

Purinergic modulation of microglial cell activation

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Abstract Microglial cells are resident macrophages in the brain and their activation is an important part of the brain immune response and the pathology of the major CNS diseases. Microglial activation is triggered by pathological signals and is characterized by morphological changes, proliferation, phagocytosis and the secretion of various cytokines and inflammatory mediators, which could be both destructive and protective for the nervous tissue. Purines are one of the most important mediators which regulate different aspects of microglial function. They could be released to the extracellular space from neurons, astrocytes and from the microglia itself, upon physiological neuronal activity and in response to pathological stimuli and cellular damage. Microglial activation is regulated by various subtypes of nucleotide (P2X, P2Y) and adenosine (A₁, A_{2A} and A₃) receptors, which control ionic conductances, membrane potential, gene transcription, the production of inflammatory mediators and cell survival. Among them, the role of P2X₇ receptors is especially well delineated, but P2X₄, various P2Y, A₁, A_{2A} and A₃ receptors also powerfully participate in the microglial response. The pathological role of microglial purine receptors has also been demonstrated in disease models; e.g., in ischemia, sclerosis multiplex and neuropathic pain. Due to their upregulation and selective activation under pathological

conditions, they provide new avenues in the treatment of neurodegenerative and neuroinflammatory illnesses.

Key words adenosine receptors · ATP · microglia · neurodegeneration · P2X₇ receptor · P2Y receptors

Abbreviations

AD	Alzheimer's disease
A β	amyloid- β peptide
APP	amyloid precursor protein
ALS	amyotrophic lateral sclerosis
2-AG	2-arachydonoylglycerate
ABC	ATP binding cassette
LPS	bacterial lipopolisaccharide
BzATP	2', 3'-O-(4-benzoyl-benzoyl)adenosine 5'-triphosphate
CNT	concentrative nucleoside transporter
COX-2	cyclooxygenase-2
E-NTPDase	ectonucleoside triphosphate diphosphohydrolase
E-NPP	ecto-nucleotide pyrophosphatase
ENT	equilibrative nucleoside transporter
EAE	experimental autoimmune encephalomyelitis
ERK	extracellular signal regulated protein kinase
GFAP	glial fibrillary acidic protein
iNOS	inducible nitric oxide synthase
IL-1 β	interleukin-1 β
ICE	interleukin-1 convertase enzyme
IL-6	interleukin-6
MRF-1	microglial response factor-1
MCAO	middle cerebral artery occlusion
MAPK	mitogen activated protein kinase
NGF	nerve growth factor
NO	nitric oxide
NFAT	nuclear factor of activated T cells

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PD	Parkinson's disease
PI3K	phosphatidylinositol-3'-kinase
PLC	phospholipase C
PPADS	pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid
ROI	reactive oxygen intermediates
SM	sclerosis multiplex

Introduction

Microglial cells originate from monocyte/macrophage precursors and are regarded as the major immunocompetent cell type of the nervous system, constituting about 10% of all cells in the brain. The immune response of the brain is spatially segregated from the peripheral immune response by the blood brain barrier and together with astroglial cells and infiltrating peripheral immune cells, is predominantly executed by microglial cells. Thus, this cell type is rapidly activated in response to pathological signals such as ischemia or bacterial endotoxin and respond with morphological changes transforming the resting ramified microglia to an amoeboid form with phagocytic activity, proliferation and the production of a wide array of inflammatory mediators. Although microglial activation is a highly complex process, consisting of a number of interrelated extra- and intracellular pathways, it is a rather uniform response, which is triggered by any environmental challenge, which affects the functional integrity of the nervous tissue. Therefore, microglial activation is heavily implicated in the pathogenesis of virtually all CNS diseases and the following repair process, including brain and spinal cord injury, stroke, Alzheimer's (AD) and Parkinson's disease (PD), sclerosis multiplex (SM), amyotrophic lateral sclerosis (ALS) and sensory neuropathies. Apart from cytokines, growth factors, and other bioactive substances, purines are one of the most important mediators which regulate and orchestrate various aspects of microglial activation by the interaction of numerous subtypes of adenosine (P1) and nucleotide (P2) receptors.

Purine bioavailability under conditions leading to microglial activation

The participation of purines in the microglial response presumes their accumulation in the extracellular space upon signals activating microglia. Extracellular purine availability in the nervous system is basically determined by the balance of release, and removal by enzymatic degradation and uptake. Whereas ATP is released per se, adenosine is thought to be generated in the extracellular space as a breakdown product of released ATP, but it could be also

released on its own right, depending on the type of stimulus. Since ATP is ubiquitous, all metabolically active cells of the nervous system are able to synthesize ATP, which provides a potential pool for release. Therefore, the cellular source of released purines participating in microglial activation could be any cell type located in contact with microglia, i.e., nerve terminals, astrocytes, endothelial tissue and finally the microglial cells themselves as well. The majority of ATP is formed in the mitochondria by oxidative phosphorylation, which results in approximately 10 mM ATP concentration in the cytoplasm under normal metabolic conditions. In addition, ATP is also taken up and stored in synaptic vesicles of nerve terminals [1] and astrocytes [2]. On the other hand, the basal intracellular adenosine concentration is much less, in the low micromolar to high nanomolar range, and the majority of adenosine, which is taken up into cells is rapidly reincorporated into ATP stores, or deaminated by adenosine deaminase under normal metabolic conditions [3].

A wide variety of stimuli are known to release ATP and/or adenosine to the extracellular space, which theoretically could lead to purine levels sufficiently high to activate nucleotide receptors expressed on the surface of microglia (Fig. 1) [1, 3, 4]. Although the stimulation-dependent release of ATP and adenosine upon conventional [5, 6] and high frequency [7] neuronal activity is well documented, these stimuli probably result in a spatially restricted, localized increase in extracellular purine levels, which serve the synaptic transmission and the modulation of pre- and postsynaptic functions within the synaptic cleft. ATP-metabolizing ectoenzymes, present on the nerve terminal membrane, but also on the surface of microglia [8], such as ectoNTPDases, the CD73/ecto-5'-nucleotidase [9] and nucleoside transporters may strongly limit purine availability under these conditions. On the other hand, pathological events such as mechanical or metabolic stress, inflammation, cellular injury or changes in the ionic environment are known also to powerfully stimulate purine release. This might result in a purine-rich extracellular milieu leading to a more widespread activation of receptors reaching also the neighboring or distant cells such as astrocytes and microglia as well. These signals include mechanical [2, 10, 11] and hypotonic [12] stimuli, hypoxia/hypoglycemia/ischemia and consequent energy deprivation [13–17], inflammatory signals, such as bacterial lipopolysaccharide (LPS) [18, 19], concanavalin-A [20], or interleukin-1 β (IL-1 β) [21], and cytolysis, which could release purines not only from neurons but also from non-neuronal cells, i.e., from astrocytes, endothelial cells and from the microglia itself. Moreover, nucleotides and nucleosides by themselves may promote further release of purines, by a homo- or heteroexchange mechanism, if they reach relatively high concentration in the extracellular space [22].

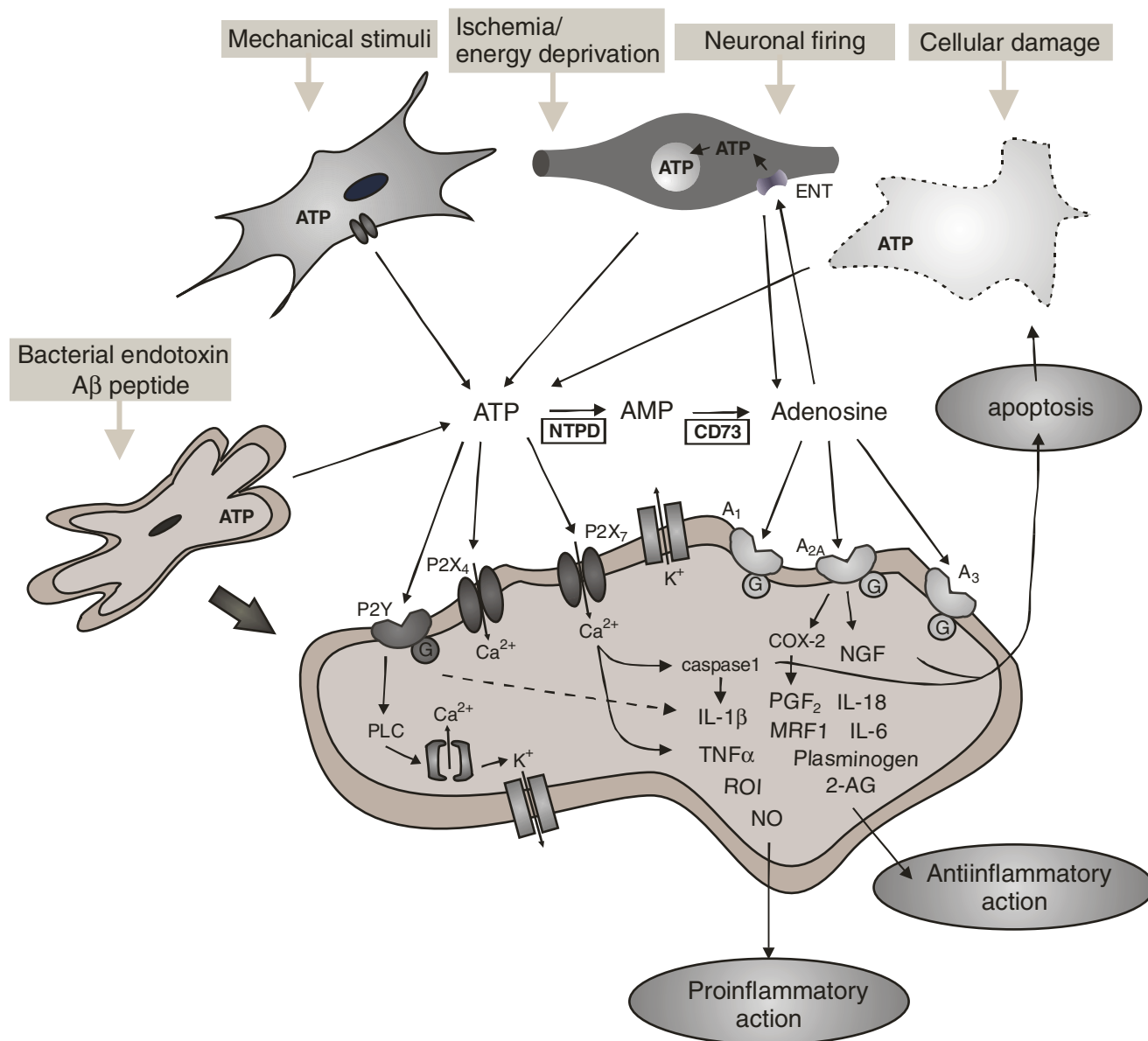


Fig. 1 A summary of purinergic pathways involved in microglial response. Since ATP is ubiquitous, it is present in the cytoplasm of nerve terminals, astrocytes, and the microglia itself. It is released to the extracellular space in response to a wide variety of signals, including neuronal firing, mechanical stimuli, ischemia/energy deprivation, bacterial endotoxin and cellular damage. Adenosine could be either released from cells, or generated from extracellular ATP via breakdown by NTPDase (*NTPD*) and CD73/5'-nucleotidase (*CD73*) enzymes. The action of adenosine is terminated by its uptake into nerve terminals by the equilibrative nucleoside transporter system (*ENT*), and the same transporters could mediate the release of adenosine under energy deprivation. ATP acts on ionotropic P2X₇ and P2X₄ receptors, and also on metabotropic P2Y receptors, whereas adenosine activates metabotropic A₁, A_{2A} and A₃ receptors, all present on the surface of activated microglia. Whilst the activation of P2X receptors triggers an inward cationic current and depolarizes the microglial membrane, the activation of P2Y receptors is coupled to the G protein-

phospholipase C (*PLC*) signal transduction pathway, both resulting in the elevation of intracellular Ca²⁺. P2X receptors are involved in the expression, posttranslational processing and secretion of various factors shaping the microglial response—i.e., IL-1β, IL-6, IL-18, TNF-α, reactive oxygen intermediates (*ROI*), plasminogen, 2-arachydonoyl glycerate (*2-AG*), and microglial response factor-1 (*MRF-1*)—and thereby contribute to both the proinflammatory and anti-inflammatory aspects of microglial activation. In addition, P2X₇ receptors eventually also mediate apoptosis by caspase 1 activation. The stimulation of P2Y receptors hyperpolarizes the microglia, via an outward K⁺ conductance and inhibits the production of proinflammatory mediators. Adenosine receptors regulate the proliferation/survival of microglia, COX-2 expression and subsequent secretion of PGF₂, and the production of nerve growth factor (*NGF*). For more details and references, see text. For the sake of clarity, details of intracellular signal transduction and transcriptional changes are not illustrated

Since ATP is a highly polarized molecule, which cannot pass freely through the cell membrane, it is also of interest to identify the mechanism, whereby it could enter the extracellular space. These include (1) vesicular exocytosis, (2) carrier-mediated release, (3) release through channels and membrane pores, (4) cytolitic release.

- (1) Vesicular exocytosis is a prototype mechanism for neurotransmitters to enter the extracellular space, which is expected to be a $[Ca^{2+}]_o$ -dependent process. Indeed, $[Ca^{2+}]_o$ -dependent ATP and adenosine release in response to neuronal stimulation appears in many areas of the central and peripheral nervous system (for further references see [1, 3, 4]). Moreover, recent findings indicate that vesicular ATP release could be derived not only from nerve terminals but also from astrocytes [2].
- (2) Although specific transporters capable for the transmembrane movement of ATP are yet to be molecularly identified in the nervous system, ABC (ATP binding cassette) proteins have been implicated as ATP transporters [12, 23, 24] in non-neuronal cells. These transporters are also expressed in glial cells [25] and mediate ATP release upon hypoosmotic challenge [26, 27]. On the other hand, transmembrane transporters for adenosine and other nucleosides are widely expressed in the nervous system. Specific nucleoside transporters consist of two families: equilibrative transporters (ENT) and concentrative transporters (CNT); the former is driven by the concentration gradient, the latter is driven by the sodium gradient [28, 29]. ENT transporters could carry different nucleosides, including adenosine and inosine, but not nucleotides, across the cell membrane in both directions, and they are regarded as the dominant nucleoside transporters of the brain. Since the intracellular adenosine level under normal metabolic state is in the low micromolar range [3], if the ENT transporter is loaded from the extracellular space by excess adenosine, it mediates adenosine uptake into the nerve terminals. When adenosine is taken up, intracellular adenosine eliminating mechanisms, the adenosine kinase and adenosine deaminase enzymes convert it to AMP and inosine, respectively, thereby maintaining the driving force of the carrier. However, the ENT transporter could also act in a reverse direction under certain circumstances, mediating the release of adenosine into the extracellular space. This could occur during energy deprivation or metabolic distress, when ATP stores are depleted and AMP is generated intracellularly. Cytosolic 5'-nucleotidase, which has a relatively high K_m for AMP (1–14 μM), becomes active under these conditions and accumulates aden-

osine intracellularly. This adenosine then flows out to the extracellular space in a transporter-mediated manner [3, 30].

- (3) Channels and pores are also potential candidates to drive the transmembrane movement of ATP. Connexin hemichannels are gap junction proteins, and their main function is to mediate electrical signaling, but they are also able to release neuroactive substances such as glutamate and ATP [31]. They have been recently identified to mediate ATP release from astrocytes and other non-neuronal cells in response to mechanical stress [32–35].
- (4) Although it has only recently received direct experimental proof [36], any kind of cellular injury is generally thought to result in high local ATP concentrations in the extracellular space. In this case the millimolar cytoplasmic ATP is expected to flow out to the extracellular space through the membrane damage.

Apart from release, the other major factor determining purine bioavailability is their removal from the extracellular space by enzymatic degradation and transporters. Several enzyme families are responsible for the extracellular degradation of ATP in the nervous system. The first step of the inactivation of ATP is mediated by the family of ectonucleoside triphosphate diphospho-hydrolases (E-NTPDases, EC 3.6.1.5, also known as ectoATPase or apyrase), which are able to hydrolyse ATP and ADP to AMP [9]. These enzymes show widespread distribution in the brain [37, 38] and have low micromolar K_m for ATP and ADP giving rise to rapid and highly effective hydrolysis of ATP in almost all neuronal tissues. In addition to the E-NTPDase family, ATP could also be dephosphorylated by ecto-nucleotide pyrophosphatases (E-NPPs) and by alkaline phosphatases, both having broader substrate specificity, but also widespread tissue distribution [9].

The next step of extracellular inactivation is the hydrolysis of AMP by the ecto-5'-nucleotidase (EC 3.1.3.5) enzyme, which is the rate-limiting step giving rise to the formation of adenosine, which is a new extracellular signal, acting on its own receptors. Ecto-5'-nucleotidase exhibits micromolar K_m for AMP and is feed-forwardly inhibited by ATP, which results in a delayed, 'burst-like' adenosine production [30]. It is also widely present in the brain, and it is predominantly associated to glial cells [39, 40], although its expression has been also demonstrated in purified nerve terminals [41, 42]. Finally, adenosine, generated by the ecto-5'-nucleotidase, or released on its own right, is then taken up to the nerve terminal by specific nucleoside transporters (see above) and rapidly reincorporated to ATP stores, or deaminated extra- or intracellularly by the adenosine deaminase enzyme.

Modulation of microglial activation by P2 receptors

It has been known for a considerable time that microglial cells respond with both ionotropic and metabotropic actions to ATP application [43–47]. Whereas the activation of ligand-gated P2X receptors produces depolarization and Ca^{2+} influx through the receptor-ion channel complex, the stimulation of the G protein coupled P2Y receptors elicits hyperpolarization via phospholipase C (PLC)- and IP_3 -mediated mobilization of Ca^{2+} from intracellular stores and subsequent opening of K^+ channels (Fig. 1) [47]. Now it is confirmed that all members of the P2 receptor family are expressed on resting and activated microglial cells kept in culture at the mRNA and/or protein level [48, 49].

Among various subtypes of the ionotropic P2 receptors, the role of P2X₇ receptors in the microglial response is especially well delineated. Within the P2X receptor family, the P2X₇ receptor has a distinguished role for several reasons. First, this receptor seems to function only in homooligomeric form, and is activated by relatively high concentrations of ATP. Second, this receptor was also known previously as P2z receptor, the ‘cell death receptor’; its prolonged activation elicits the opening of a transmembrane pore, permeable to large molecular weight molecules up to 800 Da, which finally leads to cellular death. Last but not least, in spite of being cloned originally from the rat brain, it was suggested that P2X₇ receptors are expressed predominantly on antigen-presenting immune cells and epithelia, and function as immunomodulatory receptors [50, 51]. Supporting this concept, a wealth of data confirm that P2X₇ receptors regulate many aspects of immune response in the periphery [52], but this holds true for microglial activation as well [53].

The presence of the ‘pore-forming’ ATP receptor on microglial cells has already been described before the molecular identification of P2X₇ receptors [54, 55]. The expression of P2X₇ receptor transcripts and of the corresponding protein have been confirmed later in both cultured microglial cells [51, 56, 57] and in situ microglial cells activated following middle cerebral artery occlusion [51].

Primary microglial cultures and immortalized microglial cell lines respond to ATP and 2',3'-O-(4-benzoyl-benzoyl) adenosine 5'-triphosphate (BzATP) application with an inward current [54, 58, 59], membrane depolarization, a sustained increase in intracellular free Ca^{2+} [55], the uptake of high molecular weight fluorescent dyes [55, 58], and the secretion of IL-1 β upon an LPS stimulus [55, 60, 61], with a pharmacological profile resembling that of P2X₇ receptors. The central role of P2X₇ receptors, as co-stimulators of the posttranslational processing of IL-1 β in microglial cells upon LPS challenge has been repeatedly proven [19, 61,

62]. The mechanism underlying ATP dependent IL-1 β maturation and release in this cell type involves an outwardly directed K^+ conductance and the activation of interleukin-1 convertase enzyme (ICE, also known as caspase 1) responsible for the cleavage of pro-IL1 β to the mature, 17-kDa form [61]. This mechanism appears to participate not only in the exogenous but also in the endogenous activation of P2X₇ receptors, since LPS releases ATP from microglial cells, and the P2X₇ receptor selective antagonist oxiATP prevents the LPS-induced IL-1 β release [19].

Interestingly, ADP and AMP also act as agonists of P2X₇ receptors in microglial cells in terms of membrane currents and LPS induced IL-1 β secretion, but only after ‘priming’ of the cells by ATP challenge [63]. The molecular mechanism underlying the priming effect of ATP could be a reversible conformational change leading to the modification of the agonist binding motif, or the gating properties of the P2X₇ receptor, or its activity-dependent phosphorylation. This priming effect, on the other hand, does not extend to the pore-forming property of recombinant and microglial P2X₇ receptors [63].

In addition to IL-1 β , the synthesis and release of other cytokines, are also stimulated by P2X₇ receptor activation in the microglia. Hence, ATP is a full stimulus (i.e., without the requirement of priming by LPS) to induce TNF α production via a Ca^{2+} -dependent, extracellular signal regulated protein kinase (ERK)/JNK/p38 signaling pathway [64, 65], although P2 receptors other than P2X₇ may also participate in ATP-induced ERK activation [65]. On the other hand, involvement of P2X₇ receptors in the regulation of the production of the antiinflammatory cytokine, interleukin-6 (IL-6) is more controversial. Whereas Inoue et al. [66, 67] reported that ATP-induced IL-6 production is not mediated by P2X₇ receptors, a recent study found markedly elevated levels of IL-6 in the inflamed hindpaw of P2X₇^{-/-} mice [68], implicating the participation of this receptor in shaping IL-6 levels. Moreover, Rampe et al. [69] reported that P2X₇ receptors play a role in the distinct modulation of cytokine secretory pathways not only after LPS, but also upon amyloid beta peptide (A β) pre-activation. Whereas the production of IL-1 β , IL-1 α , TNF α and IL-18 was increased, that of IL-6, the anti-inflammatory cytokine was attenuated under these conditions, implicating the involvement of P2X₇ receptors in the pathogenesis of Alzheimer's disease [69].

High concentrations of ATP induce inducible nitric oxide synthase (iNOS) mRNA expression and increase nitric oxide (NO) production from rat microglia [70], an effect potentially mediated by P2X₇ receptors. In contrast, ATP by itself does not induce iNOS expression, but enhances IFN γ -induced iNOS expression and subsequent NO production in the murine BV-2 microglial cell line through an ERK1/2

and tyrosine kinase mediated pathway [56]. ATP and BzATP also promote the generation of reactive oxygen intermediates (ROI), in particular superoxide, by interacting with P2X₇ receptors, in a way depending on extracellular Ca²⁺ and the p38 mitogen activated protein kinase (MAPK) pathway [71]. The stimulation of P2X₇ receptors elicits a pronounced increase in the secretion of the endocannabinoid, 2-arachidonoylglycerate (2-AG) in primary microglial cell cultures by the activation of diacylglycerol lipase and the simultaneous inhibition of monoacylglycerol lipase, the enzyme responsible for endocannabinoid degradation [72]. This mechanism is noteworthy, because endocannabinoids are also recognized as key regulators of microglial activation [73]. Finally, activation of P2X₇ receptors by low concentration of agonists stimulates the release of the neuroprotective mediator plasminogen from cultured microglia [74]. Therefore, regulation of the production of putatively protective (plasminogen, TNF α , 2-AG) and harmful (IL-1 β , NO) mediators by P2X₇ receptors appears to follow a highly time- and concentration-dependent pattern [67].

In addition to its role to regulate the production of inflammatory mediators, the activation of P2X₇ receptors elicits changes in microglia at the transcriptional level; it rapidly activates the transcription factor nuclear factor of activated T cells (NFAT) in a [Ca²⁺]_o-dependent manner [75] as well as causes the nuclear translocation of NF- κ B via ROIs and caspase activation leading to the transcription of a subset of NF- κ B target genes [76]. The expression of other transcription factors is regulated time-dependently upon P2X₇ receptor activation. While an acute exposition (10 min to 1 h) of microglial cells to ATP and BzATP upregulates the production of the inflammation related protein microglial response factor (MRF)-1 [77], long-term (6 h) exposure suppresses its transcription and synthesis [78]. Moreover, a conditioned medium from cerebellar granule cells undergoing apoptosis also upregulated MRF-1 release in a P2X₇ receptor-mediated manner, indicating that this mechanism plays a role in the neuron-microglia cross-talk during microglial activation in response to apoptotic signals [78].

According to its pore-forming property, the sustained activation of P2X₇ receptors leads to cytolysis in an apoptotic fashion in the microglia [57]. The P2X₇ receptor-mediated apoptosis involves the activation of the proteolytic pathway of the caspase activation, which leads to nuclear DNA damage, but is not an absolute requirement for the membrane damage and cytolysis; i.e., if caspases are inhibited, cell death proceeds through the necrotic pathway [79]. Nevertheless, the P2X₇ receptor activated IL-1 β secretion and cell death, although both processes involve caspases and both are eliminated in P2X₇^{-/-} mice, appear to be independent from each other [62].

Finally, P2X₇ receptor activation also plays an important role in Ca²⁺ signaling between astrocytes and microglial cells [11]. Astrocyte populations coordinate their functions via Ca²⁺ waves, and the spread of the Ca²⁺ signal is implemented by two ways: an intercellular pathway mediated by gap junctions, and an extracellular pathway mediated by ATP and P2 receptors [80]. Moreover, astrocytes communicate by calcium-mediated signaling not only with each other but also with neighboring cells, including neurons and microglia. Thus, astrocyte-derived ATP activates P2X₇ receptors on microglial cells and elicits Ca²⁺ signals in the microglia, a process which eventually leads to cytolysis of this cell type [11]. It is interesting to note that P2X₇ receptor-activation in astrocytes usually does not lead to cytolysis, whilst the same effect might cause cellular death in microglia. The reason for the different resistance of astrocytes and microglia against the P2X₇ receptor mediated cytolysis is unknown.

The widespread and profound effects of P2X₇ receptor activation on different aspects of microglial activation implicates the role of these receptors in the pathology of CNS and PNS diseases and provokes their applicability as therapeutic targets. Indeed, rapidly emerging knowledge supports such a role, in particular the demonstration of (1) the activity-dependent expression of P2X₇ receptors under pathological conditions, and (2) the protective role of P2X₇ receptor ligands in animal disease models. The diseases, in which microglial P2X₇ receptors may play either a harmful or protective function include neurodegenerative and neuro-inflammatory diseases, such as ischemia-reperfusion and traumatic injury, Alzheimer's disease, sclerosis multiplex, neuro- and retinopathies. In fact, the upregulation of microglial P2X₇ receptors has been observed in several pathological models, including in vivo ischemia [81], a transgenic model of Alzheimer's disease [71], as well as in human tissue samples obtained from patients suffering in proliferative vitreoretinopathy [82], and sensory nerve injury [68].

The first study showing an upregulation of P2X₇ receptors in the brain was that of Collo et al. [51], who found an increased immunostaining for P2X₇ receptors in activated microglial cells of the zona penumbra in the middle cerebral artery occlusion (MCAO) model. The microglial upregulation of P2X₇ receptors in response to oxygen deprivation has also been documented in the retinal microglia [83]. However, the ischemia-induced upregulation of P2X₇ receptors appears in different cell types in a temporally distinct manner. In a more recent study, an early (1 day after MCAO occlusion) upregulation of the P2X₇ receptor protein was observed in microglial cells, and later (4–7 days after MCAO occlusion), the receptor was also overexpressed in neurons and astrocytes of the periinfarct area in spontaneously hypertensive rats, as revealed by co-

localization studies with neuronal, astroglial and microglial markers [81]. P2X₇ receptor overexpression is also associated with microglial cells in other pathological models; e.g., P2X₇ receptor immunoreactivity appears to be upregulated around amyloid plaques in activated microglia and astrocytes in the Tg2576 transgenic mice having mutant amyloid precursor protein (APP) [71].

Altogether, these data indicate that the expression of P2X₇ receptors is strongly activity-dependent during pathological situations. Nonetheless, whether the change in their expression pattern and functional responsiveness is a simple adaptive change or plays a more active pathogenetic role, warrants further investigation. It is also a largely open question whether the final outcome of versatile actions mediated by microglial P2X₇ receptors is protective or harmful. As an example, P2X₇ receptor deficient mice show increased susceptibility to experimental autoimmune encephalomyelitis (EAE), an animal model of sclerosis multiplex [84], which is potentially due to the decreased microglial secretion of the protective endocannabinoid 2-AG [72]. On the other hand, chronic inflammatory and neuropathic pain is almost completely abolished in the same mice line, which is attributed partly to disrupted microglial production of the inflammatory cytokine IL-1 β [68]. Here we have to mention that in addition to those present on the surface of microglial cells, P2X₇ receptors on other cell types (i.e., on astroglia and neurons) may also contribute to disease pathology.

In addition to P2X₇ receptors, other subtypes of the P2 receptor family, responding to lower concentration of ATP, are also involved in different aspects of microglial activation [85]. Thus, microglial Ca²⁺ influx could be also initiated by lower agonist concentrations, effects, which are associated to a non-P2X₇ ionotropic, probably P2X₄ receptor activation, and there is also a metabotropic, P2Y receptor mediated response [59, 85]. The expression of P2X₄ receptors is enhanced in the spinal cord microglia after peripheral nerve injury [86, 87], and this receptor and the subsequent activation of the p38 MAP kinase pathway, have been shown to be involved in the generation of inflammatory and neuropathic pain [88, 89].

Early studies showed that exposure of microglial cells to ATP leads to the accumulation of immediate early gene products, c-fos, c-jun, junB and TIS11, effects most likely mediated by P2Y receptor activation [90]. P2Y₁ receptors mediate the inhibition of the LPS induced IL-1 β , and TNF- α production [91]. The level of proinflammatory cytokines therefore seems to be differentially and oppositely regulated by ionotropic and metabotropic P2 receptors. The production of the anti-inflammatory cytokine IL-6 upon LPS challenge is inhibited by P2Y receptors [91], although the involvement of pertussin toxin insensitive P2Y receptors in the induction of IL-6 production has also been reported [66]. In addition, stimulation of a G_i protein coupled,

P2Y₁₂-like receptor induces membrane ruffling and chemotaxis in cultured primary microglia [92]. Finally, a recent study showed that the activation of P2Y but not P2X receptors induces cyclooxygenase-2 (COX-2) expression in the human microglia [93].

In contrast to the plethora of experimental data obtained in glial cells kept in culture, much less data have been accumulated on the role of glial P2 receptors in more integrated systems, where the cell architecture and extracellular environment are retained. Nonselective cationic membrane currents could be recorded from identified resting microglial cells in acute brain slices in response to ATP (1 mM) and BzATP (0.2 mM) application, which presumably reflects the activation of P2X₇ receptors, although the underlying receptor subtype has not been identified pharmacologically [94]. BzATP also activates microglial P2X₇ receptors in the isolated rat inner retina, which leads to the permeabilization of this cell type [95]. Moreover, intrastriatal administration of ATP promotes cell death in the striatum *in vivo*, which extends to both neurons, astrocytes and microglial cells, as confirmed by immunostaining by cell-type specific markers NeuN, glial fibrillary acidic protein (GFAP) and OX-42, respectively [96]. However, this effect was mimicked by α,β -methylene-ATP and ATP γ S, and antagonized by reactive blue 2, but not by pyridoxal-phosphate-6-azophenyl-2',4'-disulphonic acid (PPADS), and therefore is mediated by an unidentified subtype of P2 receptors.

Modulation of microglial activation by adenosine receptors

Although relatively limited data have been accumulated on the adenosinergic modulation, microglial cells undoubtedly express functional A₁, A_{2A} and A₃ but not A_{2B} adenosine receptors [97], which regulate different aspects of the microglial response [98].

The pivotal role of A₁ adenosine receptors in neuroinflammation is underscored by a recent study showing that A₁ receptor null mice develop a severe form of experimental autoimmune encephalomyelitis (EAE), which indicates that endogenous adenosine might have a protective role in the defense against this disease [99]. The involvement of endogenous adenosine in the development of the disease is supported by the downregulation of A₁ adenosine receptors in microglia of A₁^{+/+} mice and by the beneficial effect of caffeine, which upregulates A₁ receptors and reduces disease severity concomitantly [99]. Although the identification of cellular effects underlying the protective action of endogenous adenosine awaits further investigation, these findings offer a novel opportunity for the treatment of sclerosis multiplex and other neuroinflammatory diseases.

Activation of A_{2A} adenosine receptors differentially regulates the transcription and the de novo synthesis of various subtypes of K⁺ channels, via cAMP- and PKC-mediated pathways in cultured rat microglial cells; whereas the expression of Kv1.3 type K⁺ channels are enhanced at both the mRNA and the protein level, only the mRNA but not the protein synthesis of ROMK1 channels seems to be regulated by A_{2A} receptors [100]. This mechanism could participate in the transition of the microglia from the resting state to the activated form, in which the function of K⁺ channels seems to play a pivotal role [47]. The A_{2A} receptor agonist CGS21680 also increases the mRNA expression of nerve growth factor (NGF) and promotes the release of the NGF protein [101] as well as upregulates COX-2 and induces subsequent PGE₂ release in rat microglia again by interacting with A_{2A} receptors and downstream activation of the cAMP pathway [97].

Selective stimulation of A₃ adenosine receptors induces p38 and ERK1/2 phosphorylation in primary murine microglia and in the N13 microglial cell line [102, 103]. This effect is mediated by G_i protein coupling and the phosphatidylinositol-3'-kinase (PI3K) [103], and is absent in microglial cells derived from A₃ receptor deficient transgenic mice [102].

Adenosinergic stimulation also influences the proliferation/survival of microglial cells; however, the clear-cut identity of the underlying receptor subtype involved in these effects is still uncertain. Thus, early studies indicate that only the simultaneous activation of A₁ and A_{2A} receptors could promote microglia proliferation, but this action can be prevented by the A₁ selective antagonist DPCPX [104]. On the other hand, the adenosine receptor agonist 2-chloroadenosine also induces apoptosis in rat microglial cells; however, the identity of the adenosine receptor responsible for this latter effect remains unknown [105].

Conclusions

It is now increasingly clear that ATP is one of the key players of microglial activation, which regulates numerous elements of microglial response from membrane potential to long-lasting transcriptional changes and also affects cell survival. Nevertheless, there are a number of aspects which need further investigation. Despite of the wealth of data on ATP mediated signaling obtained in cultured microglia, the present knowledge is poorly extrapolated to in vivo and disease conditions. The pathways of endogenous activation of purine receptors during the microglial response also need to be better explored. Finally, the mechanisms underlying the protective action of adenosine in the process of microglial activation awaits further investigation. The

progress along this line is essential for the therapeutic utilization of purinergic signaling system in neurodegenerative diseases.

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References

1. Sperl gh B, Vizi ES (1996) Neuronal synthesis, storage and release of ATP. *Semin Neurosci* 8:175–186
2. Coco S, Calegari F, Pravettoni E et al (2003) Storage and release of ATP from astrocytes in culture. *J Biol Chem* 278:1354–1362
3. Latini S, Pedata F (2001) Adenosine in the central nervous system: release mechanisms and extracellular concentrations. *J Neurochem* 79:463–484
4. Sperl gh B, Vizi ES (2000) Regulation of purine release. In: Abbracchio MP, Williams M (eds) *Handbook of experimental pharmacology*, vol 151. Springer, Berlin Heidelberg New York, pp 179–209
5. Sperl gh B, Kittel A, Lajtha A et al (1995) ATP acts as fast neurotransmitter in rat habenula: neurochemical and enzyme-cytochemical evidence. *Neuroscience* 66:915–920
6. Cunha RA, Vizi ES, Ribeiro JA et al (1996) Preferential release of ATP and its extracellular catabolism as a source of adenosine upon high- but not low-frequency stimulation of rat hippocampal slices. *J Neurochem* 67:2180–2187
7. Wieraszko A, Goldsmith G, Seyfried TN (1989) Stimulation-dependent release of adenosine triphosphate from hippocampal slices. *Brain Res* 485:244–250
8. Braun N, Sevigny J, Robson SC et al (2000) Assignment of ecto-nucleoside triphosphate diphosphohydrolase-1/cd39 expression to microglia and vasculature of the brain. *Eur J Neurosci* 12:4357–4366
9. Zimmermann H (2000) Extracellular metabolism of ATP and other nucleotides. *Naunyn Schmiedebergs Arch Pharmacol* 362:299–309
10. Wang X, Arcuino G, Takano T et al (2004) P2X7 receptor inhibition improves recovery after spinal cord injury. *Nat Med* 10:821–827
11. Verderio C, Matteoli M (2001) ATP mediates calcium signaling between astrocytes and microglial cells: modulation by IFN-γ. *J Immunol* 166:6383–6391
12. Wang Y, Roman R, Lidofsky SD et al (1996) Autocrine signaling through ATP release represents a novel mechanism for cell volume regulation. *Proc Natl Acad Sci USA* 93:12020–12025
13. Hagberg H, Andersson P, Lacarewicz J et al (1987) Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. *J Neurochem* 49:227–231
14. Hisanaga K, Onodera H, Kogure K (1986) Changes in levels of purine and pyrimidine nucleotides during acute hypoxia and recovery in neonatal rat brain. *J Neurochem* 47:1344–1350
15. Juranyi Z, Sperl gh B, Vizi ES (1999) Involvement of P2 purinoceptors and the nitric oxide pathway in [³H]purine outflow evoked by short-term hypoxia and hypoglycemia in rat hippocampal slices. *Brain Res* 823:183–190

16. Pedata F, Latini S, Pugliese AM et al (1993) Investigations into the adenosine outflow from hippocampal slices evoked by ischemia-like conditions. *J Neurochem* 61:284–289
17. Lutz PL, Kabler S (1997) Release of adenosine and ATP in the brain of the freshwater turtle (*Trachemys scripta*) during long-term anoxia. *Brain Res* 769:281–286
18. Sperlagh B, Hasko G, Nemeth Z et al (1998) ATP released by LPS increases nitric oxide production in raw 264.7 macrophage cell line via P2Z/P2X7 receptors. *Neurochem Int* 33:209–215
19. Ferrari D, Chiozzi P, Falzoni S et al (1997) Purinergic modulation of interleukin-1 β release from microglial cells stimulated with bacterial endotoxin. *J Exp Med* 185:579–582
20. Filippini A, Taffs RE, Sitkovsky MV (1990) Extracellular ATP in T-lymphocyte activation: possible role in effector functions. *Proc Natl Acad Sci USA* 87:8267–8271
21. Sperlagh B, Baranyi M, Hasko G et al (2004) Potent effect of interleukin-1 β to evoke ATP and adenosine release from rat hippocampal slices. *J Neuroimmunol* 151:33–39
22. Sperlagh B, Szabo G, Erdelyi F et al (2003) Homo- and heteroexchange of adenine nucleotides and nucleosides in rat hippocampal slices by the nucleoside transport system. *Br J Pharmacol* 139:623–633
23. al-Awqati Q (1995) Regulation of ion channels by ABC transporters that secrete ATP. *Science* 269:805–806
24. Schwiebert EM (1999) ABC transporter-facilitated ATP conductive transport. *Am J Physiol* 276:C1–C8
25. Ballerini P, Di Iorio P, Ciccarelli R et al (2002) Glial cells express multiple ATP binding cassette proteins which are involved in ATP release. *Neuroreport* 13:1789–1792
26. Abdipranoto A, Liu GJ, Werry EL et al (2003) Mechanisms of secretion of ATP from cortical astrocytes triggered by uridine triphosphate. *Neuroreport* 14:2177–2181
27. Darby M, Kuzmiski JB, Panenka W et al (2003) ATP released from astrocytes during swelling activates chloride channels. *J Neurophysiol* 89:1870–1877
28. Cass CE, Young JD, Baldwin SA et al (1999) Nucleoside transporters of mammalian cells. *Pharm Biotechnol* 12:313–352
29. Thorn JA, Jarvis SM (1996) Adenosine transporters. *Gen Pharmacol* 27:613–620
30. Cunha RA (2001) Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: different roles, different sources and different receptors. *Neurochem Int* 38:107–125
31. Stout C, Goodenough DA, Paul DL (2004) Connexins: functions without junctions. *Curr Opin Cell Biol* 16:507–512
32. Stout CE, Costantin JL, Naus CC et al (2002) Intercellular calcium signaling in astrocytes via ATP release through connexin hemichannels. *J Biol Chem* 277:10482–10488
33. Bahima L, Aleu J, Elias M et al (2006) Endogenous hemichannels play a role in the release of ATP from *Xenopus* oocytes. *J Cell Physiol* 206:95–102
34. Pearson RA, Dale N, Llaudet E et al (2005) ATP released via gap junction hemichannels from the pigment epithelium regulates neural retinal progenitor proliferation. *Neuron* 46:731–744
35. Gomes P, Srinivas SP, Van Driessche W et al (2005) ATP release through connexin hemichannels in corneal endothelial cells. *Invest Ophthalmol Vis Sci* 46:1208–1218
36. Cook SP, McCleskey EW (2002) Cell damage excites nociceptors through release of cytosolic ATP. *Pain* 95:41–47
37. Wang TF, Rosenberg PA, Guidotti G (1997) Characterization of brain ecto-ATPase: evidence for only one ecto-ATPase (CD39) gene. *Brain Res Mol Brain Res* 47:295–302
38. Wang TF, Guidotti G (1998) Widespread expression of ecto-ATPase (CD39) in the central nervous system. *Brain Res* 790:318–322
39. Grondal EJ, Janetzko A, Zimmermann H (1988) Monospecific antiserum against 5'-nucleotidase from Torpedo electric organ: immunocytochemical distribution of the enzyme and its association with Schwann cell membranes. *Neuroscience* 24:351–363
40. Schoen SW, Graeber MB, Reddington M et al (1987) Light and electron microscopical immunocytochemistry of 5'-nucleotidase in rat cerebellum. *Histochemistry* 87:107–113
41. Cunha RA, Sebastiao AM, Ribeiro JA (1992) Ecto-5'-nucleotidase is associated with cholinergic nerve terminals in the hippocampus but not in the cerebral cortex of the rat. *J Neurochem* 59:657–666
42. James S, Richardson PJ (1993) Production of adenosine from extracellular ATP at the striatal cholinergic synapse. *J Neurochem* 60:219–227
43. Norenberg W, Langosch JM, Gebicke-Haerter PJ et al (1994) Characterization and possible function of adenosine 5'-triphosphate receptors in activated rat microglia. *Br J Pharmacol* 111:942–950
44. Walz W, Iltschner S, Ohlemeyer C et al (1993) Extracellular ATP activates a cation conductance and a K⁺ conductance in cultured microglial cells from mouse brain. *J Neurosci* 13:4403–4411
45. Langosch JM, Gebicke-Haerter PJ, Norenberg W et al (1994) Characterization and transduction mechanisms of purinoceptors in activated rat microglia. *Br J Pharmacol* 113:29–34
46. Norenberg W, Cordes A, Blohbaum G et al (1997) Coexistence of purino- and pyrimidinoceptors on activated rat microglial cells. *Br J Pharmacol* 121:1087–1098
47. Illes P, Norenberg W, Gebicke-Haerter PJ (1996) Molecular mechanisms of microglial activation. B. Voltage- and purinoceptor-operated channels in microglia. *Neurochem Int* 29:13–24
48. Bianco F, Fumagalli M, Pravettoni E et al (2005) Pathophysiological roles of extracellular nucleotides in glial cells: differential expression of purinergic receptors in resting and activated microglia. *Brain Res Brain Res Rev* 48:144–156
49. Xiang Z, Burnstock G (2005) Expression of P2X receptors on rat microglial cells during early development. *Glia* 52:119–126
50. Surprenant A, Rassendren F, Kawashima E et al (1996) The cytolytic P2Z receptor for extracellular ATP identified as a P2X₇ receptor (P2X₇). *Science* 272:735–738
51. Collo G, Neidhart S, Kawashima E et al (1997) Tissue distribution of the P2X₇ receptor. *Neuropharmacology* 36:1277–1283
52. Di Virgilio F, Chiozzi P, Ferrari D et al (2001) Nucleotide receptors: an emerging family of regulatory molecules in blood cells. *Blood* 97:587–600
53. Di Virgilio F, Sanz JM, Chiozzi P et al (1999) The P2Z/P2X₇ receptor of microglial cells: a novel immunomodulatory receptor. *Prog Brain Res* 120:355–368
54. Haas S, Brockhaus J, Verkhatsky A et al (1996) ATP-induced membrane currents in amoeboid microglia acutely isolated from mouse brain slices. *Neuroscience* 75:257–261
55. Ferrari D, Villalba M, Chiozzi P et al (1996) Mouse microglial cells express a plasma membrane pore gated by extracellular ATP. *J Immunol* 156:1531–1539
56. Gendron FP, Chalimoniuk M, Strosznajder J et al (2003) P2X₇ nucleotide receptor activation enhances IFN γ -induced type II nitric oxide synthase activity in BV-2 microglial cells. *J Neurochem* 87:344–352
57. Ferrari D, Chiozzi P, Falzoni S et al (1997) ATP-mediated cytotoxicity in microglial cells. *Neuropharmacology* 36:1295–1301
58. Chessell IP, Michel AD, Humphrey PP (1997) Properties of the pore-forming P2X₇ purinoceptor in mouse NTW8 microglial cells. *Br J Pharmacol* 121:1429–1437

59. Visentin S, Renzi M, Frank C et al (1999) Two different ionotropic receptors are activated by ATP in rat microglia. *J Physiol* 519:723–736
60. Ferrari D, Chiozzio P, Falzoni S et al (1997) Extracellular ATP triggers IL-1 beta release by activating the purinergic P2_Z receptor of human macrophages. *J Immunol* 159:1451–1458
61. Sanz JM, Di Virgilio F (2000) Kinetics and mechanism of ATP-dependent IL-1β release from microglial cells. *J Immunol* 164:4893–4898
62. Brough D, Le Feuvre RA, Iwakura Y et al (2002) Purinergic (P2X₇) receptor activation of microglia induces cell death via an interleukin-1-independent mechanism. *Mol Cell Neurosci* 19:272–280
63. Chakfe Y, Seguin R, Antel, JP et al (2002) ADP and AMP induce interleukin1-β release from microglial cells through activation of ATP-primed P2X₇ receptor channels. *J Neurosci* 22:3061–3069
64. Hide I, Tanaka M, Inoue A et al (2000) Extracellular ATP triggers tumor necrosis factor-α release from rat microglia. *J Neurochem* 75:965–972
65. Suzuki T, Hide I, Ido K et al (2004) Production and release of neuroprotective tumor necrosis factor by P2X₇ receptor-activated microglia. *J Neurosci* 24:1–7
66. Shigemoto-Mogami Y, Koizumi S, Tsuda M et al (2001) Mechanisms underlying extracellular ATP-evoked interleukin-6 release in mouse microglial cell line, MG-5. *J Neurochem* 78:1339–1349
67. Inoue K (2002) Microglial activation by purines and pyrimidines. *Glia* 40:156–163
68. Chessell IP, Hatcher JP, Bountra C et al (2005) Disruption of the P2X₇ purinoceptor gene abolishes chronic inflammatory and neuropathic pain. *Pain* 114:386–396
69. Rampe D, Wang L, Ringheim GE (2004) P2X₇ receptor modulation of β-amyloid- and LPS-induced cytokine secretion from human macrophages and microglia. *J Neuroimmunol* 147:56–61
70. Ohtani Y, Minami M, Satoh M (2000) Expression of inducible nitric oxide synthase mRNA and production of nitric oxide are induced by adenosine triphosphate in cultured rat microglia. *Neurosci Lett* 293:72–74
71. Parvathani LK, Tertyshnikova S, Greco CR et al (2003) P2X₇ mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. *J Biol Chem* 278:13309–13317
72. Witting A, Walter L, Wacker J et al (2004) P2X₇ receptors control 2-arachidonoylglycerol production by microglial cells. *Proc Natl Acad Sci USA* 101:3214–3219
73. Cabral GA, Marciano-Cabral F (2005) Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. *J Leukoc Biol* 78:1192–1197
74. Inoue K, Nakajima K, Morimoto T et al (1998) ATP stimulation of Ca²⁺-dependent plasminogen release from cultured microglia. *Br J Pharmacol* 123:1304–1310
75. Ferrari D, Stroh C, Schulze-Osthoff K (1999) P2X₇/P2_Z purinoreceptor-mediated activation of transcription factor NFAT in microglial cells. *J Biol Chem* 274:13205–13210
76. Ferrari D, Wesselborg S, Bauer MK et al (1997) Extracellular ATP activates transcription factor NF-κB through the P2_Z purinoreceptor by selectively targeting NF-κB p65. *J Cell Biol* 139:1635–1643
77. Kaya N, Tanaka S, Koike T (2002) ATP selectively suppresses the synthesis of the inflammatory protein microglial response factor (MRF)-1 through Ca²⁺ influx via P2X₇ receptors in cultured microglia. *Brain Res* 952:86–97
78. Tanaka S, Koike T (2002) Selective inflammatory stimulations enhance release of microglial response factor (MRF)-1 from cultured microglia. *Glia* 40:360–371
79. Ferrari D, Los M, Bauer MK et al (1999) P2_Z purinoreceptor ligation induces activation of caspases with distinct roles in apoptotic and necrotic alterations of cell death. *FEBS Lett* 447:71–75
80. Guthrie PB, Knappenberger J, Segal M et al (1999) ATP released from astrocytes mediates glial calcium waves. *J Neurosci* 19:520–528
81. Franke H, Gunther A, Grosche J et al (2004) P2X₇ receptor expression after ischemia in the cerebral cortex of rats. *J Neuropathol Exp Neurol* 63:686–699
82. Bringmann A, Pannicke T, Moll V et al (2001) Upregulation of P2X₇ receptor currents in Muller glial cells during proliferative vitreoretinopathy. *Invest Ophthalmol Vis Sci* 42:860–867
83. Morigiwa K, Quan M, Murakami M et al (2000) P2 Purinoceptor expression and functional changes of hypoxia-activated cultured rat retinal microglia. *Neurosci Lett* 282:153–156
84. Chen I, Raine CS, Brosnan C (2005) Mice with a non-functional P2X₇ receptor are more susceptible to experimental autoimmune encephalomyelitis than WT controls. *Society Neuroscience Abstracts* 902.1
85. McLarnon JG (2005) Purinergic mediated changes in Ca²⁺ mobilization and functional responses in microglia: effects of low levels of ATP. *J Neurosci Res* 81:349–3456
86. Guo LH, Trautmann K, Schluesener HJ (2005) Expression of P2X₄ receptor by lesional activated microglia during formalin-induced inflammatory pain. *J Neuroimmunol* 163:120–127
87. Schwab JM, Guo L, Schluesener HJ (2005) Spinal cord injury induces early and persistent lesional P2X₄ receptor expression. *J Neuroimmunol* 163:185–189
88. Tsuda M, Shigemoto-Mogami Y, Koizumi S et al (2003) P2X₄ receptors induced in spinal microglia gate tactile allodynia after nerve injury. *Nature* 424:778–783
89. Inoue K, Tsuda M, Koizumi S (2004) ATP- and adenosine-mediated signaling in the central nervous system: chronic pain and microglia: involvement of the ATP receptor P2X₄. *J Pharmacol Sci* 94:112–114
90. Priller J, Haas CA, Reddington M et al (1995) Calcitonin gene-related peptide and ATP induce immediate early gene expression in cultured rat microglial cells. *Glia* 15:447–457
91. Ogata T, Chuai M, Morino T et al (2003) Adenosine triphosphate inhibits cytokine release from lipopolysaccharide-activated microglia via P2Y receptors. *Brain Res* 981:174–183
92. Honda SY, Sasaki K, Ohsawa et al (2001) Extracellular ATP or ADP induce chemotaxis of cultured microglia through G_{i/o}-coupled P2Y receptors. *J Neurosci* 21:1975–1982
93. Choi HB, Hong SH, Ryu JK et al (2003) Differential activation of subtype purinergic receptors modulates Ca²⁺ mobilization and COX-2 in human microglia. *Glia* 43:95–103
94. Boucsein C, Zacharias R, Farber K et al (2003) Purinergic receptors on microglial cells: functional expression in acute brain slices and modulation of microglial activation in vitro. *Eur J Neurosci* 17:2267–2276
95. Innocenti B, Pfeiffer S, Zrenner E et al (2004) ATP-induced non-neuronal cell permeabilization in the rat inner retina. *J Neurosci* 24:8577–8583
96. Ryu JK, Kim J, Choi SH et al (2002) ATP-induced in vivo neurotoxicity in the rat striatum via P2 receptors. *Neuroreport* 13:1611–1615
97. Fiebich BL, Biber K, Gyufko K et al (1996) Adenosine A_{2b} receptors mediate an increase in interleukin (IL)-6 mRNA and IL-6 protein synthesis in human astroglia cells. *J Neurochem* 66:1426–1431

98. Hasko G, Pacher P, Vizi ES et al (2005) Adenosine receptor signaling in the brain immune system. *Trends Pharmacol Sci* 26:511–516
99. Tsutsui S, Schnermann J, Noorbakhsh F et al (2004) A1 adenosine receptor upregulation and activation attenuates neuroinflammation and demyelination in a model of multiple sclerosis. *J Neurosci* 24:1521–1529
100. Kust BM, Biber K, van Calker D et al (1999) Regulation of K⁺ channel mRNA expression by stimulation of adenosine A_{2A}-receptors in cultured rat microglia. *Glia* 25:120–130
101. Heese K, Fiebich BL, Bauer J et al (1997) Nerve growth factor (NGF) expression in rat microglia is induced by adenosine A_{2a}-receptors. *Neurosci Lett* 231:83–86
102. Hammarberg C, Schulte G, Fredholm BB (2003) Evidence for functional adenosine A₃ receptors in microglia cells. *J Neurochem* 86:1051–1054
103. Hammarberg C, Fredholm BB, Schulte G (2004) Adenosine A₃ receptor-mediated regulation of p38 and extracellular-regulated kinase ERK1/2 via phosphatidylinositol-3'-kinase. *Biochem Pharmacol* 67:129–134
104. Gebicke-Haerter PJ, Christoffel F, Timmer J et al (1996) Both adenosine A₁- and A₂-receptors are required to stimulate microglial proliferation. *Neurochem Int* 29:37–42
105. Ogata T, Schubert P (1996) Programmed cell death in rat microglia is controlled by extracellular adenosine. *Neurosci Lett* 218:91–94