

## Commentary & View

# UDP Facilitates Microglial Phagocytosis Through P2Y6 Receptors

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microglia, phagocytosis, P2Y6 receptors, UDP

### ABSTRACT

Microglia engage in the clearance of dead cells or dangerous debris. When neighboring cells are injured, the cells release or leak ATP into extracellular space and microglia rapidly move toward or extend a process to the nucleotides as chemotaxis through P2Y12 receptors. In the meanwhile, microglia express the metabotropic P2Y6 receptors, the activation of which by uridine 5'-diphosphate (UDP) triggers microglial phagocytosis in a concentration-dependent fashion. UDP/UTP was leaked when hippocampal neurons were damaged by kainic acid in vivo and in vitro. Systemic administration of kainic acid in rats resulted in neuronal cell death in the hippocampal CA1 and CA3 regions, where increases in mRNA for P2Y6 receptors in activated microglia. Thus, the P2Y6 receptor is upregulated when neurons are damaged, and would function as a sensor for phagocytosis by sensing diffusible UDP signals.

Accumulating findings indicate that nucleotides play an important role in neuron to glia communication through P2 purinoceptors, even though ATP is recognized primarily to be a source of free energy and nucleotides are key molecules in cells. P2 purinoceptors are divided into two families, ionotropic receptors (P2X) and metabotropic receptors (P2Y) (Fig. 1). P2X receptors (seven types; P2X<sub>1</sub>–P2X<sub>7</sub>) contain intrinsic pores that open by binding with ATP. P2Y (eight types; P2Y<sub>1,2,4,6</sub> and 11–14) are activated by nucleotides and couple to intracellular second-messenger systems through heteromeric G-proteins.<sup>1</sup> Microglia express P2X<sub>4</sub>, P2X<sub>7</sub>, P2Y<sub>2</sub>, P2Y<sub>6</sub> and P2Y<sub>12</sub><sup>1</sup> and are known as resident macrophages in CNS, accounting for 5–10% of the total population of glia.<sup>2,3</sup> When neurons are injured or dead, microglia are activated, resulting in their interaction with immune cells, active migration to the site of injury, release of pro-inflammatory substances and the phagocytosis of damaged cells or debris. For such activation of microglial motilities, extracellular nucleotides have a central role. Extracellular ATP functions as a chemoattractant. Microglial chemotaxis by ATP via P2Y<sub>12</sub> receptors was originally found by Honda et al.,<sup>4</sup> and has recently been confirmed in vivo in P2Y<sub>12</sub> receptor knockout animals.<sup>5</sup> Neuronal injury results in the release or leakage of ATP that appears to be a “find-me” signal from damaged neurons to microglia to cause chemotaxis. In addition to microglial migration by ATP, another nucleotide, UDP, an endogenous agonist of the P2Y<sub>6</sub> receptor, greatly activates the motility of microglia and orders microglia to engulf damaged neurons.<sup>6</sup>

Phagocytosis is a specialized form of endocytosis taking relatively large particles (> 1.0 μm) into vacuoles and has a central role in tissue remodeling, inflammation and the defense against infectious agents.<sup>7</sup> Phagocytosis is initiated by the activation of cell-surface phagocytosis receptors, including Fc receptors, complement receptors, integrins, endotoxin receptors (CD18, CD14), mannose receptors and scavenger receptors<sup>8</sup> which are activated by corresponding extracellular ligands called as “eat-me” signals. Since recognition is the most important step for phagocytosis, extensive studies on phagocytosis receptors have been reported. With regard to apoptotic cells, it is well known that dying cells express so called “eat-me” signals such as phosphatidylserine (PS) on their surface membrane,<sup>8</sup> by which microglia recognize the apoptotic cells in order to catch and remove them.<sup>8</sup> As for amyloid β protein (Aβ), a key molecule that mediates Alzheimer's disease, microglia remove Aβ presumably via Fc receptor-dependent phagocytosis.<sup>9,10</sup> It, however, is unclear how phagocytotic cells come to the target cells or debris. Our findings suggest that nucleotides might be the molecules to guide phagocytotic cells to the targets.

We found that exogenously applied UDP caused microglial phagocytosis through P2Y<sub>6</sub> in a concentration-dependent manner, and that neuronal injury caused by kainic

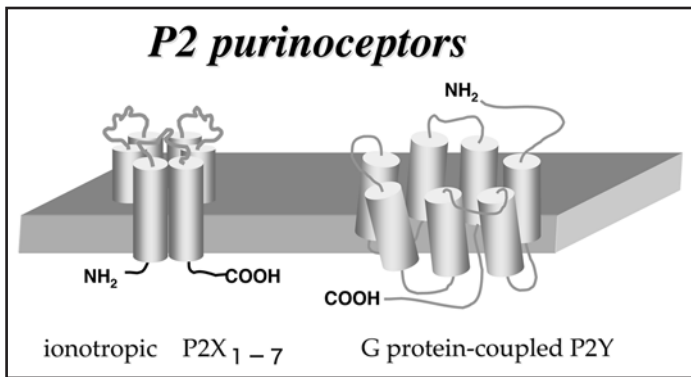


Figure 1. P2 purinergic receptors (ATP receptors).

acid (KA) upregulated P2Y<sub>6</sub> receptors in microglia, the KA evoked neuronal injury resulted in an increase in extracellular UTP, which was immediately metabolized into UDP *in vivo* and *in vitro*. We also found that UDP leaked from injured neurons caused P2Y<sub>6</sub> receptor-dependent phagocytosis *in vivo* and *in vitro*. Thus, UDP could be a diffusible molecule that signals the crisis of damaged neurons to microglia, triggering phagocytosis. Nucleotides seem to have the ability to act as “eat-us” signals for necrotic cells suffering traumatic or ischemic injury because such necrotic 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-nucleotidases, suggesting that leaked nucleotides could be transient and localized signals that alert to the crisis created by the presence of the necrotic cells. These findings suggest that microglia might be attracted by ATP/ADP<sup>4,5,11,12</sup> and subsequently recognize UDP, starting to recognize “eat-me” signals attached to the targets and engulf them (Fig. 2). It is interesting that ATP/ADP is not able to efficiently activate P2Y<sub>6</sub> receptors, nor can UDP act on P2Y<sub>12</sub> receptors. Thus, adenine and uridine nucleotides would regulate microglial motilities, i.e. chemotaxis and phagocytosis, in a coordinated fashion.

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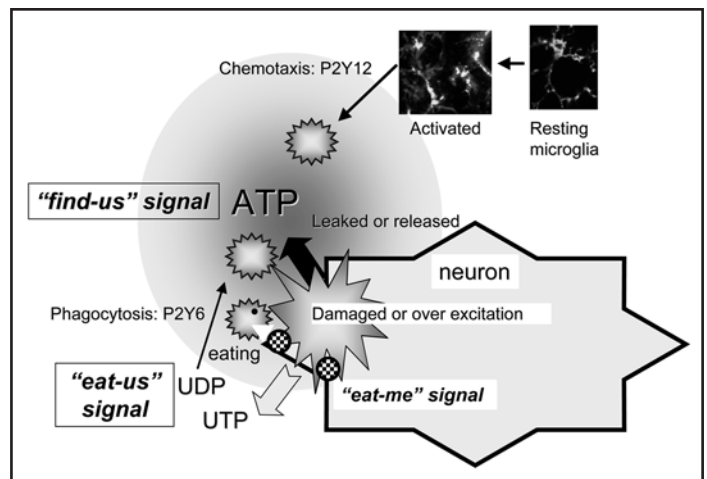


Figure 2. Illustration of nucleotide-activated microglial chemotaxis and phagocytosis. Activated microglia might be attracted by ATP/ADP is not able to efficiently activate P2Y<sub>6</sub> receptors, nor can UDP act on P2Y<sub>12</sub> receptors.

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