functional interactions between $P2Y_2Rs$ and other P2 receptors expressed in cell types of the CNS under various conditions [27, 28].

This review focuses on cell-specific and tissue-integrated functions of $P2Y_2Rs$ in the CNS with an emphasis on $P2Y_2R$ signaling pathways in neurons, glia, and endothelium that comprise the structure of the brain. In addition, we describe mechanisms whereby the $P2Y_2R$ activates cellular responses under proinflammatory conditions associated with neurodegenerative diseases, such as Alzheimer's disease (AD), and postulate a role for these receptors in the regulation of neuroprotective responses.

P2 receptors in the CNS

Purinergic receptors are expressed in many mammalian cell types and are activated by extracellular adenine and uridine nucleotides or nucleosides [6, 29–31]. Both P1 receptors for adenosine and P2 receptors (P2Rs) for adenine and/or uridine nucleotides are expressed in cells comprising the CNS and have been shown to regulate important physiological and pathophysiological functions, including neurotransmission, inflammation, cell growth, and apoptosis [11, 31–33]. The P2R agonist ATP is a neuro- and gliotransmitter released by exocytosis from neurons and by diffusion through hemichannels, pannexins, and voltage-gated channels in various cell types [12, 17, 34–37]. P2Rs (both P2X ligand-gated cation channels and P2Y G protein-coupled receptors) [1, 4, 6] are expressed in neuroglia (astrocytes, oligodendrocytes) and microglia of the CNS [14], where they regulate differentiation, nociceptive transmission, cytokine release, apoptosis, and metalloprotease-dependent degradation of amyloid precursor protein (APP) [16, 27, 31, 38–40]. Among cell types that comprise the CNS, mRNAs for P2X1–7 and $P2Y_{1,2,4,6,11,12,13,14}$ receptor subtypes have been identified in primary rat astrocytes [4, 41–44], and their expression patterns can vary with the age of the animal [45, 46]. Neurons express mRNAs for P2X3, 5–7 and $P2Y_{1,2,4,6,12,13}$ receptors [12, 47–49]. Multiple subtypes of P2Rs are expressed in monocytes (P2X1,4,7 and $P2Y_{1,2,4,6,11,12,13}$ receptors) [50] and human endothelial cells (P2X4 and $P2Y_{1,2,4,6,11}$ receptors) [51]. The role of P1 and P2 receptors in the function of immune cells (e.g., neutrophils, eosinophils, monocytes, macrophages, mast cells, and lymphocytes) has been well described [26, 52–57], and the studies suggest that these receptors regulate cellular responses associated with inflammatory diseases. P2Rs are expressed at presynaptic nerve terminals where P2X1, P2X2, and P2X3 receptors have facilitatory, whereas $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors have inhibitory roles in synaptic transmission [10, 58–62]. Studies also have shown that postsynaptic P2 receptors including P2X3, $P2Y_4$, and $P2Y_1$ receptors are involved in neuromodulation [10, 63–65], where they regulate either transmitter release or postsynaptic sensitivity to other neurotransmitters.

Among the G protein-coupled P2YRs, the G_q-coupled $P2Y_2R$ subtype is expressed in neurons and glial cells [13, 15, 48, 66–68]. Our studies using in situ hybridization and reverse transcriptase polymerase chain reaction with rodent brain slices have shown high levels of $P2Y_2R$ expression in the hippocampus and cerebellum [20], and $P2Y_2R$ expression can be significantly upregulated in mouse cortical neurons by the proinflammatory cytokine interleukin-1β (IL-1β) [48]. P2YRs have been shown to be coupled either directly or indirectly to G_q, G_i, G_o, and G_{12} protein activation and downstream signaling pathways associated with alterations in PLC or adenylyl cyclase activities [1, 20, 69–71]. The agonist selectivity of P2YR subtypes varies widely, in contrast to P2X receptors [1]; for example, the $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{14}$ receptor subtypes can be activated by uridine nucleotides or UDP-glucose that are ineffective agonists of all P2X and four P2Y receptor subtypes [1, 4, 19, 20, 56, 70, 72–74].

Interactions have been reported between different P2 receptor subtypes in cells of the CNS. For example, activation of both astrocytic P2YRs and P2X7Rs occurs in brain lesions during the functional remodeling that accompanies astrogliosis and neuroinflammation [72]. Interactions between P2X7R and $P2Y_2R$ signaling pathways mediate glial cell-dependent neuroprotective responses [15, 75, 76] in which P2X7R activation in microglial cells leads to the release of nucleotides and cytokines, including IL-1β [75], that enhance the functional expression of $P2Y_2Rs$ in neurons [48]. $P2Y_1R$ activation in astrocytes of hippocampal cultures also provides neuroprotection from oxidative stress by increasing IL-6 release [77]. $P2Y_2$ and $P2Y_6$ receptors have been suggested to play complementary roles in the regulation of apoptosis, since $P2Y_6R$ activation inhibits tumor necrosis factor-α (TNF-α) receptor signaling [78] and $P2Y_2R$ activation upregulates anti-apoptotic proteins [79] to promote survival mechanisms in astrocytic cells.

P2YR functions associated with the pathogenesis of inflammation in the CNS, a process involving astrocyte and microglial cell proliferation and migration to a site of injury (i.e., gliosis), can be induced by a variety of conditions (e.g., oxidative stress or excessive β-amyloid (Aβ) peptide production) that stimulate the release of proinflammatory mediators, including cytokines [15, 75, 76, 80–86]. Among these mediators, ATP and other nucleotides also can be released into the extracellular space due to cell damage, oxidative stress, hypoxia, ischemia, or mechanical stress [17, 81–84], whereupon the nucleotides activate P2X and P2Y receptors expressed in surrounding cells. Several studies have proposed the involvement of P2Rs, including the P2X7R and $P2Y_2R$, in proinflammatory responses mediated by glial cells that are associated with neurodegenerative diseases [15, 20, 57, 75, 76, 85]. In the absence of inflammation, $P2Y_2R$ expression levels are low in neurons, but the presence of IL-1β upregulates $P2Y_2R$ expression [48]. As mentioned above, we will focus on the role of $P2Y_2Rs$ in the regulation of neuroprotective responses associated with inflammation.

$P2Y_2$ receptor signaling pathways

Activation of the G_q-coupled $P2Y_2R$ by ATP or UTP (EC_{50} ~ 1–6 μM) [7, 74] is linked to the stimulation of PLC leading to an increase in the production of inositol 1,4,5 trisphosphate (IP_3) and diacylglycerol that elevates the intracellular Ca^{2+} concentration, $[Ca^{2+}]_i$, and activates protein kinase C (PKC), respectively [73, 87]. Recent studies have demonstrated that the $P2Y_2R$ can activate signaling pathways independent of coupling to G_q protein. Although $P2Y_2R$-mediated activation of mitogen-activated protein (MAP) kinases is stimulated by G_q-dependent increases in $[Ca^{2+}]_i$, $P2Y_2R$ activation also stimulates epidermal growth factor receptor (EGFR) phosphorylation and significantly enhances the activities of the MAP kinases ERK1/2 and related adhesion focal tyrosine kinase (RAFTK) via a mechanism involving Src and Shc/Grb2 [21, 88]. Other studies have shown that the $P2Y_2R$ contains 2 Src-homology-3 (SH3) binding domains in the intracellular C terminus that facilitate the binding of Src and the association of the $P2Y_2R$ with the EGFR, thereby enabling nucleotides to induce Src-dependent phosphorylation of the EFGR [22, 89]. This $P2Y_2R$-mediated transactivation of growth factor receptors has implications in the regulation of cell growth, motility, differentiation, and cytoskeleton-associated morphological changes [22, 89]. Studies also have shown that $P2Y_2Rs$ in the presence of nerve growth factor co-localize with tyrosine receptor kinase A via a Src-dependent event that promotes neurite outgrowth and cell division [27]. This pathway leads to the activation of p38 and ERK1/2 MAP kinases and is inhibited by siRNA directed against $P2Y_2R$ mRNA. $P2Y_2R$-mediated activation of PI3-kinase/Akt and MAP kinases has been shown to inhibit apoptosis in PC12 pheochromocytoma cells and dorsal root ganglion neurons [90]. In smooth muscle cells of human chorionic arteries, transactivation of the EGFR by the $P2Y_2R$ can activate RhoA and Rac1, a pathway that is dependent on clustering of these molecules in lipid rafts and internalization of the $P2Y_2R$ [91].

$P2Y_2Rs$ in endothelial cells can activate vascular endothelial growth factor receptor-2 (VEGFR-2) that has been shown to lead to the upregulation of vascular cell adhesion molecule-1 (VCAM-1) and an increase in the binding of monocytic cells to endothelium [89]. Deletion of the SH3-binding domains in the $P2Y_2R$ prevented nucleotides from activating VEGFR-2-dependent VCAM-1 upregulation [22, 89]. VCAM-1 expression in endothelial cells also was found to be dependent on increases in $[Ca^{2+}]_i$ and p38 and Rho kinase activation but was independent of ERK1/2 activity [92]. Similarly, lymphocyte binding to epithelium is stimulated by $P2Y_2R$ activation in epithelial cells via EGFR-dependent VCAM-1 upregulation [93]. However, this pathway was found to be Src-independent and required the release of growth factors by $P2Y_2R$-dependent activation of matrix metalloproteases (MMPs) [94].

The human and mouse $P2Y_2Rs$ contain an integrin-binding Arg-Gly-Asp (RGD) motif in the first extracellular loop that enables nucleotides to activate integrin signaling pathways [23, 24, 95]. In contrast, the rat $P2Y_2R$ homolog contains Gln-Gly-Asp (QGD) instead of RGD [96], although this is considered to be a conservative substitution that maintains integrin-binding affinity [23, 97]. Although the presence of a RGD motif in a G protein-coupled receptor is rare, its functional significance has not been extensively investigated. Studies have shown that the RGD sequence in the $P2Y_2R$ promotes its interaction with $\alpha_v\beta_{3/5}$ integrins [23], and following $P2Y_2R$ activation by UTP, there is an increase in the activation of monomeric G_o and G_{12} proteins and the subsequent stimulation of the small GTPases Rho and Rac [24, 95]. Results indicate that mutation of the RGD sequence to Arg-Gly-Glu (RGE), a motif that does not bind well to integrins [98], prevented the binding of the $P2Y_2R$ to $\alpha_v\beta_{3/5}$ integrins and inhibited nucleotide-induced G_o, G_{12}, Rho, and Rac activation [24, 95]. G_o-dependent Rac and G_{12}-dependent Rho activation are known to mediate cytoskeletal rearrangements and cell migration through a mechanism involving the activation of LIM kinase-dependent cofilin phosphorylation, a key regulator of actin polymerization [99], and studies indicate that activation of the $P2Y_2R$ promotes cytoskeletal rearrangements and cell migration that are abolished by mutation of the RGD motif to RGE [24, 95]. Thus, it appears that the ability of the $P2Y_2R$ to increase cell chemokinesis is dependent upon $P2Y_2R$ association with $\alpha_v\beta_{3/5}$ integrins that stimulates signaling pathways involved in cytoskeletal reorganization required for cell motility.

The C-terminal domain of the $P2Y_2R$ has been shown to bind filamin A (FLNa), an actin-binding protein that regulates cytoskeletal dynamics [100]. Using the yeast 2-hybrid system, an 11-amino acid stretch including the SH3-binding domains in the C-terminal tail of the $P2Y_2R$ was found to regulate FLNa binding to the $P2Y_2R$ and nucleotide-induced increases in cell migration and spreading [100]. Since both $P2Y_2R$-mediated transactivation of growth factor receptors and integrins contribute to cell migration [21, 24, 91], it is intriguing to postulate that FLNa binding to the SH3-binding domains of the $P2Y_2R$ links nucleotide-induced EGFR transactivation to the RGD-dependent integrin signaling pathway that regulates cytoskeletal rearrangements required to increase cell motility. In other studies, $P2Y_2R$-mediated monocyte diapedesis (i.e., transendothelial migration) has been shown to occur by disruption of intercellular adherens junctions, suggesting that cytoskeletal rearrangements promoted by the $P2Y_2R$ also can affect cell polarization [89, 93, 95, 101–103]. Therefore, these data suggest a mechanism whereby the tropism of monocytic cells (e.g., microglia) into damaged areas of the CNS can be induced by activation of $P2Y_2Rs$.

Activation of $P2Y_2Rs$ expressed in human astrocytoma cells or rat primary cortical neurons (rPCNs) stimulates α-secretases, i.e., the MMPs adamalysin 10/17 (ADAM10/17), that mediate the proteolytic processing of APP to generate the non-amyloidogenic soluble APPα (sAPPα) peptide [38, 48]. $P2Y_2R$-mediated α-secretase activity is dependent on activation of the PI3-kinase/Akt pathway and partially dependent on activation of PKC and ERK1/2. Recent data with rat cortical astrocytes indicate that both $P2Y_2$ and $P2Y_4$ receptor activation can increase the production and release of APP via activation of ERK and p38 [40]. In rPCNs, $P2Y_2R$ expression is relatively low but is significantly upregulated by IL-1β via a pathway that involves the activation of the transcription factor NF-κB [48]. Indeed, it has been shown that the $P2Y_2R$ promoter contains a NF-κB binding sequence that is required for inflammation-induced $P2Y_2R$ upregulation, a pathway that can be blocked by Bay-11-7085, a specific inhibitor of the phosphorylation of IκB-α, the endogenous regulator of NF-κB activity [104]. Thus, it seems likely that the co-release of IL-1β and nucleotides mediated by ATP-induced P2X7R activation in microglia [16, 75, 105] provides an in vivo mechanism for both the upregulation and activation of $P2Y_2Rs$ in neurons and other cell types. ATP release also occurs from activated microglia and astrocytes under oxidative stress [15] and following neuronal excitation [17, 106] via volume-activated anion channels [106], P2X7Rs [107], and pannexin hemichannels [37, 108] or upon exposure to fibrillar or oligomeric $A\beta_{1–42}$ [14, 75, 109]. Clearly, the proinflammatory effects of cytokine and ATP release in the CNS can be coordinately regulated by the P2X7 and $P2Y_2$ receptors.

The $P2Y_2R$ is known to desensitize and internalize following activation [110, 111], which can be inhibited by deletion of segments of the C terminus of the receptor [110]. Depletion of intracellular calcium stores is another mechanism by which further G protein-coupled receptor (GPCR)-induced elevations in $[Ca^{2+}]_i$ in microglia can be desensitized [112]. Following agonist-induced GPCR desensitization, receptor internalization occurs, a process that is regarded both as a resensitization step and as a means to link a GPCR to intracellular signaling pathways [113]. GPCR internalization often requires arrestin binding to the desensitized receptor that provides a scaffold for multiple protein–protein interactions [113, 114]. A role for arrestin-2 in cell migration has been reported [115], and arrestin has been shown to associate with LIM kinase/cofilin [116] and FLNa [117] providing a mechanism for activation of arrestins by a GPCR, such as the $P2Y_2R$. The β1-adrenergic receptor-mediated transactivation of the EGFR is mediated by arrestin 1 and 2 following G protein receptor kinase 5/6-dependent phosphorylation of the β1 receptor [118]. These interactions lead to Src-dependent activation of MMPs and consequent release of the HB-EGF ligand to enable autocrine activation of the EGFR [119]. Since G_q-coupled $P2Y_2R$ activation can induce Src-dependent activation of the EGFR [21, 22, 88, 89], activation of the MMPs α-secretases [38], and integrin-dependent increases in cell motility [24, 95], it is intriguing to speculate that arrestins and receptor internalization play a role in these processes. Signaling pathways known to be coupled to $P2Y_2R$ activation are shown in Fig. 1.

$P2Y_2$ receptors in glial cells

The major glial cells in the brain are astrocytes, oligodendrocytes, and microglia. Astrocytes are derived from the ectoderm and contribute to the maintenance of the blood–brain barrier (BBB) [120–122], which prevents invasion of pathogenic substances into the brain from the circulation [123]. Astrocytes also release neurotrophic factors that play an important role in neuronal survival and sprouting and supply energy substrates to neurons [124]. Oligodendrocytes are involved in the insulation of axons in the CNS, and it has been shown that oligodendrocyte precursor cells express $P2Y_1$, $P2Y_2$, and $P2Y_4$ receptors [12], but these cells do not appear to have a significant role in glial cell activation due to brain injury [123]. $P2Y_2R$ interactions with integrins have been shown to promote migration of astrocytes [68]. $P2Y_1Rs$ and $P2Y_2Rs$ mediate astroglial calcium signals at the gliovascular interface by two distinct forms of P2R-dependent negative feedback mechanisms that differentially control Ca^{2+} signaling in astrocytes, suggesting divergent roles for these receptor subtypes in downstream signal transduction [125]. Functional studies with astrocytes and oligodendrocytes have demonstrated a role for both $P2Y_1$ and $P2Y_2$ receptors in mediating ATP-evoked and IP_3-dependent increases in $[Ca^{2+}]_i$ [20, 80]. $P2Y_2Rs$ are

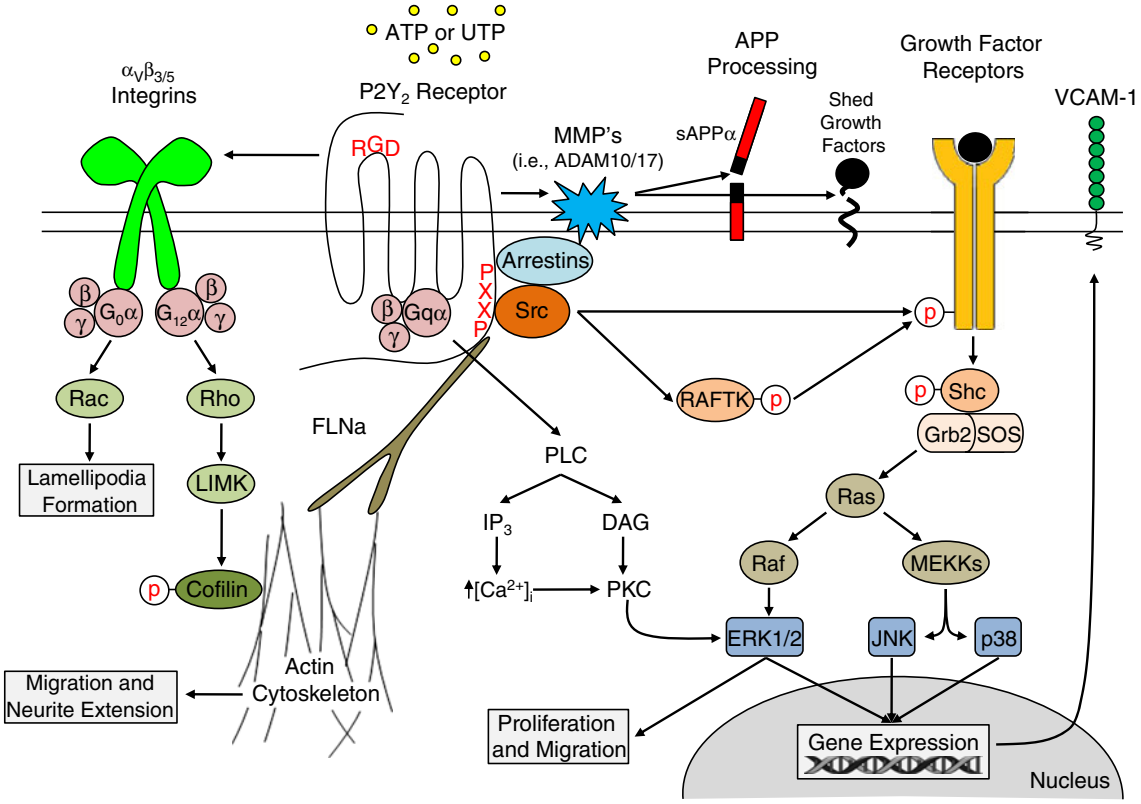

Fig. 1 P2Y$_2$R signaling pathways. Upon activation with ATP or UTP, the G_q-coupled P2Y$_2$R stimulates $G_{q\alpha}$-dependent phospholipase C (*PLC*) activity which generates inositol 1,4,5 trisphosphate (*IP$_3$*) and diacylglycerol (*DAG*) resulting in an elevation in the intracellular calcium concentration via IP$_3$-dependent calcium release from intracellular stores and DAG-dependent activation of protein kinase C (*PKC*), respectively. The P2Y$_2$R via an extracellularly oriented RGD domain can interact with $\alpha_V\beta_{3/5}$ integrins to regulate the activities of G_{12}-dependent Rho, G_o-dependent Rac, LIM kinase, and cofilin, proteins that regulate actin cytoskeletal rearrangements. Src-homology-3 binding domains (PXXP) within the C terminus of the P2Y$_2$R bind Src to enable ATP or UTP to transactivate growth factor receptors and related adhesion focal tyrosine kinase (*RAFTK*; also known as Pyk2) and downstream MAP kinases. P2Y$_2$R-mediated transactivation of growth factor receptors leads to upregulation of vascular cell adhesion molecule 1 (*VCAM-1*). The C terminus of the P2Y$_2$R also has been shown to interact with the actin-binding protein filamin A (*FLNa*). P2Y$_2$R activation can stimulate the activity of matrix metalloproteases (e.g., ADAM10 and ADAM17) leading to the α-secretase-dependent processing of APP to the non-amyloidogenic peptide sAPPα [38, 48] and release of growth factors (e.g., NRG1) [94]

upregulated in reactive astrocytes of the cortex and nucleus accumbens in rat due to mechanical injury, suggesting a role in modulating responses to trauma [126]. Furthermore, studies with astrocytic cells also suggest that P2Y$_2$Rs play a role in astrocyte survival after injury [13, 79].

Microglial cells, in contrast to astrocytes and oligodendrocytes, belong to the myelomonocytic lineage and become parenchymal cells in the CNS at early stages of embryonic development [127]. Microglial cells are originally derived from the mesoderm and possess functions similar to peripheral monocytes/macrophages [120, 128]. Microglia have important immunoregulatory functions in the CNS. Injury or other disturbances to the CNS trigger rapid transformation of ramified (quiescent) microglia into activated phenotypes that further develop into phagocytic macrophages [129, 130]. Activated microglia can be either neuroprotective [130–134] or neurotoxic [131, 135–137]. Although the CNS is considered to be an immune-privileged site because the BBB limits entry of blood-borne cells and proteins, recent findings indicate that peripheral leukocytes have important physiological and pathophysiological functions in the CNS [138]. Discrete populations of blood-borne leukocytes are recruited into the CNS by traversing the BBB under normal conditions or in response to injury or disease [139]. Similarly, hematopoietic cells can cross the BBB and enter the CNS whereupon they differentiate into microglia [140]. In fact, these peripheral macrophages have been shown to be more adept than resident microglia in the phagocytosis of neurotoxic Aβ in animal models of AD [138]. Peripheral macrophages have a dynamic life cycle and can enter and exit the CNS [141] to engulf and digest significant amounts of cellular debris and pathogens [138].

Microglial cell activation by the proinflammatory cytokines TNF-α, IL-1β, and IL-6 is accompanied by partial rounding and increased cell motility and proliferation [142]. The P2Y$_2$R agonists UTP and ATP, released from apoptotic

cells as a result of caspase 3/7 activation, also have been shown to induce cell migration of phagocytic cells [143]. In an in vivo model of cell migration, supernatants from apoptotic cells produced a three-fold greater recruitment of monocytes and macrophages than supernatants from control cells and depletion of nucleotides in the apoptotic cell supernatants diminished cell migration [143]. Extracellular nucleotides also have been shown to promote human monocyte migration in vitro by co-activation of $P2Y_2$ and $P2Y_6$ receptors that enhances TLR1/2-induced IL-8 release, whereas the innate immune response resulting from $P2Y_2R$ and $P2Y_6R$ activation in vivo was shown to be dependent upon TLR2 [56]. It has been reported that following injury or neuroinflammation, $P2Y_6Rs$ are functionally upregulated in microglial cells and their activation by UDP triggers phagocytosis [144]. Our data with mouse primary microglial cells indicate that $P2Y_2R$ activation enhances microglial cell migration (Table 1) and their uptake and degradation of neurotoxic oligomeric $A\beta_{1-42}$ (Table 2), responses that were markedly decreased in microglia from $P2Y_2R^{-/-}$ cells [145].

Microglial cell migration at early stages of local CNS injury has been suggested to be regulated by $P2Y_{12}R$ expression, which is robust in the resting stage, but decreases upon microglial cell activation [146]. It also has been shown that activated microglia attach to and engulf myelinated axons in the dorsal horn after a peripheral nerve injury, and $P2Y_{12}R$ inhibition suppresses engulfment of these myelinated axons by activated microglia [147]. $P2Y_{12}$ and $P2X_4$ receptor activation has been shown to induce migration of ramified microglia, attracting them to regions of high ATP concentrations [148, 149]. Studies using peritoneal macrophages in mice have shown that stimulation of $P2Y_2$ and $P2Y_{12}$ receptors induces the formation of lamellipodial membrane protrusions that leads to cell spreading and efficient directional motility of cells [150]. Taken together, these findings support the hypothesis that extracellular nucleotides serve as endogenous danger signals that activate microglia during the innate immune response.

$P2Y_2$ receptors in neurons

$P2Y_2Rs$ are expressed in neurons of the central and peripheral nervous systems [1], but expression levels are relatively low, as compared to other tissues [48, 151, 152]. $P2Y_2R$ expression in mouse primary cortical neurons can be upregulated in response to the proinflammatory cytokine IL-1β [16, 48], the levels of which are elevated in the AD brain [153]. Activation of the P2X7R in microglia promotes the release of IL-1β, TNF-α, and ATP [16, 75, 105, 154–156], suggesting a mechanism whereby the P2X7R regulates functional $P2Y_2R$ expression in neurons and other cells. The finding that $P2Y_2R$ expression under proinflammatory conditions is regulated by NF-κB binding to the *$P2Y_2R$* promoter [104] is consistent with the established role of NF-κB activation in the induction of inflammation [157].

Other studies suggest a role for $P2Y_2Rs$ in the regulation of neuroprotective responses. As discussed above, $P2Y_2R$/α_v integrin interaction enables nucleotides to stimulate Rac and Rho and induce cytoskeletal rearrangements [24, 95], well-established signaling pathways that regulate the outgrowth and stabilization of dendritic spines [99, 158]. The $P2Y_2R$ agonist UTP has been shown to increase levels of neurofilament M and neurofilaments that promote neurite outgrowth [159]. In neural progenitor cells isolated from the subventricular zone of adult mouse brain, $P2Y_2R$ activation was shown to induce rapid and transient activation of the EGFR, ERK1/2, and CREB [160]. $P2Y_2R$ mRNA levels also were shown to increase during the acute and chronic stages of spinal cord injury [161] and with brain ischemia, mechanical injury to the nucleus accumbens, and brain trauma, an acute inflammatory response suggested to provide neuroprotection [126]. Other potential neuroprotective responses linked to $P2Y_2R$ function in primary cortical neurons include the activation of non-amyloidogenic APP processing [48]. The $P2Y_2R$ also has been suggested to contribute to synaptic transmission through the regulation of intracellular calcium waves in astrocytes [162].

Table 1 Role of the $P2Y_2R$ in cell migration

	Control	Wild type			$P2Y_2R^{-/-}$		
$oA\beta_{1-42}$	–	+	–	–	+	–	–
ATP	–	–	+	–	–	+	–
UTP	–	–	–	+	–	–	+
Cell motility (fold over controls)		3-fold increase**	6-fold increase**	7-fold increase**	~ same as control	~ same as control	~ same as control

Briefly, primary microglial cells (1×10^6) from WT or $P2Y_2R^{-/-}$ mice were seeded in the upper chamber of Transwell inserts that were then placed in six-well plates, and cells were treated for 6 h with or without oligomeric $A\beta_{1-42}$ (1 μM) or ATP or UTP (100 μM) in the lower chamber. Cells that migrated across the membrane were counted under a microscope, and cell motility was expressed as fold increase over untreated control. Data represent means ± SEM (n=4)

**$p<0.01$ indicates a significant difference from untreated control

Table 2 Role of the $P2Y_2R$ in $A\beta_{1–42}$ uptake and degradation

	Control		Wild type		$P2Y_2R^{-/-}$	
ATP 100 μM	–	–	+	–	+	–
UTP 100 μM	–	–	–	+	–	+
$A\beta_{1–42}$ uptake (pg/mg protein)	1800	2000	3800**	4500**	2000	2100
$A\beta_{1–42}$ degradation	47 %		78 %*	80 %*	50 %	48 %

Primary microglial cells from WT or $P2Y_2R^{-/-}$ mice were treated with ATP or UTP (100 μM) followed by oligomeric $A\beta_{1–42}$ (1 μM) for 1 h. Control cells from WT $P2Y_2R^{-/-}$ were treated with oligomeric $A\beta_{1–42}$ without ATP or UTP, and the values represent average of two control groups. Cell lysates were analyzed for intracellular $A\beta_{1–42}$ uptake by ELISA. Data represent means ± SEM (n=3). To determine $P2Y_2R$-mediated $A\beta_{1–42}$ degradation, cells were incubated with $A\beta_{1–42}$ for 1 h, the medium was removed, cells were washed, and fresh media containing ATP or UTP (100 μM) was added. After 24 h, cell lysates were analyzed for intracellular $A\beta_{1–42}$ levels by ELISA. Levels of $A\beta_{1–42}$ remaining in the cell lysates after 24 h were divided by $A\beta_{1–42}$ levels in cell lysates after 1 h to calculate the percentage of $A\beta_{1–42}$ degradation. Data represent means ± SEM (n=4)

*p<0.05 indicates a significant difference from untreated control; **p<0.01 indicates a significant difference from untreated control

$P2Y_2Rs$ in peripheral neurons can regulate the sensation of bladder distension in response to stretch-induced ATP release [163]. $P2Y_1$, $P2Y_2$, and A2 adenosine receptors have been suggested to regulate smell via the plasma membrane localization of olfactory receptor M71 in olfactory neurons [164]. Interactions between activated $P2Y_2Rs$ and the capsaicin-sensitive TRPV1 channel in peripheral neurons have been suggested to modulate pain sensation [165]. $P2Y_2R$ activation in retinal neurons has been shown to stimulate subretinal fluid reabsorption, inhibit retinal folding and apoptosis, and increase the rate of retinal reattachment in rat and rabbit models of experimental retinal detachment [166]. Thus, the $P2Y_2R$ appears to play a neuroprotective and/or reparative function under a variety of conditions associated with tissue injury, such as inflammation, pain, and mechanical damage.

Glial–neuronal interactions involving $P2Y_2$ receptors

Over the past three decades, our understanding of intercellular communication in the CNS has evolved, and it has become widely accepted that glial cells act as organized networks rather than single cells. In vitro studies indicate that neurons require help from glia to form and maintain proper synaptic connections, and it is hypothesized that development of neuronal synapses is influenced by the differentiation of surrounding glial cells [167]. For example, astrocytes release gliotransmitters, including glutamate, ATP, and D-serine that exert direct effects on synaptic plasticity [168–170]. Microglial cells also have relevance to neuronal function in various types of brain injury and disease, such as ischemic trauma and AD. Activation of microglia under pathological conditions results in their transformation to amoeboid morphology, migration toward the site of injury/damage, and release of neuroactive compounds that can have either neurotoxic or neuroprotective effects [171, 172]. In vivo two-photon imaging revealed that resting microglia make brief but direct contacts with synapses without undergoing complete transformation/activation associated with a pathological phenotype [173, 174]. In contrast, prolonged microglial cell contact with neurons can initiate a cascade of events that results in synaptic stripping and functional impairment of neuronal circuits [175, 176]. Ca^{2+} waves in astrocytes can extend hundreds of micrometers from their site of initiation (e.g., an injury) and have been suggested to serve as a long range signal for recruitment of microglia from uninjured to injured areas of the brain [177]. Ca^{2+} waves initiated in astrocytes can propagate into microglia by an ATP-dependent pathway [177, 178]. Microglia–neuronal interactions are known to be mediated by release of a variety of cell signaling molecules from microglia or neurons that activate cell surface receptors [179–183]. Some of the chemoattractant signals released at synapses include glutamate, nucleosides, nucleotides, brain-derived growth factor, dopamine, noradrenaline, and chemokines [146, 184–186]. Both adenine and uridine nucleotides increase the motility of microglial cells [146, 187], and $P2Y_2$ and $P2Y_{12}$ receptors have been shown to mediate these effects [146, 184, 187]. Extracellular ATP release significantly increases process extension toward an injury site for resting or activated microglia [148]. Astrocytes under pathological conditions also can release ATP to activate P2Rs in neighboring cells [18, 188, 189], and inflammation in vivo can elevate extracellular ATP levels sufficiently to activate P2 receptors [18].

The specific contributions of individual P2R subtypes to functional responses in tissues are difficult to discern, particularly when multiple P2R subtypes are co-expressed at different levels and since individual subtypes can be activated at different agonist concentrations [190, 191]. Also, activation of individual P2R subtypes can increase $[Ca^{2+}]_i$ to differing extents [80], and there can be significant divergence in the intracellular signaling pathways coupled to

each P2R subtype. Additional sources of complexity include interactive effects of nucleotides with other ligands and variability of $P2Y_2R$-mediated responses with cell type or experimental condition. Microglia express both ionotropic P2X and metabotropic P2Y receptors [60, 66], and stimulation of these P2X and P2Y receptors by ATP increases $[Ca^{2+}]_i$ via extracellular Ca^{2+} influx or release of Ca^{2+} from intracellular stores, respectively [191]. However, responses downstream of P2X and P2Y receptor activation can vary widely. For example, Ca^{2+} influx and associated changes in membrane conductance accompanying activation of P2XRs trigger the opening of voltage-gated and Ca^{2+}-dependent K^+ channels [192], whereas P2Y receptor activation is coupled to a variety of G protein-dependent and G protein-independent signaling pathways, as described above.

Accumulation of proinflammatory cytokines and neurotoxic oligomeric Aβ peptide is associated with the progression of AD [193]. As shown in Fig. 2, Aβ exposure in glial cells causes ATP release which activates the P2X7R to increase the release of nucleotides and cytokines, including IL-1β [75], which enhances the functional expression of $P2Y_2Rs$ in neurons [48] and glial cells (unpublished results). $P2Y_2R$ activation in glial cells increases their proliferation and migration by transactivation of growth factor receptors and integrins [20, 68]. We postulate that under proinflammatory conditions, IL-1β-dependent $P2Y_2R$ upregulation in neurons and $P2Y_2R$ activation by released ATP or UTP regulates neuroprotective responses, such as the non-amyloidogenic processing of APP, rather than Aβ generation, and the stimulation of integrin-dependent dendritic spine growth (see Fig. 2). Furthermore, $P2Y_2R$ activation in microglial cells in response to released nucleotides is postulated to provide neuroprotection by increasing microglial cell migration toward sites of Aβ release followed by $P2Y_2R$-mediated Aβ phagocytosis and degradation by the activated microglia (see Fig. 3). Thus, $P2Y_2R$ upregulation in response to inflammation likely serves a neuroprotective function in the CNS that requires contributions from both glial and neuronal $P2Y_2Rs$.

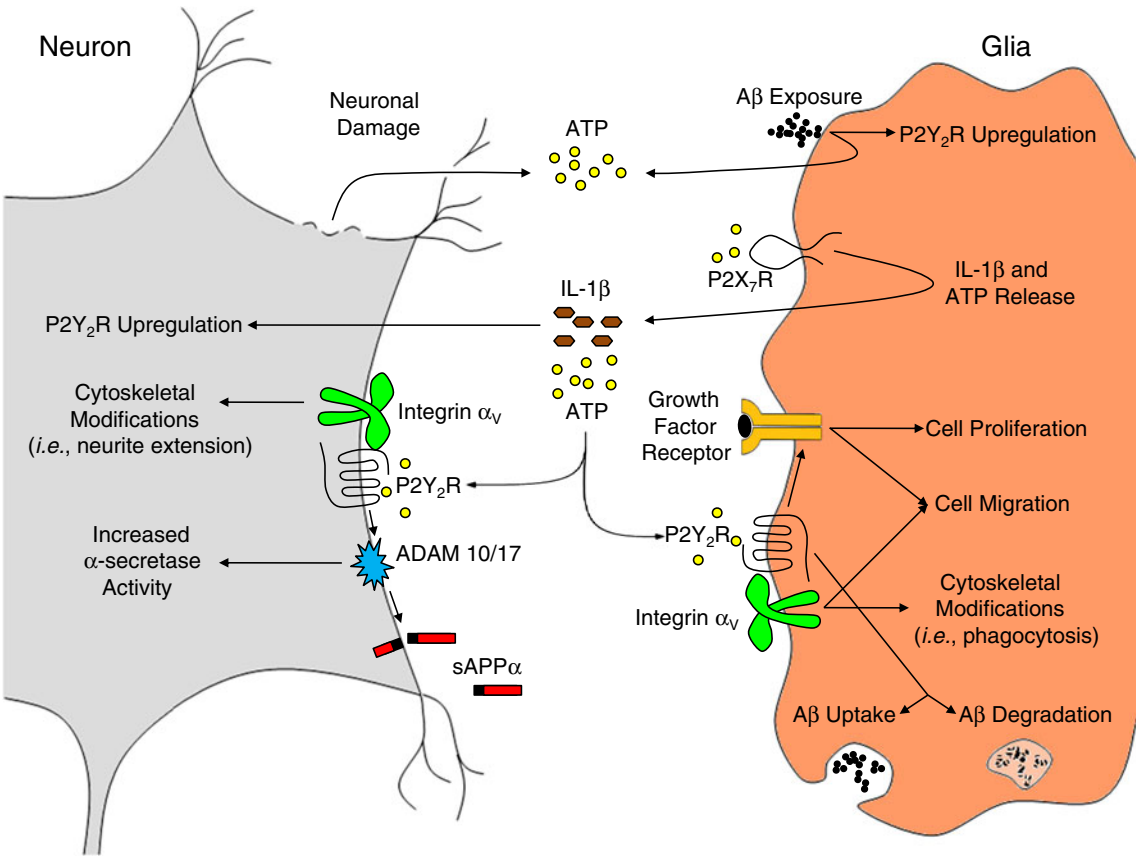

Fig. 2 Neuronal–glial cell interactions involving the $P2Y_2R$ in the CNS. Extracellular ATP, released via neuronal damage, microglial cell exposure to Aβ, or tissue inflammation or injury, activates P2X7Rs on microglial cells that stimulate the release of IL-1β and additional ATP. Exposure of neurons to IL-1β upregulates the $P2Y_2R$, whereas subsequent $P2Y_2R$ activation increases ADAM10/17 activity to increase non-amyloidogenic APP processing. $P2Y_2R$ interaction with integrins induces cytoskeletal rearrangements involved in neurite extension. $P2Y_2R$ activation in microglia increases their proliferation and migration by transactivation of growth factor receptors and integrins. $P2Y_2R$ activation in microglia also increases Aβ phagocytosis and degradation (see Fig. 3)

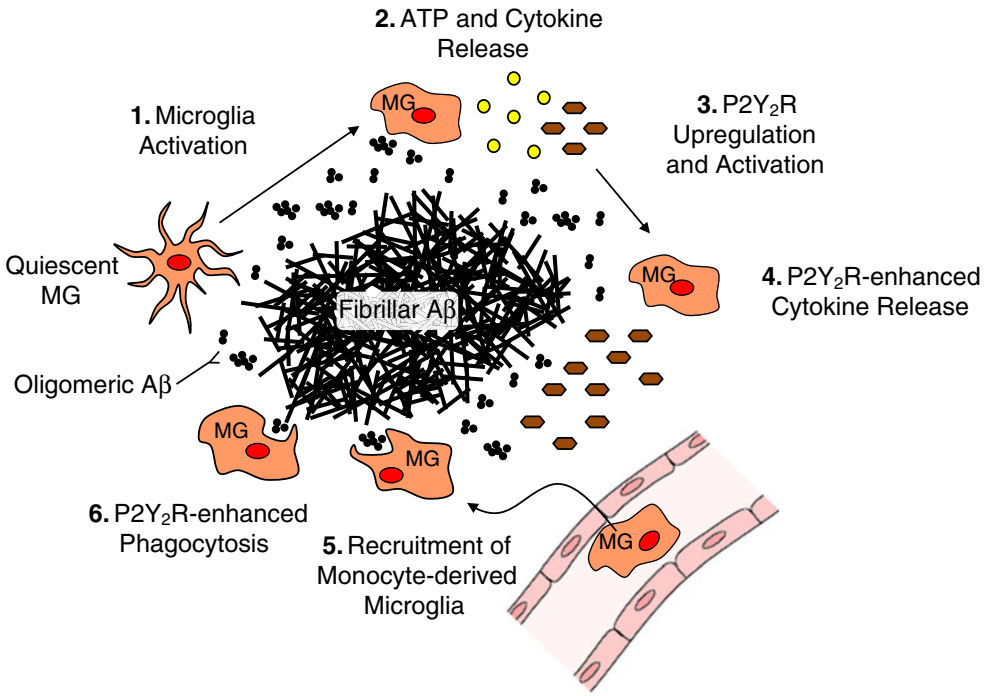

Fig. 3 The $P2Y_2R$ contributes to microglia-mediated uptake and degradation of Aβ. In response to oligomeric $A\beta_{1–42}$ exposure, microglial cells (*MG*) become activated (*1*), release cytokines and ATP (*2*), and the $P2Y_2R$ is upregulated (*3*). Subsequent $P2Y_2R$ activation by released ATP can enhance Aβ-induced cytokine release (*4*), which increases monocyte-derived microglial cell recruitment from the blood (*5*). The $P2Y_2R$ also plays a role in phagocytosis and degradation of fibrillar and oligomeric $A\beta_{1–42}$ (*6*)

$P2Y_2Rs$ in CNS inflammation

Inflammation is an early response to injury, although it remains controversial whether the inflammatory response is beneficial or detrimental to brain tissue [194, 195]. Chronic inflammation damages cells and is thought to be a key player in neurodegenerative disorders, such as AD [193]. The point at which acute inflammation turns chronic is unclear. However, it has been suggested that sustained oxidative stress on cells of the CNS, associated with activation of NADPH oxidase and production of reactive oxygen species, leads to amyloidogenic Aβ production and cell death in AD [196–199]. Early neuroinflammation is thought to have a protective effect in the brain by activating glial cells that secrete cytokines, chemokines, and growth factors at the site of injury [200], which fits well with our model on the role of ATP release and $P2Y_2R$ activation as a neuroprotective response in inflammation (see Fig. 2). In CNS injury, the upregulation and activation of MMPs, a known component of the $P2Y_2R$ signaling pathway [38], can be either beneficial or detrimental depending on the length of time after the injury, the profile of the inflammatory cells at the injury site, and the substrates present [201]. During neuroinflammation, astrocytes undergo morphological and functional changes (i.e., reactive gliosis) characterized by hypertrophy, proliferation, upregulation of the intermediate filament protein glial fibrillary acidic protein, accumulation of activated glial cells around plaques, adhesion of cells to Aβ peptides, internalization and degradation of Aβ peptides by activated glial cells, expression of proteinases required for Aβ peptide catabolism, production of arachidonic acid and related proinflammatory substances in the vicinity of plaques, and regulation of regenerative processes in the brain [202–206]. Activated glia have been shown to produce neurotrophic factors [207, 208] and stimulate neuronal outgrowth during development and repair of damaged brain cells in the adult [209]. $P2Y_2Rs$ regulate many of these responses associated with reactive gliosis [13, 15, 20, 68, 79, 126].

Purinergic signaling has been shown to influence the initiation, progression, and downregulation of an inflammatory response [210], and the $P2Y_2R$ is an important mediator of neuroinflammation [15]. As described above, IL-1β regulates the expression of the $P2Y_2R$ in neurons [48] and other proinflammatory mediators in the AD brain [134, 211], and overexpression of IL-1β has been associated with head trauma, epilepsy, genetic polymorphisms, and age-related damage [212, 213]. In AD, IL-1β increases with Aβ plaque accumulation and dystrophic neurite formation [200]. Although the endogenous expression of $P2Y_2Rs$ has been reported in mouse microglia [67, 214], it seems likely that increased levels of proinflammatory cytokines should further increase $P2Y_2R$ expression in glial cells in vivo. Our recent in vitro data show that treatment of mouse primary oligomeric/oligomeric $A\beta_{1–42}$ upregulates $P2Y_2R$ expression [145] via a pathway likely involving P2X7R-mediated IL-1β release [16, 75, 105, 154–156]. It also has been determined that P2X7R activation increases $P2Y_2R$ expression in rat astrocytes [215]. IL-1β has been shown to stimulate neuronal synthesis of APP, leading to the increased production of neurotoxic Aβ, which in turn activates microglia and further enhances IL-1β production

[216]. Since IL-1β also upregulates $P2Y_2R$ expression in neurons to promote non-neurotoxic APP processing [48], we postulate that $P2Y_2R$ upregulation can counteract the deleterious effects of increased APP synthesis induced by IL-1β. Accordingly, we suggest that a major effect of $P2Y_2R$ upregulation in the CNS is to delay the progression of neurodegeneration that occurs with chronic inflammation.

It is known that activated astrocytes and microglia internalize and degrade Aβ [217–221], a mechanism that reduces Aβ toxicity in neurons, which is postulated to cause neuronal death in AD. Recruitment of activated microglia to sites of inflammation enhances the phagocytosis of aggregated Aβ via the F_c receptor [222, 223]. Studies have shown that microglial cells exposed to Aβ release ATP [75, 224]. Our recent data indicate that primary microglial cells exposed to oligomeric $Aβ_{1–42}$ have 3–4-fold increased levels of TNF-α and IL-1β release, as compared to control cells without $Aβ_{1–42}$ treatment (Table 3). In addition, there was a significant reduction in cytokine release in response to $Aβ_{1–42}$ in microglial cells from $P2Y_2R^{-/-}$ mice (Table 3) or in apyrase-treated microglial cells (not shown).

Figure 3 summarizes the postulated role of the $P2Y_2R$ in microglial cell-mediated phagocytosis and degradation of Aβ. Phagocytosis also plays a major role in controlling inflammation and antigen-cross presentation via uptake of apoptotic bodies from dying cells. The P2R agonists ATP, ADP, α,β-methylene ATP, 3′-*O*-(4-benzoyl) benzoyl ATP, UTP, and UDP have been shown to increase phagocytosis in macrophages [225], suggesting that multiple P2Rs contribute to phagocytosis in the myelomonocytic lineage. We postulate that $P2Y_2R$s can contribute to the phagocytosis of apoptotic debris generated due to ATP release and cell apoptosis previously linked to P2X7R activation [226, 227].

Proinflammatory $P2Y_2R$ functions in endothelium

It has been shown that monocytic cell infiltration across the BBB augments the resident microglial cell population of the AD brain due to differentiation of the infiltrating monocytes into microglia [109]. Our previous studies provide strong evidence that $P2Y_2R$s in endothelial cells regulate the binding and the transendothelial migration (i.e., diapedesis) of monocytic cells. As described above, $P2Y_2R$s mediate the Src-dependent transactivation of the vascular endothelial growth factor receptor-2 (VEGFR-2) in endothelial cells that promotes upregulation of monocyte-binding proteins (e.g., VCAM-1) and a decrease in endothelial adherens junction integrity [22, 89, 92, 101, 102]. Other studies have shown that microglia are attracted to and surround Aβ plaques in both human AD brain and rodent transgenic models that develop AD-like symptoms [228–237]. Although the role of microglial cells in AD (i.e., neurotoxic vs. neuroprotective) is controversial, recent work has shown that the majority of microglia that surround amyloid plaques in an AD mouse model are derived from monocytes originating in bone marrow [233, 238] and thus must pass from the bone marrow into the bloodstream through the vasculature and across the BBB to reach sites of plaque formation in the brain. Furthermore, these bone marrow-derived microglia (to a greater extent than resident microglia in the brain) were shown to eliminate Aβ deposits by phagocytosis in AD mice [238], strongly suggesting that bone marrow-derived microglia serve a neuroprotective role in restricting AD progression. Previous in vivo work by us and others indicates that the $P2Y_2R$ is important for the recruitment of leukocytes (monocytes, neutrophils, and eosinophils) to sites of sterile surgical injury [101] and tissue infected with allergens or bacteria [239–241]. The process of leukocyte recruitment involves several steps: the emigration of leukocytes from bone marrow into the circulation, adhesion of circulating leukocytes to vascular endothelial cells, and diapedesis of leukocytes towards chemoattractants released at the site of injury or infection. Although the leukocyte $P2Y_2R$ is important for controlling the leukocyte migration step [143, 239, 240], other studies indicate that the endothelial $P2Y_2R$ promotes both the leukocyte adhesion step, by increasing the expression of VCAM-1 in endothelial cells [22, 89, 92] and the diapedesis step [102]. A postulated pathway for the regulation of leukocyte diapedesis by the $P2Y_2R$ is shown in Fig. 4.

It is known that signal transduction in endothelial cells (e.g., increases in $[Ca^{2+}]_i$, phosphorylation of myosin light chain, and RhoA activation) occurs in response to adhesion of activated leukocytes and that these events are required for leukocyte diapedesis [242, 243]. However, mechanisms by which endothelial cells promote leukocyte diapedesis are less clear. In a recent in vitro diapedesis study, expression of the endothelial $P2Y_2R$ was found to be important for the transendothelial migration of neutrophils toward lipopolysaccharide (LPS), a chemoattractive component of gram-negative bacteria [102]. This study also demonstrated that

Table 3 Role of the $P2Y_2R$ in proinflammatory cytokine release

	$oAβ_{1–42}$ (1 μM)	TNF-α (pg/ml)	IL-1β (pg/ml)
Control	–	20	28
Wild type	+	95**	115**
$P2Y_2R^{-/-}$	+	35	41

WT and $P2Y_2R^{-/-}$ mouse primary microglial cells were treated with $oAβ_{1–42}$ (1 μM), incubated for 24 h, and supernatants were collected and analyzed for TNF-α and IL-1β by ELISA. Data represent means ± SEM (n=3)

**p<0.01 indicates a significant difference from untreated control

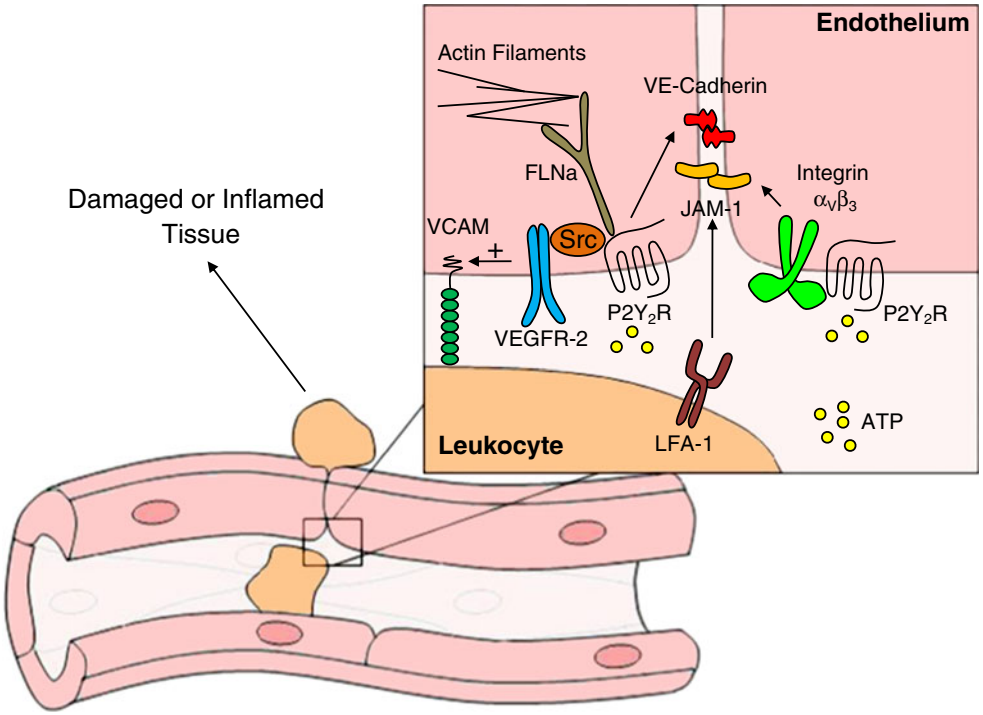

Fig. 4 Proposed role of the endothelial $P2Y_2R$ in paracellular leukocyte diapedesis. Tissue damage or infection causes the local release of inflammatory signals that upregulate the endothelial $P2Y_2R$ as well as adhesion molecules required for leukocyte adhesion to vascular endothelium. These leukocytes begin migrating toward chemoattractants released from the damaged/infected site and, in so doing, release a burst of ATP from their leading edge [239]. We hypothesize that ATP released from adherent leukocytes activates the endothelial $P2Y_2R$ causing trafficking or relocation of this receptor to endothelial adherens junctions, possibly through interaction of the $P2Y_2R$ with Src, VEGFR-2, and the actin-binding protein, filamin A [22, 89, 100]. We also hypothesize that endothelial $P2Y_2R$ relocation to adherens junctions assists in the transient disruption of endothelial junctions and escorts leukocytes to endothelial borders for paracellular diapedesis, possibly through association of the $P2Y_2R$ with the $\alpha_V\beta_3$ integrin. This integrin is known to form a complex with the $P2Y_2R$ [23] and with JAM-1 [276], a junctional adhesion molecule in endothelial cells that interacts with the leukocyte integrin, LFA-1, and is important for both leukocyte adhesion and diapedesis [277]

diapedesis of neutrophils toward LPS required Rho kinase activity and was potentiated by treatment with UTP [102]. Nucleotides are released from leukocytes migrating toward chemoattractants [239], including $A\beta_{1-42}$ [224], and increased expression of $P2Y_2Rs$ in vascular endothelium of damaged tissue [244] regulates nucleotide-induced increases in $[Ca^{2+}]_i$, phosphorylation of myosin light chain, and activation of Rho kinases [24, 95]. Thus, it seems plausible that the $P2Y_2R$ in microvascular endothelium of the brain should contribute to monocyte binding, diapedesis, and accumulation of bone marrow-derived microglia around brain tissue burdened with Aβ plaques whereupon the loss of this mechanism should limit Aβ clearance and proinflammatory neuroprotection in the AD brain.

Leukocyte diapedesis and the increase in microvascular permeability to macromolecules that occurs during an inflammatory event are controlled by the formation and dissociation of endothelial cell adhesion structures comprised of adherens junctions, tight junctions, and gap junctions [245]. It is well-known that Rho family GTPases regulate the endothelial permeability barrier by affecting the stability of these junctional structures [246]. For example, endothelial Rho GTPase and Rho kinase promote the sealing of intercellular junctions by controlling phosphorylation of myosin light chain [247], whereas dominant negative Rac enhances thrombin-induced permeability of macromolecules [248]. Many compounds, including thrombin, VEGF, TNF-α, and histamine, have been found to alter the endothelial permeability barrier due to modulation of the activities of Rho GTPases and protein distribution in adherens junctions [249–256]. Extracellular nucleotides also have been shown to play a role in regulating blood vessel permeability properties [257, 258], and the $P2Y_2R$, in particular, has been shown to stimulate leukocyte recruitment [101, 239] and Rho GTPase activity through a mechanism involving $P2Y_2R$ interaction with $\alpha_v\beta_{3/5}$ integrins [24, 95]. In addition, the endothelial $P2Y_2R$ has been linked to other proinflammatory responses, including vasodilation of rat cerebral arteries through a Ca^{2+}-dependent mechanism involving the production of nitric oxide and endothelium-derived hyperpolarizing factor [259]; production of prostacyclin, an effective vasodilator and inhibitor of platelet

activation [73]; and the upregulation of tissue factor, an initiator of platelet aggregation [260]. Recently, we found that activation of the $P2Y_2R$ in human coronary artery endothelial cells causes a rapid translocation of the receptor to cell–cell junctional zones where it interacts with VE-cadherin (unpublished results), a protein found specifically in endothelial adherens junctions that is important for maintaining the vascular permeability barrier. Furthermore, we found that luminal application of UTP increases microvascular permeability of albumin in wild type, but not $P2Y_2R^{-/-}$, mice (unpublished results), indicating that the $P2Y_2R$ controls the microvascular barrier function in vivo. Therefore, we hypothesize that the endothelial $P2Y_2R$ assists in monocyte diapedesis by interacting with VE-cadherin and disrupting endothelial junctions localized at the site of leukocyte passage. VE-cadherin has been well recognized for its role in regulating the endothelial permeability barrier and leukocyte recruitment [261–267]. The N-terminal extracellular domain of VE-cadherin provides tight adhesion between endothelial cells through Ca^{2+}-dependent homophilic interaction, whereas the cytoplasmic domain interacts with various intracellular binding partners, including α-, β-, and p120 catenins, providing a linkage to the actin cytoskeleton [268]. Modulation of cell–cell contacts that regulate cell adhesion and cell motility likely requires interactions between cadherins and catenins, and it has been shown that p120 catenin regulates actin cytoskeletal organization and cell motility by activation of Rho GTPases [269–271]. In addition, VE-cadherin associates with VEGFR-2, known to be transactivated by the $P2Y_2R$ [20, 89], and also with Src, Shc, Csk [272, 273], and the vascular endothelial protein tyrosine phosphatase, VE-PTP [274]. VEGFR-2 activation in endothelial cells has been shown to stimulate the tyrosine phosphorylation of VE-cadherin, β- and p120-catenins, plakoglobin, and PECAM-1 [254]. These interactions may be important for regulating cell–cell contacts, cell adhesion, and growth factor signaling [275].

Conclusion

This review evaluates the role of $P2Y_2Rs$ in the CNS with an emphasis on brain functions. $P2Y_2Rs$ are expressed in glial cells (i.e., astrocytes and microglia), neurons, and endothelium, primary cell types comprising the CNS. The $P2Y_2R$ has been shown to play a role in the activation of astrocytes and microglia and the phagocytosis of apoptotic cell debris. $P2Y_2R$ expression is upregulated under proinflammatory conditions in neurons and glial cells. The G_q-coupled $P2Y_2R$ has structural motifs that have been shown to facilitate interactions with growth factor receptors, integrins, and filamin A that activate signaling pathways beyond those regulated by G_q protein activation alone. Results indicate that under proinflammatory conditions associated with neurodegenerative diseases, such as AD, the release of cytokines, including IL-1β, upregulates $P2Y_2R$ expression through activation of NF-κB and its binding to the *P2Y2R* promoter. Upregulation of the $P2Y_2R$ by proinflammatory cytokines in neurons enables $P2Y_2R$ activation to promote neuroprotective responses, such as the metalloprotease-dependent non-amyloidogenic processing of APP and integrin-dependent neurite outgrowth. In addition, activation of $P2Y_2Rs$ expressed in glial cells can increase cell migration and phagocytosis and degradation of neurotoxic Aβ. Furthermore, $P2Y_2Rs$ in endothelium promote the binding of monocytes and their diapedesis, which is postulated to increase the neuroprotective microglial cell population in the brain. Taken together, current research suggests that the $P2Y_2R$ plays a neuroprotective role during inflammation in the CNS and indicates mechanisms that should be further investigated as promising targets for the treatment of neurodegenerative diseases, including AD.

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