Cell. Mol. Life Sci. 65 (2008) 3074 – 3080
1420-682X/08/193074-7
DOI 10.1007/s00018-008-8210-3

Cellular and Molecular Life Sciences

Review

Purinergic systems in microglia

K. Inoue

Department of Molecular and System Pharmacology, Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi, Fukuoka 812 – 8582 (Japan), Fax: +81 92 642 4729,
e-mail: inoue@phar.kyushu-u.ac.jp

Received 19 April 2008; received after revision 20 May 2008; accepted 23 May 2008
Online First 20 June 2008

Abstract. Accumulating findings indicate that nucleotides play an important role in microglia through P2 purinoceptors. P2 purinoceptors are divided into two families, ionotropic receptors (P2X) and metabotropic receptors (P2Y). P2X receptors (7 types; $P2X_1$ – $P2X_7$) contain intrinsic pores that open by binding with ATP. P2Y receptors (8 types; $P2Y_{1, 2, 4, 6, 11, 12, 13 \text{ and } 14}$) are activated by nucleotides and couple to intracellular second-messenger systems through heteromeric G-proteins. Nucleotides are released or leaked from non-excitable cells as well as neurons in physiological and pathophysiological conditions. Microglia express many types of P2 purinoceptors and are known as resident macrophages in the CNS. ATP and other nucleotides work as 'warning molecules' especially through activating microglia in pathophysiological conditions. Microglia play a key role in neuropathic pain, chemotaxis and phagocytosis through nucleotide-evoked activation of $P2X_4$, $P2Y_{12}$ and $P2Y_6$ receptors, respectively. These findings indicate that extracellular nucleotides are important players in the central stage of microglial function.

Keywords. ATP receptors, microglia, pain, phagocytosis, chemotaxis.

Introduction

Accumulating evidence suggests that nucleotides are released or leaked from non-excitable cells as well as neurons and play a role in cell-to-cell communication in physiological and pathophysiological conditions [1]. One of the most interesting cell types in non-excitable cells is glia, which make up over 70 % of the total cell population in the central nervous system (CNS) and are classified into astrocytes, oligodendrocytes and microglia. Microglia express many types of P2 purinoceptors and are known as resident macrophages in the CNS, accounting for 5 – 10 % of the total population of glia [2, 3]. P2 purinoceptors are divided into two families, ionotropic receptors (P2X) and metabotropic receptors (P2Y) [1]. P2X receptors (7 types; $P2X_1$ – $P2X_7$) contain intrinsic channels allowing ions to flow that switch conformation from closed to open on binding ATP [1]. P2Y (8 types; $P2Y_{1,2,4,6,11,12,\ 13 \text{ and } 14}$) are activated by purine or pyrimidine nucleotides or sugar nucleotides subtype-dependently and couple to intracellular second-messenger systems through heteromeric G-proteins [1].

In this article, I describe how ATP and other nucleotides work as 'warning molecules' especially through activating microglia in pathophysiological conditions. Activated microglia play a key role in neuropathic pain, chemotaxis and phagocytosis through nucleotide-evoked activation of $P2X_4$, $P2Y_{12}$ and $P2Y_6$ receptors, respectively.

Chemotaxis of microglia

The initial microglial responses that occur after brain injury and in various neurological diseases are char-

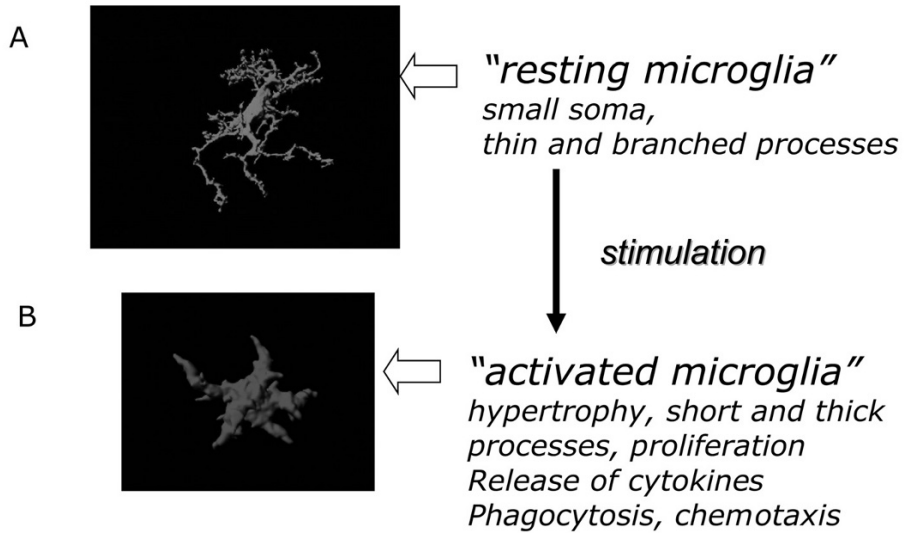

Figure 1. Resting (*A*) and activated (*B*) microglia. Microglia are ubiquitously distributed throughout the CNS and represent a morphologically unique type of cell which has a small soma bearing thin and branched processes under normal conditions (*A*). Pathophysiological conditions, including peripheral nerve injury, brain damage, trauma, virus infections and microglia show an activated phenotype (*B*); the small soma of microglia become hypertrophic, and the long and thin processes withdraw.

acterized by microglial accumulation in the affected sites of the brain, which results from the migration and proliferation of these cells. The early-phase signal responsible for this accumulation is likely to be transduced by rapidly diffusible factors. Honda et al. examined the possibility that ATP released from injured neurons and nerve terminals affects cell motility in rat primary cultured microglia [4]. They found that extracellular ATP and ADP induce membrane ruffling and markedly enhance chemokinesis in a Boyden chamber assay. Further analyses using the Dunn chemotaxis chamber assay, which allows direct observation of cell movement, revealed that both ATP and ADP induce chemotaxis of microglia. The elimination of extracellular calcium or treatment with PPADS or suramin does not inhibit ATP- or ADP-induced membrane ruffling, whereas AR-C69931MX, a P2Y12 and P2Y13 receptor blocker [5, 6], or pertussis toxin (PTX) treatment clearly inhibits the ruffling. As an intracellular signaling molecule underlying these phenomena, the small G-protein Rac is activated by ATP and ADP stimulation, and its activation is also inhibited by pretreatment with PTX. These findings suggested that the membrane ruffling and chemotaxis of microglia induced by ATP or ADP are mediated by G(i/o)-coupled P2Y receptors (P2Y12 and/or P2Y13).

Haynes et al. recently confirmed the finding by Honda et al. *in vivo* that extracellular ATP functions as a chemoattractant for microglia via $P2Y_{12}$ receptors using $P2Y_{12}$ receptor knockout animals [7]. In the adult, microglia are ubiquitously distributed throughout the CNS and represent a morphologically unique type of cell which, under normal conditions, has a small soma bearing thin and branched processes (Fig. 1a). Such microglia were considered to be 'resting', but recent studies using a transgenic mouse line that expressed green fluorescent protein in microglia together with two-photon microscopy have revealed that microglia processes are highly dynamic in the brain [8, 9]. The processes of microglia rapidly move toward the site of injury, an effect that is mimicked by local injection of ATP and can be inhibited either by the ATP-hydrolyzing enzyme apyrase or by blockers of P2YRs [8]. Furthermore, Haynes et al. have shown that microglia in $P2Y_{12}$R-deficient mice exhibit normal basal motility but diminished directional branch extension toward nucleotides or sites of cortical damage *in vivo* [7]. Thus, microglia show chemotaxis through the $P2Y_{12}$R signaling system.

Neuropathic pain

Spinal microglial P2X4 in neuropathic pain

Pain in response to noxious stimuli has an important role as an early warning device. This normal pain usually goes away after the nociceptive stimulus is removed or the tissue damage is repaired, and even if pain persists for awhile, it can generally be managed by treatment with analgesics. However, there is a type of pain that does not go away even though the tissue has already healed. One such type is neuropathic pain, which typically develops when peripheral nerves are damaged such as through surgery, bone compression in cancer, diabetes or infection. This type of pain provides no known physiological advantage. Neuropathic pain is a major factor causing impaired quality of life in millions of people worldwide and, unfortunately, is frequently resistant to all known analgesics. It has been understood that neuropathic pain is not just a symptom of disease but a consequence of

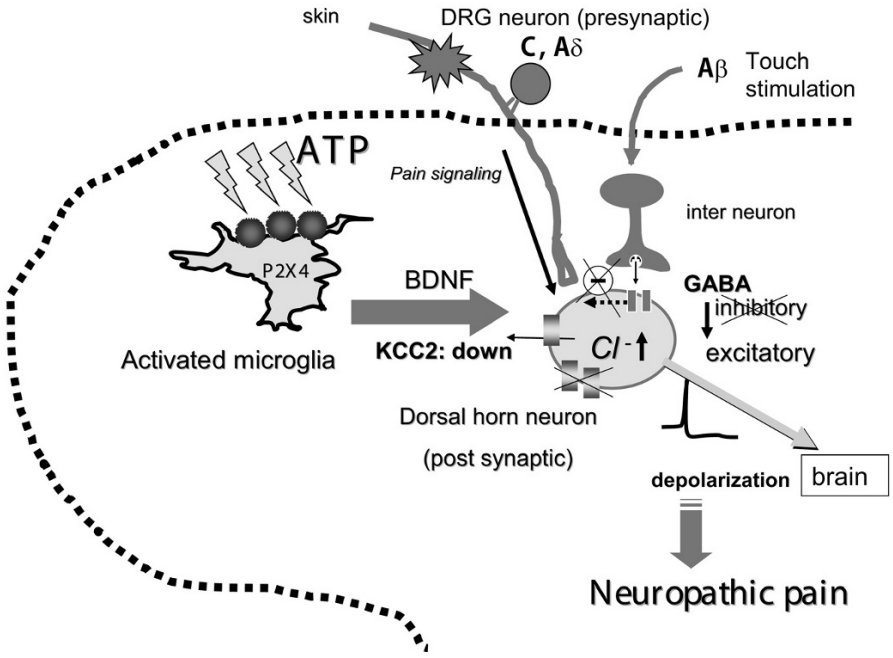

Figure 2. Schematic illustration of potential mechanisms by which $P2X_4R$ in activated microglia modulate pain signaling in the dorsal horn. On the normal side of the dorsal horn normal microglia have a small soma bearing thin and branched processes and are homogeneously distributed. After peripheral nerve injury, microglia in the spinal cord ipsilateral to the nerve injury become transformed to the activated phenotype that is characterized by hypertrophy, proliferation, and the expression of cell-surface molecules. Activated microglia after nerve injury increase the expression of $P2X_4R$. The $P2X_4R$ are activated by ATP, which is presumably released from primary sensory neurons (C-fiber and/or Aδ-fiber) (or astrocytes), and in turn cause a rise in intracellular calcium. $P2X_4R$ activation leads to the release of bioactive diffusible factors such as BDNF. BDNF causes collapse of the transmembrane anion gradient in dorsal horn lamina I neurons presumably through downregulation of KCC2, which in turn renders GABA and glycine effects depolarizing in these neurons. Thus, touch stimulation through Aβ may cause the release of GABA (and/or glycine) from inhibitory interneurons, and GABA activates the dorsal horn neurons, evoking neuropathic pain.

disordered functioning of the nervous system [10–12]. Accumulating evidence concerning how peripheral nerve injury creates neuropathic pain suggests that nerve injury produces molecular and cellular alterations that result in multiple forms of neuronal plasticity and anatomical reorganization in the dorsal horn of the spinal cord. These alterations have been proposed to be crucial in the pathogenesis of neuropathic pain. Besides the role of neurons in pain, there is a rapidly growing body of evidence indicating that spinal glial cells, in particular microglia, play a critical role in the pathogenesis of neuropathic pain.

Evidence from studies using animal models of neuropathic pain reveal that peripheral nerve damage leads to a dramatic change in microglia within the spinal dorsal horn, converting these cells to an activated phenotype (Fig. 1b) through a step-by-step process [13]. Within the first 24 h after peripheral nerve injury, the first signs of microglial activation can already be observed: the small soma become hypertrophic, and the long and thin processes withdraw [14]. This is followed by a burst in proliferation of microglia with a peak around 2~3 days after the nerve injury[15]. Activated microglia upregulate the expression of the complement receptor 3, leading to enhanced OX-42 labeling [14, 16–19]. The temporal pattern of OX-42 upregulation in the dorsal horn correlates with that of the development of tactile allodynia [18], which is an abnormal hypersensitivity to innocuous stimuli and is a hallmark of neuropathic pain syndrome in humans. In all animal models for studying neuropathic pain, the activation of microglia in the dorsal horn is observed after nerve injury [14, 16, 18–22], bone cancer [23] and diabetes [24].

There are abundant findings indicating the activation of spinal microglia in a neuropathic pain state, but until recently it remained an open question whether spinal microglia play a causal role in neuropathic pain behavior. Tsuda et al. directly implicated activated microglia in the pathogenesis of neuropathic pain by determining the role of the purinoceptor $P2X_4$ receptors (Rs) [20]. A clue to identifying $P2X_4Rs$ in the spinal cord as being required for neuropathic pain first came from a pharmacological investigation of pain behavior after nerve injury, using the P2X receptor antagonists 2′,3′-O-(2,4,6-trinitrophenyl)adenosine 5′-triphosphate (TNP-ATP) and pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) [20]. The

marked tactile allodynia after injury of a spinal nerve was found to be reversed by acutely administering TNP-ATP intrathecally but was unaffected by administering PPADS. From the pharmacological profiles of TNP-ATP and PPADS, it was inferred that the tactile allodynia depends upon $P2X_4Rs$ in the spinal cord. The expression of $P2X_4R$ protein progressively increased in the days following nerve injury, the time course of which was parallel to that of the development of tactile allodynia. In the immunohistochemical analysis, it was found that increase of the expression of $P2X_4R$ protein was seen only in activated microglia rather than neurons or astrocytes. Moreover, it was found that reducing the upregulation of $P2X_4R$ protein in spinal microglia by $P2X_4R$ antisense oligodeoxynucleotide prevented development of the nerve injury-induced tactile allodynia. Together, this evidence implied that $P2X_4Rs$ activation in spinal microglia is necessary for neuropathic pain. The sufficiency of $P2X_4R$ activation in microglia for the development of allodynia was demonstrated by intrathecal administration of activated, cultured microglia in which these receptors had been stimulated *in vitro* by ATP [20]. In otherwise naïve animals, allodynia develops progressively over a period of 3–5 h following the administration of $P2X_4R$-stimulated microglia. Moreover, in rats in which tactile allodynia was caused by the ATP-stimulated microglia, this allodynia was reversed by administering TNP-ATP. Thus, the allodynia caused by ATP-stimulated microglia is pharmacologically similar to that caused by peripheral nerve injury. These findings indicate that $P2X_4R$ stimulation of microglia is not only necessary for tactile allodynia but is also sufficient to cause allodynia (Fig. 2).

BDNF release from microglia by activation of $P2X_4Rs$

The first evidence that microglia may participate in the hyperexcitability in dorsal horn neurons was revealed by Coull et al. [25]. They used spinal cord slices taken from rats that had displayed allodynia by intrathecal administration of $P2X_4R$-stimulated microglia, and found that ATP-stimulated microglia positively shifted the anion reversal potential (E_{anion}) in spinal lamina I neurons and rendered the GABA effects depolarizing in these neurons [25]. TNP-ATP, which can reverse nerve injury-induced allodynia, acutely reverses the depolarizing shift in E_{anion} in lamina I neurons after peripheral nerve injury [25]. Together with the findings that the depolarizing shift in E_{anion} and the excitatory response to GABA are key events in dorsal horn neurons in neuropathic pain [26], these results suggest that spinal microglia stimulated by $P2X_4R$ cause neuropathic pain through a rise in intracellular Cl^- in spinal lamina I neurons. Coull et al. also determined the role of brain-derived neurotrophic factor (BDNF) as a signaling factor between microglia and dorsal horn lamina I neurons [25]. It was found that intrathecal application of BDNF mimicked the tactile allodynia and the depolarizing shift in E_{anion} in lamina I neurons by peripheral nerve injury or by intrathecally administering $P2X_4R$-stimulated microglia. Furthermore, interfering with the signaling between BDNF and its receptor (TrkB) either by a function-blocking TrkB antibody or by a BDNF-sequestering fusion protein (TrkB-Fc) prevented the tactile allodynia caused by peripheral nerve injury or by intrathecally administering $P2X_4R$-stimulated microglia. Applying ATP to microglia caused the release of BDNF, which was prevented by TNP-ATP. Therefore, these results indicate that $P2X_4R$-stimulated microglia release BDNF as a crucial factor in the signaling to lamina I neurons, causing a collapse of their transmembrane anion gradient and subsequent neuronal hyperexcitability (Fig. 2). $GABA_AR$-mediated depolarization might also produce excitation through voltage-sensitive Ca^{2+} channels and NMDA receptors. There is evidence that several proinflammatory cytokines that are known to be released from microglia [27, 28] modulate excitatory synaptic transmission. Interleukin-1β was reported to enhance NMDA receptor-mediated Ca^{2+} response [29]. Long-term treatment with interferon-γ produced an increase in neuronal excitability in dorsal horn neurons [30]. Thus, net enhanced transmission in the dorsal horn pain network by these factors might be responsible for nerve injury-induced neuropathic pain.

Possible mechanism of the upregulation of $P2X_4R$ expression in microglia

Nasu-Tada et al. reported the role of fibronectin, an extracellular matrix protein, as a potential candidate molecule in $P2X_4R$ upregulation in microglia (Fig. 2) [31]. It was found that microglia cultured on fibronectin-coated dishes showed a marked increase in $P2X_4R$ expression both at the mRNA and protein levels [31]. The upregulated $P2X_4R$ protein by fibronectin on microglia might be functional since the $P2X_4$ R-mediated Ca^{2+} response was enhanced in fibronectin-treated microglia. Furthermore, intrathecal delivery of ATP-stimulated microglia to the rat lumber spinal cord revealed that microglia treated with fibronectin more effectively induce allodynia than control microglia. More recently, we found that fibronectin-integrin signaling and integrin-Lyn (tyrosine kinase) signaling is involved in the upregulation of $P2X_4Rs$ in spinal microglia after peripheral nerve injury *in vivo* [32, 33]. Microglia cultured on dishes coated with fibronectin expressed a higher level of $P2X_4R$ protein as compared with those cultured on

control dishes. The increase was suppressed by echistatin, a peptide that selectively blocks β1s- and β3-containing integrins, and with a function-blocking antibody of β1 integrin. In *in vivo* studies, the upregulation of $P2X_4$Rs in the spinal cord after spinal nerve injury was significantly suppressed by intrathecal administration of echistatin. Tactile allodynia in response to nerve injury and intrathecal administration of ATP- and fibronectin-stimulated microglia was inhibited by echistatin. Furthermore, intrathecal administration of fibronectin in normal rats increased the level of $P2X_4$R protein in the spinal cord and produced tactile allodynia. The fibronectin-induced allodynia was not observed in mice lacking $P2X_4$R. These results suggest that the fibronectin-integrin signaling system participates in the upregulation of $P2X_4$R expression after nerve injury and subsequent neuropathic pain [32]. We identified Lyn as the predominant Src family kinase (SFK) amongst the five members (Src, Fyn, Yes, Lck and Lyn) in spinal microglia. The level of Lyn expression was increased after nerve injury. Using mice lacking lyn (lyn–/–), we found that these mice exhibit a striking reduction of the phospho-SFK-IR and of tactile allodynia after nerve injury without any change in basal mechanical sensitivity or inflammatory pain. The lyn-/- mice displayed impaired upregulation of $P2X_4$R in the spinal cord after nerve injury, which is crucial for tactile allodynia. Microglial cells from lyn-/- mice showed a deficit in increasing $P2X_4$R expression caused by fibronectin. These findings suggest that Lyn may be a critical kinase mediating nerve injury-induced $P2X_4$R upregulation and neuropathic pain [33].

It was also reported that activating both TLRs and NOD2 (another pattern-recognition receptor) in cultured microglia increased the expression of $P2X_4$R at the mRNA level [34], thus suggesting the involvement of these receptors in the regulation of $P2X_4$R. The functional relevance of these receptors *in vivo* is still unclear.

Neuropathic pain via $P2Y_{12}$ activation

As mentioned before, $P2Y_{12}$R in microglia is implicated in ATP-induced membrane ruffling and chemotaxis [4, 7, 35]. A recent study has demonstrated that $P2Y_{12}$R is required for the extension of microglial processes to engulf injured cells *in vivo* [7, 8, 36]. Moreover, whereas the microglial ATP receptors (for example, $P2X_4$R, $P2X_7$R and $P2Y_6$R) are expressed in both microglia and peripheral macrophages [37], the $P2Y_{12}$R subtype is unique in that its expression is restricted to microglia [7, 38]. These observations suggest that microglia are key sensors of adverse conditions in the CNS, detecting nucleotides via $P2Y_{12}$Rs. Despite rapid progress in elucidating the physiological functions of microglia mediated by $P2Y_{12}$R, relatively little insight has been gained concerning the *in vivo* role of $P2Y_{12}$Rs in pathophysiological conditions in the CNS. We investigated the role of microglial $P2Y_{12}$Rs in the spinal cord in neuropathic pain and discovered that activation of $P2Y_{12}$Rs in spinal microglia is a critical in the pathogenesis of neuropathic pain, using selective antagonists for $P2Y_{12}$R and mice lacking $P2Y_{12}$R. The level of $P2Y_{12}$R mRNA expression was markedly increased in the spinal cord ipsilateral to the nerve injury, and this expression was highly restricted to activated microglia [39]. An increase in the immunofluorescence of $P2Y_{12}$R protein in the ipsilateral spinal cord was also observed after nerve injury, and $P2Y_{12}$R-positive cells were double-labeled with the microglial marker OX-42. The intrathecal administration of $P2Y_{12}$R antagonist AR-C69931MX prevented the development of tactile allodynia, a hallmark of neuropathic pain syndrome. Furthermore, mice lacking *P2ry12* (*P2ry12*$^{-/-}$) displayed impaired tactile allodynia after nerve injury without any change in basal mechanical sensitivity. Moreover, a single intrathecal administration of AR-C69931MX or oral administration of clopidogrel, a $P2Y_{12}$R blocker clinically in use, to nerve-injured rats produced a striking alleviation of existing tactile allodynia. These findings suggest that activation of $P2Y_{12}$Rs in spinal microglia is a critical event in the pathogenesis of neuropathic pain [40], and that blocking microglial $P2Y_{12}$R might be a viable therapeutic strategy for treating neuropathic pain.

Phagocytosis by microglia

Phagocytosis is the uptake system by the cell of relatively large (>1.0 μm) particles into vacuoles and has a central role in tissue remodeling, inflammation and defense against infectious agents [41]. Phagocytosis is initiated by the activation of various cell-surface phagocytosis receptors, including Fc receptors, complement receptors, integrins, endotoxin receptors (CD18, CD14), mannose receptors and scavenger receptors [42], which are activated by corresponding extracellular ligands. In the CNS, a full innate immune system, i.e., Fc receptors, complement system, scavenger receptors and Toll-like receptors etc., has been described, and microglia reveal related roles as dedicated phagocytes. It is well-known that dying cells express so-called 'eat-me' signals such as phosphatidylserine (PS) on their surface membrane [42], by which microglia recognize the apoptotic cells in order to catch and remove them [42]. As for amyloid β protein (Aβ), a key molecule that mediates Alzheimer's disease, microglia remove Aβ presumably via Fc receptor-dependent phagocytosis [43, 44].

We first found that exogenous UDP caused microglial phagocytosis in a concentration-dependent manner, which was $P2Y_6$ receptor-dependent [45]. Neuronal injury caused by kainic acid (KA) upregulated $P2Y_6$ receptors in microglia, and the KA-evoked neuronal injury resulted in an increase in extracellular UTP, which was immediately metabolized into UDP *in vivo* and *in vitro* [45]. Moreover, UDP leaked from injured neurons caused $P2Y_6$ receptor-dependent phagocytosis *in vivo* and *in vitro*. Nucleotides seem to have the ability to act as 'eat-me' signals for necrotic cells suffering traumatic or ischemic injury because these cells cause swelling, followed by shrinkage, leading to the leakage of cytoplasmic molecules, including a large amount of ATP and UTP; and extracellular nucleotides are immediately degraded by ecto-nucleotideases, suggesting that leaked nucleotides could be transient and localized signals that alert to the crisis created by the presence of the necrotic cells. It should be noted that nucleotides could be both 'find-me' and 'eat-me' signals. Cells release ATP, and we also found that KA causes an increase in extracellular UTP/UDP [45]. Therefore, microglia might be attracted by ATP/ADP and subsequently recognize UDP, leading to the removal of the dying cells and their debris. It is interesting that ATP/ADP is not able to efficiently activate $P2Y_6$ receptors, nor can UDP act on $P2Y_{12}$ receptors.

Conclusion

We have described how nucleotides work as 'warning molecules' through microglia in a pathophysiological condition. Microglia play a key role in chemotaxis, neuropathic pain and phagocytosis through the nucleotide-evoked activation of $P2Y_{12}$, $P2X_4$ and $P2Y_6$ receptors respectively, expressed on cells. These findings for extracellular nucleotides in microglia during physiological and pathophysiological processes suggest that nucleotide signalling has an important role in the regulation of the functions of microglia.

1 Burnstock, G. and Knight, G. E. (2004) Cellular distribution and functions of P2 receptor subtypes in different systems. Int. Rev. Cytol. 240, 301–304.
2 Kreutzberg, G. W. (1996) Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 19, 312–318.
3 Stoll, G. and Jander, S. (1999) The role of microglia and macrophages in the pathophysiology of the CNS. Prog. Neurobiol. 58, 233–247.
4 Honda, S., Sasaki, Y., Ohsawa, K., Imai, Y., Nakamura, Y., Inoue, K. and Kohsaka, S. (2001) Extracellular ATP or ADP induce chemotaxis of cultured microglia through Gi/o-coupled P2Y receptors. J. Neurosci. 21, 1975–1982.
5 Hollopeter, G., Jantzen, H. M., Vincent, D., Li, G., England, L., Ramakrishnan, V., Yang, R. B., Nurden, P., Nurden, A., Julius, D. and Conley, P. B. (2001) Identification of the platelet ADP receptor targeted by antithrombotic drugs. Nature 409, 202–207.
6 Fumagalli, M., Trincavelli, L., Lecca, D., Martini, C., Ciana, P. and Abbracchio, M. P. (2004) Cloning, pharmacological characterisation and distribution of the rat G-protein-coupled P2Y(13) receptor. Biochem. Pharmacol. 68, 113–124.
7 Haynes, S. E., Hollopeter, G., Yang, G., Kurpius, D., Dailey, M. E., Gan, W. B. and Julius, D. (2006) The P2Y(12) receptor regulates microglial activation by extracellular nucleotides. Nat. Neurosci. 9, 1512–1519.
8 Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., Littman, D. R., Dustin, M. L. and Gan, W. B. (2005) ATP mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. 8, 752–758.
9 Nimmerjahn, A., Kirchhoff, F. and Helmchen, F. (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318.
10 Woolf, C. J. and Mannion, R. J. (1999) Neuropathic pain: aetiology, symptoms, mechanisms, and management. Lancet 353, 1959–1964.
11 Woolf, C. J. and Salter, M. W. (2000) Neuronal plasticity: increasing the gain in pain. Science 288, 1765–1769.
12 Scholz, J. and Woolf, C. J. (2002) Can we conquer pain? Nat. Neurosci. 5 Suppl., 1062–1067.
13 Tsuda, M., Inoue, K. and Salter, M. W. (2005) Neuropathic pain and spinal microglia: a big problem from molecules in 'small' glia. Trends Neurosci. 28, 101–107.
14 Eriksson, N. P., Persson, J. K., Svensson, M., Arvidsson, J., Molander, C. and Aldskogius, H. (1993) A quantitative analysis of the microglial cell reaction in central primary sensory projection territories following peripheral nerve injury in the adult rat. Exp. Brain Res. 96, 19–27.
15 Gehrmann, J. and Banati, R. B. (1995) Microglial turnover in the injured CNS: activated microglia undergo delayed DNA fragmentation following peripheral nerve injury. J. Neuropathol. Exp. Neurol. 54, 680–688.
16 Stuesse, S. L., Cruce, W. L., Lovell, J. A., McBurney, D. L. and Crisp, T. (2000) Microglial proliferation in the spinal cord of aged rats with a sciatic nerve injury. Neurosci. Lett. 287, 121–124.
17 Liu, L., Tornqvist, E., Mattsson, P., Eriksson, N. P., Persson, J. K., Morgan, B. P., Aldskogius, H. and Svensson, M. (1995) Complement and clusterin in the spinal cord dorsal horn and gracile nucleus following sciatic nerve injury in the adult rat. Neuroscience 68, 167–179.
18 E., C. D. (1998) Partial peripheral nerve injury leads to activation of astroglia and microglia which parallels the development of allodynic behavior. Glia 23, 75–83.
19 Colburn, R. W., Rickman, A. J. and DeLeo, J. A. (1999) The effect of site and type of nerve injury on spinal glial activation and neuropathic pain behavior. Exp. Neurol. 157, 289–304.
20 Tsuda, M., Shigemoto-Mogami, Y., Koizumi, S., Mizokoshi, A., Kohsaka, S., Salter, M. W. and Inoue, K. (2003) P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 424, 778–783.
21 Beggs, S. and Salter, M. W. (2006) Stereological and somatotopic analysis of the spinal microglial response to peripheral nerve injury. Brain Behav. Immun. 21, 624–633.
22 Zhang, J. and De Koninck, Y. (2006) Spatial and temporal relationship between monocyte chemoattractant protein-1 expression and spinal glial activation following peripheral nerve injury. J. Neurochem. 97, 772–783.
23 Zhang, R. X., Liu, B., Wang, L., Ren, K., Qiao, J. T., Berman, B. M. and Lao, L. (2005) Spinal glial activation in a new rat model of bone cancer pain produced by prostate cancer cell inoculation of the tibia. Pain 118, 125–136.
24 Tsuda, M., Ueno, H., Kataoka, A., Tozaki-Saitoh, H. and Inoue, K. (2008) Activation of dorsal horn microglia contributes to diabetes-induced tactile allodynia via extracellular signal-regulated protein kinase signaling. Glia 56, 378–386.
25 Coull, J. A., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., Gravel, C., Salter, M. W. and De Koninck, Y. (2005)

BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438, 1017–1021.
26 Coull, J. A., Boudreau, D., Bachand, K., Prescott, S. A., Nault, F., Sik, A., De Koninck, P. and De Koninck, Y. (2003) Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. Nature 424, 938–942.
27 Inoue, K. (2002) Microglial activation by purines and pyrimidines. Glia 40, 156–163.
28 Inoue, K. (2006) The function of microglia through purinergic receptors: neuropathic pain and cytokine release. Pharmacol. Ther. 109, 210–226.
29 Viviani, B., Bartesaghi, S., Gardoni, F., Vezzani, A., Behrens, M. M., Bartfai, T., Binaglia, M., Corsini, E., Di Luca, M., Galli, C. L. and Marinovich, M. (2003) Interleukin-1beta enhances NMDA receptor-mediated intracellular calcium increase through activation of the Src family of kinases. J. Neurosci. 23, 8692–8700.
30 Vikman, K. S., Hill, R. H., Backstrom, E., Robertson, B. and Kristensson, K. (2003) Interferon-gamma induces characteristics of central sensitization in spinal dorsal horn neurons in vitro. Pain 106, 241–251.
31 Nasu-Tada, K., Koizumi, S., Tsuda, M., Kunifusa, E. and Inoue, K. (2006) Possible involvement of increase in spinal fibronectin following peripheral nerve injury in upregulation of microglial P2X(4), a key molecule for mechanical allodynia. Glia 53, 769–775.
32 Tsuda, M., Toyomitsu, E., Komatsu, T., Masuda, T., Kunifusa, E., Nasu-Tada, K., Koizumi, S., Yamamoto, K., Ando, J. and Inoue, K. (2008) Fibronectin/integrin system is involved in P2X(4) receptor upregulation in the spinal cord and neuropathic pain after nerve injury. Glia 56, 579–585.
33 Tsuda, M., Tozaki-Saitoh, H., Masuda, T., Toyomitsu, E., Tezuka, T., Yamamoto, T. and Inoue, K. (2008) Lyn tyrosine kinase is required for P2X(4) receptor upregulation and neuropathic pain after peripheral nerve injury. Glia 56, 50–58.
34 Guo, L. H., Guo, K. T., Wendel, H. P. and Schluesener, H. J. (2006) Combinations of TLR and NOD2 ligands stimulate rat microglial P2X4R expression. Biochem. Biophys. Res. Commun. 349, 1156–1162.
35 Ohsawa, K., Irino, Y., Nakamura, Y., Akazawa, C., Inoue, K. and Kohsaka, S. (2007) Involvement of P2X(4) and P2Y(12) receptors in ATP-induced microglial chemotaxis. Glia 55, 604–616.
36 Kurpius, D., Nolley, E. P. and Dailey, M. E. (2007) Purines induce directed migration and rapid homing of microglia to injured pyramidal neurons in developing hippocampus. Glia 55, 873–884.
37 Di Virgilio, F., Chiozzi, P., Ferrari, D., Falzoni, S., Sanz, J. M., Morelli, A., Torboli, M., Bolognesi, G. and Baricordi, O. R. (2001) Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood 97, 587–600.
38 Sasaki, Y., Hoshi, M., Akazawa, C., Nakamura, Y., Tsuzuki, H., Inoue, K. and Kohsaka, S. (2003) Selective expression of Gi/o-coupled ATP receptor P2Y12 in microglia in rat brain. Glia 44, 242–250.
39 Tozaki-Saitoh, H., Tsuda, M., Miyata, H., Ueda, K., Kohsaka, S. and Inoue, K. (2008) P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. J. Neurosci. 28, 4949–4956.
40 Kobayashi, K., Yamanaka, H., Fukuoka, T., Dai, Y., Obata, K. and Noguchi, K. (2008) P2Y12 receptor upregulation in activated microglia is a gateway of p38 signaling and neuropathic pain. J. Neurosci. 28, 2892–2902.
41 Tjelle, T. E., Lovdal, T. and Berg, T. (2000) Phagosome dynamics and function. Bioessays 22, 255–263.
42 Lauber, K., Bohn, E., Krober, S. M., Xiao, Y. J., Blumenthal, S. G., Lindemann, R. K., Marini, P., Wiedig, C., Zobywalski, A., Baksh, S., Xu, Y., Autenrieth, I. B., Schulze-Osthoff, K., Belka, C., Stuhler, G. and Wesselborg, S. (2003) Apoptotic cells induce migration of phagocytes via caspase-3-mediated release of a lipid attraction signal. Cell 113, 717–730.
43 Schenk, D., Barbour, R., Dunn, W., Gordon, G., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D., Lee, M. et al. (1999) Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. Nature 400, 173–177.
44 Bard, F., Cannon, C., Barbour, R., Burke, R. L., Games, D., Grajeda, H., Guido, T., Hu, K., Huang, J., Johnson-Wood, K., Khan, K., Kholodenko, D. et al. (2000) Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. Nat. Med. 6, 916–919.
45 Koizumi, S., Shigemoto-Mogami, Y., Nasu-Tada, K., Shinozaki, Y., Ohsawa, K., Tsuda, M., Joshi, B. V., Jacobson, K. A., Kohsaka, S. and Inoue, K. (2007) UDP acting at P2Y6 receptors is a mediator of microglial phagocytosis. Nature 446, 1091–1095.