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REVIEW ARTICLE

Extracellular ATP and other nucleotides—ubiquitous triggers of intercellular messenger release

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Abstract Extracellular nucleotides, and ATP in particular, are cellular signal substances involved in the control of numerous (patho)physiological mechanisms. They provoke nucleotide receptor-mediated mechanisms in select target cells. But nucleotides can considerably expand their range of action. They function as primary messengers in intercellular communication by stimulating the release of other extracellular messenger substances. These in turn activate additional cellular mechanisms through their own receptors. While this applies also to other extracellular messengers, its omnipresence in the vertebrate organism is an outstanding feature of nucleotide signaling. Intercellular messenger substances released by nucleotides include neurotransmitters, hormones, growth factors, a considerable variety of other proteins including enzymes, numerous cytokines, lipid mediators, nitric oxide, and reactive oxygen species. Moreover, nucleotides activate or co-activate growth factor receptors. In the case of hormone release, the initially paracrine or autocrine nucleotide-mediated signal spreads through to the entire organism. The examples highlighted in this commentary suggest that acting as ubiquitous triggers of intercellular messenger release is one of the major functional roles of extracellular nucleotides. While initiation of messenger release by nucleotides has been unraveled in many contexts, it may have been overlooked in others. It can be anticipated that additional nucleotide-driven messenger functions will be uncovered with relevance for both understanding physiology and development of therapy.

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Introduction

The external control of cellular functions is typically executed by signal substances of varying chemical nature—which may act alone, in parallel, consecutively, or also in a hierarchical manner. This can complicate the identification of the primary signal pathway relevant for initiating a particular cellular response. One signal substance may induce the synthesis and release of another signal substance, which may subsequently be held singly responsible for a specific effect. Or one signal substance may activate the receptor of another signal substance via receptor crosstalk and transactivation and thus mimic its function. When analyzing the functional control of (patho)physiological processes, it is thus important to identify the hierarchical contribution of individual extracellular signaling cues. This is of vital importance for choosing the target most relevant for therapeutic interference.

This commentary makes a case for ATP and other extracellular nucleotides as primary signals in releasing other signal molecules (messengers) in intercellular communication. It does not discuss the multiple additional cellular functions which are directly elicited by extracellular nucleotides, including exocrine secretion. Nor does it discuss other extracellular signal substances which may—in specific cases—elicit similar or identical functions as extracellular nucleotides or adenosine which also induces multiple cellular functions via its own receptors [1]. Rather, it specifically highlights the role of extracellular nucleotides in inducing the cellular release of other, secondary, signal substances or in mimicking their function.

Within the past years numerous studies have demonstrated that the nucleotides ATP, UTP, ADP, UDP, and also UDP glucose can act as ubiquitous triggers of intercellular messenger release. Extracellular nucleotides are outstanding for several reasons. They are omnipresent and thus represent ubiquitously available signal substances. Essentially, every cell of an organism can release nucleotides and expresses nucleotide receptors [2, 3]. Moreover, many cells express several subtypes of nucleotide receptors (P2 receptors, P2Rs) with differing ligand specificity and/or affinity, allowing for a large variety of intracellular responses in both physiological and pathological contexts. This article highlights examples of nucleotide-mediated cellular release of a considerable variety of messenger substances of differing chemical nature, cellular origin, and function. It suggests that one of the major physiological roles of extracellular nucleotides is to act as primary signals in the release of other messenger substances thereby controlling multiple physiological and pathological mechanisms.

Nucleotide signaling in a nutshell

Physiological mechanisms controlled by extracellular nucleotides, and by ATP in particular, include neurotransmission and neuromodulation [4], glial and glial-neuron interactions [5], the development of the nervous system [6], pain [7], control of hormone secretion [8], sensory transmission [9], the function of a variety of organ systems such as the special senses [10], kidney and urinary tract [11, 12], liver [13], the cardiovascular system [14, 15], the immune system [16, 17], the musculoskeletal system [18, 19], the airways [20], the gastrointestinal system [21], or pancreatic and salivary gland epithelia [22]. Signaling via extracellular nucleotides is even more prominent in multiple pathological conditions, including neuropathic pain, a considerable number of disorders of the central nervous system, such as trauma, cerebral ischemia, multiple sclerosis, Parkinson's disease, or Alzheimer's disease [23], and diseases of essentially every peripheral organ system, including cardiovascular disorders, cancer, and inflammation [13, 24–27].

Extracellular nucleotides impact specific cell surface-located receptors functioning either as ionotropic P2X receptors (P2XRs, [28]) or metabotropic G protein-coupled receptors P2Y receptors (P2YRs, [29]) (Table 1). P2XRs are activated by ATP and ensure rapid signaling. The seven P2XR subunits (P2X1 to P2X7) form trimeric, often heteromeric ATP channels permeable to Na^+, K^+, and Ca^{2+} ions. The Na^+ influx leads to membrane depolarization and the Ca^{2+} influx can, in addition, trigger a variety of intracellular events, partly through activation of mitogen-activated protein kinases (MAPKs), protein kinase C (PKC), and calmodulin [34]. Among the P2XRs, the multifunctional P2X7R plays a special role and has become a therapeutic target for several diseases [35]. It carries a number of unique structural and functional features. Its requirement for high ATP concentrations biases it towards mediating (patho)physiological situations such as cell stress or inflammation, which are accompanied by massive nucleotide release, whereas it is minimally activated under physiological conditions. Prolonged or repeated stimulation can induce dilation of the P2X7R pore to allow molecules up to 900 Da to diffuse into and out of the cells. The extended C-terminus bears several structural motifs suggested to serve as docking sites for intracellular proteins and in the regulation of receptor function and cellular localization [35–37]. The P2X7R can thus trigger multi-pathway signaling including activation of caspase 1, phospholipases A_2 and D, MAPKs, PKC, Src, glycogen synthase kinase-3 (GSK-3), or phosphatases. Of note, the functional properties of the P2X7R can vary between species and cell type which hampers the comparison of results obtained from different animal or cellular sources [36, 38].

The seven P2YRs fall into two subgroups. $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ activate Gq and phospholipase Cβ, generating the two second messengers inositol 1,4,5-trisphosphate (IP3) which increases intracellular Ca^{2+} via release from intracellular stores and diacylglycerol which activates PKC. $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ activate G_i, inhibiting adenylyl cyclase and reducing intracellular cyclic adenosine monophosphate (cAMP) levels. Activating the P2Y11R (which is absent in rodents) increases both intracellular Ca^{2+} and cAMP by activating both Gq and Gs. Additional intracellular signaling pathways have been described for P2YRs, including the involvement of the Gβγ subunit, resulting in the activation of a diverse group of effectors including phosphatidylinositol-4,5-bisphosphate 3-kinase γ (PI3K-γ), phospholipase C-β2 and -β3, inward rectifying K^+ (GIRK) channels, G protein-coupled receptor (GPCR) kinases 2 and 3, Rho, and MAP kinases [29, 30]. These examples highlight the enormous potential of nucleotide-stimulated intracellular signaling. P2YRs differ in agonist specificity but there is redundancy of nucleotide ligands. ADP is the preferred ligand of the $P2Y_1R$ (in rodents also ATP), $P2Y_{12}R$, and $P2Y_{13}R$. Both UTP and ATP are agonists of the $P2Y_2R$, whereas the $P2Y_4R$ is activated by UTP only. UDP is the agonist of the $P2Y_6R$ and ATP activates the $P2Y_{11}R$. Importantly, agonist affinities vary considerably between receptor subtypes, ranging from nanomolar in some P2YRs to micromolar (most P2XRs) and even hundred micromolar (P2X7R) (Table 1). The amount and type of extracellular nucleotide released thus determines which type of nucleotide receptor will be activated.

Agonist activity is rapidly terminated by cell surface-located enzymes (ectonucleotidases), which hydrolyze nucleoside triphosphates successively to the respective nucleoside. ADP and UDP can be produced as intermittent hydrolysis

Table 1 Properties of mammalian P2 nucleotide receptors

P2X receptors				P2Y receptors			
Subtype	Native agonist	EC_{50} (μM)	Function	Subtype	Native agonist	EC_{50} (μM)	G protein
P2X1	ATP	1	Na^+, K^+, Ca^{2+} permeable	$P2Y_1$	ADP (also ATP in rodents)	8.1	G_q
P2X2	ATP	10	Na^+, K^+, Ca^{2+} permeable	$P2Y_2$	UTP, ATP	0.085 0.06	G_q
P2X3	ATP	1	Na^+, K^+, Ca^{2+} permeable	$P2Y_4$	UTP	0.09	G_q
P2X4	ATP	7	Na^+, K^+, Ca^{2+} permeable	$P2Y_6$	UDP	0.53	G_q
P2X5	ATP	0.5	Na^+, K^+, Ca^{2+} permeable	$P2Y_{11}$ (lacking in rodents)	ATP	17.3	G_q, G_s
P2X6	ATP	0.5	Na^+, K^+, Ca^{2+} permeable	$P2Y_{12}$	ADP	0.06	G_i
P2X7	ATP	100	Na^+, K^+, Ca^{2+} permeable, pore formation, induction of intracellular signal pathways	$P2Y_{13}$	ADP	0.011	G_i
Potential heteromeres: P2X1/2; P2X1/4, P2X1/5, P2X2/3; P2X2/6, P2X4/6	ATP	0.6–32	Na^+, K^+, Ca^{2+} permeable	$P2Y_{14}$	UDP, UDP glucose	0.16 0.4	G_i

P2X receptors are trimeric ATP-gated ion channels. P2Y receptors display a subtype-specific agonist profile and couple to trimeric G proteins. Data from [28–31]. Several P2XRs and P2YRs can be activated by dinucleotides, typically dinucleoside tri- and diphosphates [32, 33]

products of ATP and UTP, resulting in elimination of ligands of nucleoside triphosphate receptors and generation of ligands for nucleoside diphosphate receptors. Additional ectoenzymes can interconvert extracellular nucleotides and may in certain cell types significantly contribute to increase the pericellular concentrations of ATP via phosphotransfer reactions [39].

Multiple mechanisms of nucleotide release exist. Depending on the cellular and physiological context, ATP may be released by organelle-mediated exocytosis, by surface-located hemichannels (connexins, pannexins), channel proteins (P2X7Rs, maxichannels, calcium homeostasis modulator 1), and also cell damage or lysis. Notably, there is also basal or constitutive release from resting cells. In near membrane environment, this may be sufficient to activate P2Rs [40–43]. Presumably, exocytotic and conductive/transport mechanisms operate to a variable extent in many cell types [41]. For astrocytes, ATP release has been reported to be mediated by connexins 43 and 30 hemichannels, the P2X7R, pannexin 1 hemichannels, and exocytosis [44]. Stimuli inducing cellular release of ATP and other physiologically active nucleotides include activation of secretory cells including neurons and glia, mechanical insults such as shear stress and osmotic swelling, infective agents, trauma, or a variety of messenger molecules, including Ca^{2+}-mobilizing agonists such as bradykinin, histamine, thrombin—and ATP itself [41].

Extracellular nucleotides stimulate the release of other messengers in several ways. Activation of P2Rs can directly stimulate the release of another messenger (Fig. 1a) or enhance the release of a messenger induced through another signal pathway (Fig. 1b). Nucleotide-mediated release may require a conditioning stimulus initiated through another signal pathway (Fig. 1c). The nucleotide co-released with another messenger may act as an autocrine/paracrine signal to enhance its own release as well as that of another messenger (Fig. 1d). Through an autocrine mechanism, release of the nucleotide can be regenerative. ATP-mediated ATP release may serve the propagation of Ca^{2+} signals between cells (Fig. 1e). Or the nucleotide activates or co-activates the receptor of another messenger, thus mimicking its function (Fig. 1d). Hybrid versions between these possibilities exist. In many cases, the action of nucleotides is locally restricted to autocrine and paracrine signaling. It is important to note that, by inducing the release of hormones, nucleotides also control distant physiological mechanisms.

Ubiquity of nucleotide signaling does not exclude specificity of action. Ubiquity of nucleotide signaling is secured by the omnipresence of nucleotide release and nucleotide receptors. Specificity is ensured by locally restricted nucleotide release in specific target tissues and cellular niches and within specific (patho)physiological contexts. In addition, the type of nucleotide released and type and affinity of nucleotide receptor are key determinants of the physiological outcome of nucleotide signaling.

This commentary focuses on select examples highlighting the multiplicity of extracellular messengers whose release is initiated by extracellular ATP and other nucleotides. The functional consequences of nucleotide-stimulated messenger release are only briefly addressed. Similarly, detailing the nucleotide-induced intracellular signal pathways and the mechanisms underlying messenger release are beyond the

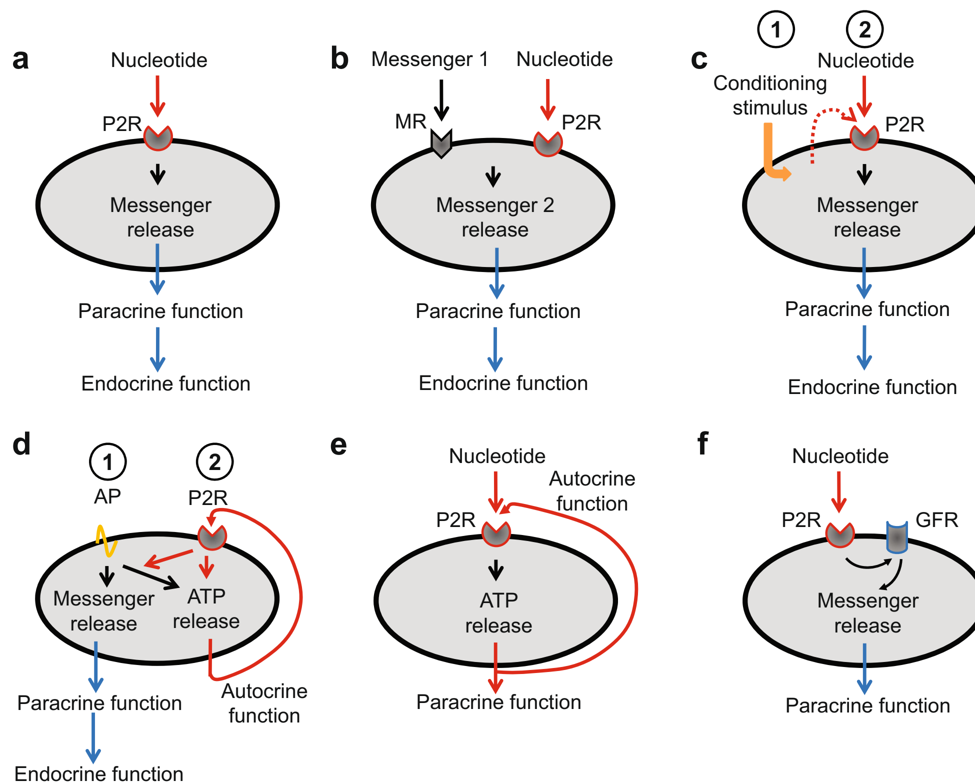

Fig. 1 Possible modes of nucleotide-mediated extracellular messenger release. **a** The nucleotide directly elicits release of a messenger substance which may exert paracrine or endocrine functions. Examples include testosterone, luteinizing hormone, cathepsins, prostaglandin E2, tumor necrosis factor, and CXCL16. **b** Nucleotides enhance the release of a messenger induced by another messenger molecule. This applies to many hormones such as testosterone, luteinizing hormone, prolactin, cortisol, or also interleukins. There is overlap with **a** since some of the hormones can also be released by nucleotide alone. **c** A conditioning stimulus (*1*) is essential for nucleotides (*2*) to induce messenger release. Typical examples are insulin release whereby pancreatic β cells first require a glucose stimulus or interleukin 1-β release whereby macrophages require stimulation through pathogen-associated molecular pattern molecules (PAMPs). PAMP-induced ATP release (*dotted arrow*) activates interleukin 1-β release in an autocrine manner. **d** A stimulus (*1*) induces co-release of ATP and another extracellular messenger molecule. The releasing cell expresses P2Rs allowing autocrine enhancement (*2*) of both messenger and ATP release. Action potential-induced co-release of noradrenaline or vasopressin with ATP would be typical examples. **e** ATP induces its own release resulting in regenerative ATP release through an autocrine pathway. In addition, released ATP can exert paracrine functions such as propagation of Ca^{2+} signals between cells. Examples include endothelial cells or astrocytes. **f** Nucleotides elicit growth factor receptor transactivation, resulting in messenger release induced by the growth factor. Examples include VEGFR or EGFR. *AP* action potential, *GFR* growth factor receptor, *MR* receptor for messenger molecule, *P2R* P2 receptor

scope of this commentary. Not in all studies was the subtype of P2R involved in messenger release defined. This applies particularly to the earlier literature and the time before the P2R subtypes were identified in molecular terms. Moreover, differences between species and cell type sometimes limit the assignment of P2R subtypes to nucleotide-mediated messenger release. The repertoire of expressed P2Rs may vary between cells in situ, cells in primary culture, and immortalized cells. Culturing may lead to reprogramming of the cellular proteome. Even in tissue slices, the cellular expression of receptors and other proteins may be altered within a short time after preparation [45]. On the other hand, experiments on cultured cells or tissue preparations often provide principle information on signal pathways and may guide further experimentation in situ in the living animal. For this reason, information on nucleotide-induced messenger release in model cellular systems are equally included in this overview.

Release of intercellular messenger directly elicited or enhanced by nucleotide

There are numerous examples for direct stimulation or enhancement of intercellular messenger release by ATP and other nucleotides (Table 2). These include neurotransmitters, hormones, growth factors, various enzymes, cytokines, lipid mediators, nitric oxide, and reactive oxygen species. While individual types of extracellular messengers may be released through different cellular mechanisms they have in common that they transfer the nucleotide stimulus to other cells.

Amino acid neurotransmitters

In general, in the nervous system, nucleotides may be released from neurons or glial cells either alone or together with other neurotransmitters. They function as widespread signal

Table 2 Release of intercellular messengers directly elicited or enhanced by nucleotide

Messenger category	Type of messenger released	Nucleotide/receptor implicated	Outcome	References
Neurotransmitters	Glutamate	ATP/P2, P2X, P2X2, P2X4, P2X7, $P2Y_1$	Increased neuronal mEPSC frequency, enhancement of excitatory transmission; glial glutamate release	[46–59]
	Aspartate	ATP/P2, $P2Y_1$	Glial aspartate release	[54, 55, 58, 60, 61]
	D-Serine	ATP/P2X7	Glial D-serine release	[62]
	Glycine	ATP/P2X	Increased neuronal mIPSC frequency, enhancement of inhibitory transmission	[63–65].
	GABA	ATP, AP_4A/P2, P2X2, P2X4, P2X7	Increased neuronal mIPSC frequency, enhancement of inhibitory transmission; glial GABA release	[65–72]
	Noradrenaline	ATP/P2X, P2Y	Neuronal noradrenaline release	[73–85]
	Dopamine	ATP/P2X, P2Y	Neuronal dopamine release	[86–89]
	Serotonin	ATP/P2X4	Dopamine release in mouse taste bud system	[90, 91]
	ATP	ATP/P2R, $P2Y_1$, $P2Y_2$	ATP release from neurons, astrocytes	[49, 50, 80, 92–99]
Hormones	Vasopressin	ATP/P2X, P2X2, P2X3, P2X4, P2X7, $P2Y_1$	Vasopressin release from neurohypophyseal nerve terminals	[100–108]
	Oxytocin	ATP/PX2/3, P2X7, P2Y1	Oxytocin release following activation of neurosecretory cell somata	[100, 105, 106, 108]
	Luteinizing hormone (LH)	ATP, UTP/P2X2, P2X5, P2Y	LH release from gonadotrophs	[109–111]
	LH-releasing hormone	ATP/P2X	LH-releasing hormone release from hypothalamic neurons	[112, 113]
	Prolactin	ATP, ADP/P2X4, $P2Y_1$	Prolactin release from lactotrophs	[114–116]
	Neuropeptide Y	ATP, UTP/P2X7, $P2Y_2$	Neuropeptide Y release in olfactory epithelium	[117–119]
	Cortisol	ATP, ADP, UTP/P2Y, P2Y1	Cortisol release from bovine adrenal zona fasciculata, human adrenal cortex cell line	[120–125]
	Testosterone	ATP, UTP/P2	Testosterone release from rat Leydig cells	[126]
	Estradiol	ATP, UTP/P2X, P2Y	Estradiol release from rat Sertoli cells	[127]
Growth factors	Fibroblast growth factor 2 (FGF2)	ATP, UTP/P2	FGF2 release from mouse olfactory epithelium	[128]
	Transforming growth factor α (TGFα)	ATP, UTP/P2	TGFα release from mouse olfactory epithelium	[128]
	Vascular endothelial growth factor (VEGF)	ATP, UTP/$P2Y_2$	VEGF release from metastatic cancer cells	[129]
	Brain-derived neurotrophic factor (BDNF)	ATP/P2X4, P2X7	BDNF release from synoviocytes, microglia	[130–135]
Enzymes / Proteins	Cathepsins B, K, L, S	ATP/P2X7	Cathepsin release from macrophages, microglia	[136–138]
	Eosinophil cationic protein (ECP)	ATP/$P2Y_2$	ECP release from human eosinophil granulocytes	[139]
	Lysyl oxidase	ATP, UTP/$P2Y_2$	Lysyl oxidase release from breast cancer cells	[140]
Cytokines	CXCL8 (Interleukin 8)	ATP, UTP, UDP glucose/P2X1, P2X7, $P2Y_2$, $P2Y_6$, $P2Y_{14}$	CXCL8 release from various cell types	[139, 141–150]
	CCL2	ATP, UDP/P2X7, $P2Y_6$	CCL2 release from microglia, glioma cells	[146, 151–153]
	CCL3	ATP/P2X7	CCL3 release from microglia	[151–153]
	CCL20	ATP/P2X7	CCL20 release from stem cells derived from human periodontal ligament cells	[147]
	CXCL2	ATP/P2X7	CXCL2 release from microglia	[151–153]

Table 2 (continued)

Messenger category	Type of messenger released	Nucleotide/receptor implicated	Outcome	References
	Interleukin-6 (IL-6)	ATP, UTP/P2X7, P2Y, $P2Y_1$, $P2Y_2$, $P2Y_{11}$, $P2Y_{13}$	IL-6 release from various cell types	[151, 154–166]
	Interleukin-10 (IL-10)	ATP, ADP, URP/$P2Y_1$, $P2Y_{11}$	IL-10 release from microglia	[166–168]
	Leukemia inhibitory factor (LIF)	ATPγS, UTP/$P2Y_2$	LIF release from cultured mouse and rat astrocytes	[169, 170]
	Tumor necrosis factor (TNF)	ATP/P2X7, $P2Y_1$	TNF release from microglia	[56, 171–175]
Proteins released by membrane shedding	CXCL16	ATP/P2X7	ADAM10-mediated CXCL16 shedding from myeloma cells	[176]
	CD62L	ATP/P2X7	CD26L shedding from leucocytes	[177–181]
	CD23	ATP / P2X7	ADAM 10-mediated shedding of CD23 from immune cells	[176, 179, 182, 183]
	CD21	ATP/P2X7	CD21 shedding from peripheral blood mononuclear cells	[177]
	Metalloprotease-9	ATP/P2X7	Metalloproteinase-9 release from peripheral blood mononuclear cells	[184]
	Soluble amyloid precursor protein α (sAPPα)	UTP/$P2Y_2$	sAPPα release from P2Y2R-transfected human 1321N1 astrocytoma cells by α-secretase-mediated shedding	[185]
Lipids	Arachidonic acid (AA)	ATP, UTP/$P2Y_2$	AA release from various cell types	[186–189]
	Prostaglandin E2 (PGE2)	ATP, ATPγS/P2X7, $P2Y_1$, $P2Y_2$	PGE2 release from various cell types	[56, 190–194]
	Prostacyclin	ATP, UTP/P2	Prostacyclin release from endothelial cells	[195–198]
	Thromboxane A2 (TXA2)	ATP, ADP/$P2Y_{12}$	TXA2 release from platelets, astrocytes	[199–201]
	Thromboxane B2 (TXB2)	ATP/P2X7	TXB2 release from primary human peritoneal macrophages and blood monocytes	[202]
	Leukotriene B4 (LTP4)	ATP/P2X7	LTP4 release from primary human peritoneal macrophages and blood monocytes	[202]
	Sphingosine 1-phosphate	ATP via ABCA family transporter	Sphingosine 1-phosphate release from rat platelets	[203]
Nitric oxide (NO)/reactive oxygen species (ROS)	NO	ATP, UTP/P2X, P2X7, $P2Y_2$, $P2Y_4$	NO release from numerous cell types	[204–216]
	ROS	ATP/P2X7, $P2Y_1$	ROS release from numerous cell types	[217–220]

substances activating P2XRs and P2YRs [221]. In peripheral synapses, ATP can act as fast neurotransmitter through its own postsynaptic P2XRs. But also for the central nervous system, there is evidence that ATP functions as a fast neurotransmitter and elicits neuronal excitatory postsynaptic potentials (EPSPs) [222]. In addition, many studies suggest that ATP increases the release probability of excitatory and inhibitory neurotransmitters, thereby enhancing excitatory or inhibitory transmission, respectively—typically via presynaptic P2XRs allowing influx of Ca^{2+} [46, 222].

P2XR activation can directly facilitate glutamate release and increase action potential-independent spontaneous miniature excitatory postsynaptic current (mEPSC) frequency [47, 48]. Blocking Na^{+}-dependent action potential propagation by tetrodotoxin allowed recording of mEPSCs in isolation and excluded effects of P2XR agonists through other intermediate neurons. An analysis of the caudal part of the rat nucleus of the solitary tract showed that P2XR agonists, by triggering direct Ca^{2+} entry through P2XRs, not only increased mEPSC frequency but also elicited mEPSCs of larger amplitude than basal mEPSCs. Temporal summation of the miniature events was sufficient to directly evoke glutamate-mediated postsynaptic action potentials [49]. In rat dorsal root ganglion (DRG)—dorsal horn co-culture, P2XRs of non-specified subtype located at presynaptic sites of DRG neurons increased the frequency of spontaneous glutamate release. Moreover, activation of P2XRs at or near presynaptic DRG nerve terminals elicited action potentials which caused evoked glutamate release [50]. The authors suggest that activation of P2XRs at DRG central terminals can modify sensory signal throughput, and might even initiate sensory signals at central synapses without direct peripheral input. Several studies show that glutamate release is due to activation of neuronal P2X7Rs. These include cultured hippocampal neurons [47], cerebellar granule neurons [51], or cerebrocortical nerve terminals [52]. Location of P2X7Rs on the nerve terminals was demonstrated for isolated synaptosomes [52, 53] or in electrophysiological studies [46, 47, 49].

ATP also enhances the presynaptic release of inhibitory neurotransmitters. The frequency of spontaneous miniature inhibitory postsynaptic currents (mIPSCs) was increased by P2XR-mediated mechanisms in several preparations. In dissociated rat dorsal horn interneuron synapses, ATP, via a P2XR, facilitated action potential-independent glycine release from nerve terminals resulting in increased frequency of mIPSCs, presumably leading to inhibition of rat substantia gelatinosa neurons which conduct nociceptive signals to the CNS [63]. Similarly, ATP increased the frequency of glycinergic mIPSCs via P2XRs in a preparation of dissociated trigeminal nucleus pars caudalis neurons [64].

In GABAergic synaptic terminals isolated from rat midbrain, GABA (γ-minobutyric acid) release could be stimulated by both ATP and P^1,P^5-di(adenosine-5) pentaphosphate (Ap_5A), a nucleotide which presumably acts on P2Rs [66]. In isolated neurons cultured from the laminae I–II of the dorsal horn the ATP-induced increase in action-potential-independent GABAergic mIPSC frequency was mediated through receptors with a pharmacological profile dominated by the P2X2R subunit. It was thought that the presynaptic receptors facilitating GABA release might underlie the modulatory role of ATP on a subset of GABAergic interneurons involved in spinal processing of nociceptive information [67]. In slices of the hypothalamic suprachiasmatic nuclei (SCN) of the rat application of ATP increased the frequency of spontaneous GABAergic IPSCs without changes in their amplitudes, a presynaptic effect presumably mediated through P2X2Rs. Extracellular ATP may thus be involved in the regulation of electrical activity of the circadian pacemaker cells of the SCN [68]. In cerebrocortical cultures P2X7R agonists acting on GABAergic terminals enhanced mIPSC frequency, an effect increased by ischemic conditions [69].

ATP can simultaneously enhance release of the excitatory neurotransmitter glutamate and of the inhibitory neurotransmitter GABA. In hippocampal slices, neuronal outflow of both GABA and glutamate was observed as a result of P2X7R activation [70], an effect abolished in P2X7R knockout mice [223]. In neurons of the hypothalamic supraoptic nucleus, the ATP-mediated increase in frequency of GABAergic or glutamatergic spontaneous synaptic currents was attributed to the activation of both presynaptic and extrasynaptic P2XRs, possibly P2X2Rs and P2X4R2, thus controlling the electrical excitability of these neurons. GABAergic and glutamatergic inputs were from different neurons [71]. Moreover, ATP can simultaneously increase GABAergic and glycinergic transmission. In rat brain stem slices, P2XR activation increased both glycinergic and GABAergic mIPSC frequency to parasympathetic cardiac vagal neurons, thus enhancing inhibitory neurotransmission [65].

These examples show that ATP increases the neuronal release of excitatory and inhibitory amino acid neurotransmitters in the CNS, thus enhancing excitatory or inhibitory transmission, respectively. It is intriguing that the presynaptic release of these fast neurotransmitters is mediated through P2XRs, fast ATP-gated ion channels.

Glial amino acid release

In addition, Schwann cells and astrocytes are sources of ATP-evoked amino acid release. In Schwann cells cultured from rat DRGs, ATP stimulated glutamate and aspartate release via P2Rs by releasing Ca^{2+} from internal stores [60]. Similarly, ATP induced release of glutamate and aspartate from astrocytes isolated from neonatal rat cerebral cortex [54] or mouse hippocampus [55] via a Ca^{2+}-dependent mechanism involving intracellular Ca^{2+} stores. In astrocyte cultures from newborn

rats and mice release of glutamate via Ca^{2+} release from internal stores was elicited through $P2Y_1Rs$ [56]. In turn, inhibition of $P2Y_1Rs$ in astrocytes cultured from rat dorsal spinal cord inhibited ATP-induced glutamate release and Ca^{2+} mobilization [57]. Interestingly, in mixed hippocampal cultures, $P2Y_1R$-evoked glutamate release was restricted to astrocytes and did not occur in neurons or microglia, the resident macrophages of the brain [56]. In another scenario, in mouse cortical astrocyte cultures, the P2X7R was implicated in ATP-mediated release of glutamate and aspartate [58]. Also D-serine, an N-methyl-D-aspartate receptor (NMDA) receptor co-agonist was released from primary astrocyte cultures through the P2X7R [62]. In rat hippocampal slices, activation of P2X7Rs mediated sustained astrocyte glutamate efflux, generating a tonic current in CA1 neurons [59]. Gliosomes, glial subcellular re-sealed particles, preloaded with [^{3}H]D-aspartate released aspartate on stimulation with ATP in a Ca^{2+}-dependent manner [61]. The inhibitory neurotransmitter GABA could be released from an astrocytic cell line through activation of P2X7Rs [72]. Additional studies emphasize the involvement of volume-regulated anion channels (VRACs) and of cell swelling in P2YR-dependent excitatory amino acid release from cultured astrocytes [224–226].

These studies suggest that glial amino acid release can be stimulated via both P2YRs and P2XRs, including P2X7Rs. Possibly, the culture conditions impact P2R subtype expression or ATP-induced astrocyte release can be elicited by more than one mechanism. Since astrocytes are in intimate contact with both neuronal cell bodies and synaptic contacts, ATP-mediated release of gliotransmitters is now considered a major mechanism for the modulation of neural function [227, 228]. The impact of ATP on astrocytes and astrocyte-neuron interaction is further enhanced under pathological conditions such as trauma, inflammation, hypoxia, or ischemia which are accompanied by enhanced ATP release from damaged cells [229].

Biogenic amines

ATP also stimulates the release of biogenic amines. Early on, it was shown that electrically evoked release of previously incorporated [^{3}H] noradrenaline from cultured chick [73] or rat [74] sympathetic neurons was enhanced by ATP or an ATP analog acting on P2Y-like receptors. In a later study on cultured rat sympathetic neurons, both ATP and UTP induced [^{3}H]noradrenaline release, albeit via different mechanisms. ATP-induced release depended on influx of Ca^{2+} through voltage-gated Ca^{2+} channels and probably a P2XR. The UDP-induced release was elicited via the generation of action potentials followed by Ca^{2+} influx through voltage-gated channels [75]. Several later studies report that ATP enhances noradrenaline release via presynaptic P2XRs. This includes a guinea pig heart synaptosomal preparation [76, 77], the prostatic portion of the vas deferens [78], the isolated right atrium of the guinea pig [79], and also central catecholaminergic terminals in rat hippocampal slices [80]. Since noradrenaline and ATP are co-released from sympathetic nerve terminals, the released ATP acts via a positive feedback mechanism in an autocrine manner amplifying its action. ATP also induced secretion of [^{3}H]noradrenaline from prelabeled PC12 cells, a cell line derived from the adrenal medulla and serving as a model system for catecholaminergic neurons [81, 82]. This release depended on cellular Ca^{2+} influx [83, 84] presumably through P2XR channels [85].

An in vivo microdialysis study in rat striatum demonstrated ATP-induced and Ca^{2+}-dependent dopamine release, presumably via stimulation of P2YRs [86]. In the nucleus accumbens, dopamine release was facilitated by endogenous ATP via stimulation of P2YRs [87, 88]. Further evidence for ATP-induced dopamine release comes from studies on PC12 cells, initiated via Ca^{2+} influx through ATP-activated P2XR channels [85, 89]. In the taste bud system, which is not of neural origin, ATP released from receptor (type II) cells was suggested to activate neighboring presynaptic (type III) taste cells via $P2Y_4Rs$, inducing vesicular release of serotonin [90, 91].

These examples illustrate that ATP can enhance the release of biogenic amines, including noradrenaline, dopamine, and serotonin. Depending on the preparation, P2XRs or P2YRs are involved.

ATP

ATP can stimulate its own release by an autocrine mechanism and, in addition, act on neighboring cells to propagate the cellular state of activation. This aspect will be discussed in a separate section.

Neuropeptides from the hypothalamo-posterior pituitary unit

The endocrine hypothalamic-neurohypophysial system controls diuresis and parturition through the release of the neuropeptide hormones vasopressin and oxytocin [100]. Vasopressin and oxytocin are synthesized in the magnocellular neurons of the hypothalamus and released in the posterior pituitary (neurohypophysis). ATP is co-released with the neuropeptides from the activated neurosecretory terminals. In isolated rat neurohypophysial nerve terminals, ATP stimulated the release of vasopressin (but only of minor amounts of oxytocin) via an increase in intracellular Ca^{2+} concentrations, presumably through P2X2Rs [101–103]. In a later study, it was suggested that co-released ATP potentiates vasopressin release through the activation of P2X2Rs, P2X3Rs, P2X4Rs, and P2X7Rs whereas oxytocin release is mediated only through the P2X7R [100]. Using electrical stimulation of isolated and

intact mouse posterior pituitaries, the concept was supported that the co-released endogenous ATP acts as a paracrine-autocrine messenger, enhancing vasopressin secretion from neurohypophysial nerve terminals [104].

In perifused explants of the rat hypothalamo-neurohypophysial system, containing cell bodies of neurosecretory neurons, application of ATP elicited vasopressin and oxytocin release presumably involving P2XRs [105]. In a more recent study on the perifused rat and mouse hypothalamo-neurohypophysial system (excluding the paraventricular nucleus), activation of $P2Y_1Rs$ increased intracellular Ca^{2+} concentrations in supraoptic nucleus neurons and release of vasopressin and oxytocin, an effect abrogated in $P2Y_1R-/-$ explants [106]. The vasopressin-synthesizing neurons in the supraoptic nucleus received a dense innervation from noradrenergic fibers co-releasing noradrenaline and ATP which synergistically stimulate vasopressin release [107]. In another series of experiments on rat hypothalamo-neurohypophysial system explants, co-exposure to ATP and an α1-adrenergic receptor agonist induced an extended elevation of vasopressin and oxytocin release. This involved P2XRs (P2X2/3 and P2X7) but not $P2Y_1Rs$ [108].

Despite the apparent differences in receptor profiles implicated, the results demonstrate that ATP can stimulate neuropeptide release in the hypothalamo-neurohypophysial system both at the level of the hypothalamic nuclei and the neurosecretory nerve endings.

Hormones from the hypothalamo-anterior pituitary unit

Gonadotrophs in the anterior pituitary are excitable endocrine cells and express P2Rs. They release ATP together with gonadotrophins such as the follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on activation with gonadotropin-releasing hormone (GnRH). ATP or UTP induced exocytotic release of LH from superfused enzymatically dispersed pituitary glands from female rats, presumably involving P2YRs [109]. The authors also provided evidence that this was accompanied by an increase in intracellular Ca^{2+} concentrations and that pituitary cells can themselves release ATP, implicating an autocrine and/or paracrine action of ATP on pituitary cells. Similarly, in anterior pituitary cells from normal and ovariectomized female rats, activation of ATP-gated channels (in this case, possibly P2X2Rs and P2X5Rs) lead to an increase in Ca^{2+} influx and cell depolarization. LH release was stimulated in the absence of GnRH. In addition, ATP was shown to enhance GnRH-induced LH secretion. It was co-released on stimulation of pituitary cells with GnRH, further supporting the notion that ATP release acts as an autocrine positive feedback system serving the self-potentiation of LH signaling and secretion [110]. In a more recent study on anterior pituitary cells from normal female rats, it was shown that gonadotrophs exclusively express P2X2Rs, and that these channels have the capacity to contribute to the control of pacemaking activity and IP3-dependent Ca^{2+} release. The ATP-induced depolarization of gonadotrophs lead to initiation of firing in quiescent cells, an increase in the frequency of action potentials in spontaneously active cells, and a transient stimulation of LH release. The P2XRs in gonadotrophs would thus operate as modulators of GnRH-controlled electrical activity and hormone secretion [111].

Additional evidence suggests that also the upstream LH-releasing hormone neurons (LHRH neurons) can be activated by ATP. In an early study on hypothalamic slices or explants from the rat median eminence, extracellular ATP was found to facilitate the copper stimulation of LHRH release [112]. LHRH neurons derived from the olfactory placode region of monkey embryos responded to ATP with an increase in intracellular Ca^{2+} and LHRH release. The action of ATP was mediated through P2XRs (possibly P2X2Rs and P2X4Rs, but not through P2YRs) [113].

Prolactin is another hormone produced in the anterior pituitary. It is synthesized in lactotrophs and its release is stimulated by thyrotropin-releasing hormone (TRH) produced in the hypothalamus. In an early study on dispersed cells derived from the rat anterior pituitary, addition of ATP increased prolactin secretion. ATP and TRH exerted partially additive effects. Since ATP is locally released, it was suggested that it subserves an autocrine and/or paracrine role in the regulation of prolactin release [114]. In mixed anterior pituitary cells from female rats, ATP and ADP, but not UTP and UDP, triggered Ca^{2+} signaling in lactotrophs and prolactin release. The analysis of the P2Rs involved suggested that Ca^{2+}-mobilizing $P2Y_1Rs$ and cation-conducting P2X4Rs played a major role in ATP- and ADP-induced calcium signaling and prolactin release [115]. In a more recent study on the same cell preparation confirming the ATP-induced release of prolactin, P2X4Rs were implicated in the facilitation of Ca^{2+} influx and prolactin secretion [116].

These data show that hormone release from the hypothalamo-anterior pituitary unit can be stimulated by ATP both at the level of the anterior pituitary secretory cells and at the hypothalamus. P2XRs and P2YRs have been implicated.

Neuropeptide Y

Neuropeptide Y (NPY), is widely distributed in the central and peripheral nervous system and also in the peripheral olfactory system. In the mouse olfactory epithelium ATP- or UTP-activated purinergic receptors evoked release of NPY which mediates neuroproliferation [117, 118]. This presumably involved P2X7Rs and $P2Y_2Rs$, intracellular Ca^{2+} increase and the IP3 receptor [119].

Secretion of steroid hormones

The glucocorticoid cortisol is generated in the zonal fasciculata of the adrenal gland from where it is typically released by the adrenocorticotropic hormone of the anterior pituitary. Several reports described nucleotide-mediated stimulation of steroidogenesis and cortisol secretion from cells of the bovine adrenal zona fasciculata. Cortisol secretion could be induced by ATP, ADP, and UTP through P2YRs, involving an increase in intracellular Ca^{2+} and cAMP [120–122]. Accordingly, ATP potentiated steroidogenesis induced by adrenocorticotrophic hormone by accelerating cAMP production [123]. Another study revealed that activation of P2YRs in the adrenal zona fasciculata cells by purine and pyrimidine nucleotides was coupled to membrane depolarization through inhibition of a specific K^+ channel, suggesting a mechanism for ATP-stimulated corticosteroid secretion which depends on depolarization-dependent Ca^{2+} entry [124]. This type of nucleotide-induced cortisol secretion appears to be absent from rat tissue. A recent study on the human adrenal cortex cell line NCI-H295R, which expresses all the key enzymes necessary for steroidogenesis, linked the $P2Y_1R$ to Ca^{2+} influx and cortisol secretion which may also apply to steroidogenesis and corticoid release in the human adrenal cortex [125].

Leydig cells in the semiferous tubules of the testicles produce the hormone testosterone, typically as a result of stimulation by LH released from the anterior pituitary. Leydig cells isolated from rat testes express P2Rs. Extracellular ATP or UTP stimulated testosterone secretion via a mechanism dependent on Ca^{2+} influx from the extracellular medium. Possibly, autonomic nerve fibers are the source of nucleotides stimulating testosterone release from Leydig cells [126]. Estradiol, the primary female sex hormone, is produced also by Sertoli cells, a cell type in the semiferous tubule of the testicles. ATP and UTP stimulated estradiol secretion from Sertoli cells isolated from young rats via P2Rs belonging to the P2XR and P2YR family. This involved a rise in intracellular Ca^{2+} and Na^+ concentrations and Na^+-dependent plasma membrane depolarization. As for the release of testosterone from Leydig cells, nucleotide release from autonomic nerve fibers could be involved in estradiol release from Sertoli cells [127]. These results show that—in addition to proteinaceous or peptide hormones—nucleotides can elicit release of small hydrophobic and membrane permeable hormones.

Growth factors

In the adult mouse olfactory epithelium, ATP induced cell proliferation by promoting the synthesis and release of two growth factors, fibroblast growth factor 2 (FGF2) and transforming growth factor alpha (TGFα) and activation of their receptors [128]. ATP or UTP stimulated release of vascular endothelial growth factor (VEGF) from highly metastatic breast cancer cells, implicating $P2Y_2Rs$ in cancer progression [129].

Brain-derived neurotrophic factor (BDNF) is the most widely expressed neurotrophin in the central nervous system. It has important functions in brain development and plasticity-related processes and it is a crucial neuromodulator in central nociceptive hypersensitivity [230, 231]. ATP-mediated increased expression and/or cellular release has been demonstrated for BDNF in a variety of cell types. In BDNF-expressing synoviocytes of osteoarthritis and rheumatoid arthritis patients, release of BDNF was driven by ATP through the P2X4R and activation of p38-MAPK [130]. Activation of the P2X4R initiated the phosphorylation of p38-MAPK and subsequent synthesis and exocytotic release of BDNF from microglia. There, released BDNF induces disinhibition of pain-transmitting spinal lamina I neurons [131–133]. Following peripheral nerve injury, P2X4Rs were expressed by activated microglia whose stimulation lead to BDNF release. BDNF release and mechanical hyperalgesia induced by peripheral nerve injury were abrogated in P2X4R-deficient mice. These results suggest that ATP, via activation of P2X4Rs and release of BDNF, contributes to chronic pain through a central inflammatory pathway [134]. On the other hand, in Schwann cells co-cultured with inner ear vestibular neurons, vesicular exocytosis of BDNF was triggered by activation of the P2X7R. Released BDNF in turn supports vestibular neuron survival and growth [135]. These studies reveal that stimulation of growth factor release considerably broadens the impact of ATP on cell proliferation, differentiation and development.

Cathepsins

Cathepsins are lysosomal proteases involved in several pathologies including osteoporosis, rheumatoid arthritis, osteoarthritis, bronchial asthma, and cancer. While they act in lysosomal protein turnover, they can also be released from cells via lysosomal exocytosis and exert various extracellular functions [232]. In resting human lung macrophages and mouse bone marrow-derived macrophages, ATP, through the P2X7R, induced the rapid release of cathepsin B, K, L, and S. Cathepsin release was absent in P2X7R-deficient mouse macrophages. Since the released cathepsins degrade collagen extracellular matrix, it has been suggested that the ATP-induced cathepsin release contributes to tissue damage such as joint disease pathology [136]. Microglial cathepsin S is a major player in microglial-neuronal communication. In mixed glial cultures from postnatal rats, activation of the P2X7R induced cathepsin S release, which was associated with p28 mitogen-activated kinase phosphorylation and phospholipase A2-mediated translocation of cathepsin S-containing lysosomes to the plasma membrane. Since cathepsin S expressed by

spinal microglia is vital for the full expression of neuropathic pain, the authors suggest that P2X7R antagonists can contribute to reducing neuropathic pain by inhibition of cathepsin S release [137]. In microglia from cerebral cortex of neonatal mice, ATP-induced release of cathepsin B through activation of the P2X7R contributed to glial migration and may thus play a role in injury—or cell damage-induced migration of glia in the brain [138].

Eosinophil cationic protein

Secretion of eosinophil cationic protein (ECP), a protein released from human eosinophil granulocytes during degranulation of eosinophils, is induced by nucleotides via P2YRs, presumably $P2Y_2Rs$. This cytotoxic protein can cause respiratory, epithelial, and cardiovascular tissue damage and consequently inflammation [139].

Lysyl oxidase

By releasing enzymes which induce environmental changes facilitating metastasis nucleotides can play a role in tumor metastasis. ATP or UTP released from hypoxia-treated MDA-MB-231 breast cancer cells induced hypoxia inducible factor-1α expression and secretion of lysyl oxidase by activating $P2Y_2Rs$. Released lysyl oxidase induces collagen crosslinking. Recruitment of hematopoietic bone marrow-derived cells to cross-linked collagen at distant sites facilitates environmental changes which promote cancer cell colonization and metastasis [140].

Inflammatory chemokines(C-C motif) ligands

Inflammatory chemokines (C-C motif) ligands (CCLs) are one example of nucleotide-mediated control of inflammatory mediator release. Their main function is to direct immune cells to the site of inflammation via chemotaxis. Numerous studies report nucleotide-mediated release of chemokine (C-X-C motif) ligand 8, (CXCL8, previously interleukin-8, IL-8). CXCL8 is produced by a variety of cell types, including monocytes, lymphocytes, granulocytes, fibroblasts, epithelial cells, and endothelial cells. It is an inflammatory chemokine which functions as a neutrophil chemoattractant and activation factor. It also attracts eosinophils, basophils, and a subpopulation of lymphocytes [141, 233, 234]. Several studies have implicated UDP and the $P2Y_6R$ in the stimulation of CXCL8 production and release. Antimicrobial human neutrophil peptides (HNPs) selectively induced CXCL8 production in human lung epithelial cells, predominantly through UDP and a $P2Y_6R$ signaling pathway. HNPs exhibit antimicrobial activity against a variety of microorganisms, such as Gram-positive and Gram-negative bacteria, viruses, and fungi [141]. Activation of the $P2Y_6R$ was also found to induce CXCL8 gene expression and release in human monocytic THP-1 cells. The study showed that LPS-induced CXCL8 production can be mediated at least in part by autocrine $P2Y_6R$ activation [142]. Both UTP and UDP were found to stimulate the expression and release of CXCL8 by the human colonic epithelial cell line Caco-2/15, involving the $P2Y_6R$ [143, 144].

In other studies, additional P2Rs were found to be involved. The secretion of CXCL8 from human eosinophil granulocytes could be triggered by several nucleotides acting via $P2Y_6Rs$ (UDP), P2X1Rs (ATP), or P2X7Rs (ATP). Eosinophils are terminally differentiated effector cells involved in the defensive response against parasites as well as in the pathogenesis of allergic diseases and immunological disorders [139]. In the human retinal pigment epithelial cell line ARPE-19 ATPγS, UTP, and UDP stimulated both basal and TNF-induced secretion of CXCL8 through an ERK-dependent pathway, an effect mediated through $P2Y_2Rs$ and $P2Y_6Rs$ [145]. P2R activation controlled both basal and LPS-induced CXCL8 secretion from human glioma cells involving $P2Y_6Rs$ and P2X7Rs. Knockdown of $P2Y_6R$ expression decreased LPS-induced CXCL8 release as well as spontaneous release of CXCL8, suggesting endogenous basal release of nucleotides. Since glioblastoma multiforme is one of the most aggressive brain tumors, these data suggest that $P2Y_6R$ and/or P2X7R antagonists may be suitable candidates for controlling glioma progression [146].

Human periodontal ligament stem cells (hPDLCs) released CXCL8 following extended stimulation of the P2X7R, involving an increase in intracellular Ca^{2+} concentrations [147]. CXCL8 release from human neutrophils was stimulated synergistically by toll-like receptors and P2Rs. Addition of nucleotides released CXCL8 from neutrophil-like human promyelocytic leukemia (HL60) cells. In contrast, release from human blood neutrophils required inhibition of the ectonucleotidase nucleoside triphosphate diphosphohydrolase 1 (NTPDase1), suggesting that endogenous ectonucleotidase activity can abrogate the nucleotide-mediated effect. In this case, the ATP and UTP-activated $P2Y_2R$ was identified as the major nucleotide receptor involved [148]. Similarly, the release of CXCL8 from a human uroepithelial cell line could be stimulated via the $P2Y_2R$ [149]. In another cellular context, in A549 and BEAS-2B cell airway epithelial cells, release of CXCL8 was induced by UDP-glucose and $P2Y_{14}Rs$. Receptor activation evoked stimulation of Ca^{2+} transients, suggesting that UDP-glucose can play an important role in activation of airway epithelial cells and regulation of immune responses [150]. These data suggest that the subtype of P2R involved in nucleotide-mediated release of CXCL8 considerably depends on tissue/cell source and animal type.

CCL2 (also monocyte chemotactic protein 1), CCL3 (also macrophage inflammatory protein-1α), and CXCL2 (also macrophage inflammatory protein 2-α) play an important role in inflammatory processes. As for CXCL8, P2R activation

controlled both basal and LPS-induced CCL2 secretion from human glioma cells. Both P2X7Rs and $P2Y_6Rs$ appeared to be involved [146]. In microglia, ATP induced messenger RNA (mRNA) expression and release of CCL2, CCL3, and CXCL2 mainly through activation of P2X7Rs [151–153]. Chemokine production was mediated via both calcineurin-dependent nuclear factor of activated T cells (NFAT) and PKC/MAPK signaling pathways. Similar to CXCL8, stem cells derived from human periodontal ligament cells released CCL20 (also macrophage inflammatory protein-3, MIP3A), a chemoattractant for lymphocytes and neutrophils, through P2X7R activation [147].

Interleukin-6-type of cytokines

Interleukins represent a large group of immunomodulatory proteins involved in both inflammatory and anti-inflammatory actions. They are released from cells and act via cell surface-located high affinity receptors in an autocrine and paracrine manner, similar to nucleotides. During an immune response, they can modulate cell proliferation, maturation, migration, and adhesion [235]. Extracellular nucleotides such as ATP represent constitutive signals which alert the immune system. Excessive release of ATP can lead to excessive inflammatory conditions. There are numerous examples that nucleotides stimulate production and release of interleukins [236]. They do so through different nucleotide receptors and in varying (patho)physiological contexts. Select examples for nucleotide-mediated interleukin release are provided in the following section. Interleukins whose nucleotide-induced release requires a conditioning stimulus are discussed in a later subchapter.

Numerous studies describe nucleotide-evoked release of Interleukin-6 (IL-6). Depending on tissue, cell type, and experimental conditions, different P2Rs can be involved. IL-6 is a member of the IL-6-type of cytokines. It is produced by a variety of cells including endothelial cells, fibroblasts, monocytes, and macrophages in response to different stimuli during systemic inflammation and involved in the regulation of immune response, hematopoiesis, and inflammation [235]. The P2X7R was shown to mediate IL-6 release in several cell types. Human skin fibroblasts pre-treated with bacterial endotoxin released IL-6 when stimulated with a P2X7R agonist [154]. ATP stimulated the release of IL-6 in mouse peritoneal macrophages, an effect greatly reduced in P2X7R-deficient mice [155]. P2X7R agonists increased mRNA expression and release of IL-6 in primary cultured mouse microglia. Antagonists blocked the P2X7R-dependent release of IL-6 [151]. P2X7R activation alone induced IL-6 release without the need for co-stimulatory activation through LPS. In contrast to primary cultured microglia [151], IL-6 release from the mouse microglial cell line MG-5 was stimulated via phospholipase C-linked P2YRs [156]. Another study reports increased P2X7R-dependent release of IL-6 in macrophages exposed to glioma-conditioned medium. The macrophages were differentiated into a M2-like phenotype associated with tumor growth [157]. Elevated free fatty acids have been recognized as a major cause of nervous system damage in diabetes. High fatty acids increased P2X7R expression in PC12 cells and activation of the P2X7R increased IL-6 release which may in turn induce pathogenic changes in the nervous system [158].

In other scenarios, IL-6 production or release was found to be mediated primarily by metabotropic purinergic receptors, as for example in human keratinocytes [159, 160]. In astrocytes from neonatal rat hippocampus, ATP induced the release IL-6 via the $P2Y_1R$ which in turn produced a neuroprotective effect on co-cultured neurons [161]. IL-6 release from a human uroepithelial cell line was elicited by ATP via the $P2Y_2R$ [149]. In cultured normal human keratinocytes, urate crystals released IL-6 through the $P2Y_6R$ [162]. Treatment with ATP and activation of the $P2Y_{11}R$ increased LPS-induced IL-6 release in THP-1 human acute monocytic leukemia cells. But ATP induced IL-6 production also without LPS-treatment [163]. A silica nanoparticle-primed human keratinocyte cell line (HaCaT) equally released IL-6 by a $P2Y_{11}R$-dependent mechanism [164]. LPS-induced release of IL-6 from mouse liver Kupffer cells was enhanced by the ADP-activated $P2Y_{13}R$ and possibly by additional P2YRs, leading to liver inflammation. Kupffer cells are macrophages resident in the liver and play important roles in liver inflammation induced by various toxic agents, including LPS [165].

These data show that nucleotides can activate cellular release of IL-6 both in the presence and in the absence of a conditioning stimulus and that IL-6 release can be activated via different, perhaps sometimes overlapping nucleotide signal pathways.

The anti-inflammatory cytokine interleukin-10 (IL-10) is released from rat microglial cells following stimulation with either ATP or ADP in an autocrine fashion, possibly involving $P2Y_1Rs$ and $P2Y_{11}Rs$. This suggests that P2Rs also have a function which counteracts the effect of proinflammatory mediators [167, 168].

Leukemia-inhibitory factor

The cytokine leukemia-inhibitory factor (LIF) is released from cultured mouse astrocytes in response to ATP. In a mixed neuron-glia culture system, ATP liberated from axons firing action potentials induced astrocytic LIF release, which in turn, promoted myelination of mature oligodendrocytes [169]. In a subsequent study, LIF could be released from primary cultures of mouse hippocampal astrocytes by treatment with ATPγS and UTP. The pharmacological analysis suggested that $P2Y_2Rs$ were involved [170]. The study further provided evidence that LIF can be released through ATP from electrically

active neurons via a $P2Y_2R$-dependent mechanism. The released LIF promoted astrocyte differentiation.

Tumor necrosis factor

Tumor necrosis factor (TNF, formerly tumor necrosis factor-α) is a major cytokine released from activated macrophages involved in systemic inflammation. It has earlier been reported that extracellular ATP alone was able to promote TNF release from RAW 264.7 macrophages and potentiate its production induced by low doses of LPS [171]. ATP stimulated the release of TNF via the $P2Y_1R$ from murine hippocampal astrocytes [56] or from astrocyte cultures derived from mouse spinal cord [172]. In other studies on primary cultured rat microglia, ATP stimulated the de novo synthesis and release of TNF via activation of P2X7Rs. This involved the three MAP kinase family members ERK, c-Jun N-terminal kinase (JNK), and p38. While a cytotoxic function is often attributed to TNF in the brain, investigations on co-cultures of neurons and microglia implied a neuroprotective effect [173, 174]. In a later study using the microglial cell line MG5, a more complex and time-dependent mechanism of ATP-mediated TNF release was suggested. This involved initially both metabotropic P2YRs, P2X7Rs, an initial increase in cytosolic Ca^{2+} levels, and store-operated channels which, following inactivation of purinergic receptors, eventually triggered a second phase of cytosolic Ca^{2+} increase and TNF release [175].

Shedding of membrane-bound proteins

ATP via the P2X7R can induce ectodomain cleavage of plasma membrane-bound proteins via activation of membrane metalloproteases. This way, the presence of these surface molecules can be regulated and physiologically active ectodomains are generated. Some examples are presented below. CXCL16 exists in both transmembrane and soluble forms and is expressed by numerous cell types. The membrane-bound cell surface-expressed form of CXCL16 is a scavenger receptor and adhesion molecule. The cleaved and soluble ectodomain of CXCL16 acts as a proinflammatory chemokine [237]. Activation of P2X7Rs induced the rapid shedding of CXCL16 from RPMI 8226 human myeloma cells involving the metalloprotease a disintegrin and metalloproteinase 10 (ADAM10) [176]. The adhesion molecule CD62L (L-selectin) is constitutively expressed on leukocytes and interacts with carbohydrate ligands mediating the initial rolling of leukocytes on the endothelium. Agonists of the P2X7R released the soluble form of CD62L into the extracellular milieu [177–180]. Soluble CD62L was found to inhibit lymphocyte attachment to cytokine-activated endothelium via binding to CD62L ligands [181]. Similarly, CD23, the low-affinity receptor for immunoglobulin E (IgE) exists in membrane-bound and soluble forms. The protein molecule is expressed on the surface of B cells, on monocytes, and to a lower extent, on T cells, eosinophils, platelets, and a subset of dendritic cells. P2X7R-mediated shedding of CD23 has been reported for B cell lymphocytes [179], human dendritic cells [182], RPMI 8226 human myeloma cells [176], or human and murine B cells [183], again via activation of ADAM 10 metalloproteinase. Soluble CD23 has been shown to control IgE synthesis and homeostasis in human B cells [238]. Human CD21 (complement receptor type 2, CR2) is a transmembrane glycoprotein strongly expressed on mature B cells and follicular dendritic cells and weakly on immature thymocytes and T lymphocytes. Similar to CD62L, CD21 was released from human peripheral blood mononuclear cells on activation of P2X7Rs [177]. By inducing release of proinflammatory cytokines and upregulating expression of molecules involved in antigen presentation, soluble CD21 modulates critical monocyte functions which may be relevant to allergic and inflammatory disorders [239]. Activation of the P2X7R by ATP also augmented extracellular matrix metalloprotease-9 activity by rapidly increasing its release from human peripheral-blood mononuclear cell. Digestion of the extracellular matrix would help recruitment of monocytes by assisting their emigration from blood into the tissue [184].

UTP enhanced the release of soluble alpha-amyloid precursor protein (sAPPα), the non-amyloidogenic soluble cleavage product of amyloid precursor protein (APP). In $P2Y_2R$-transfected human 1321N1 astrocytoma cells, the mechanism involved activation of α-secretase [185]. While proteolytic processing of APP by α-secretase precludes the formation of the amyloidogenic and neurodegenerative Aβ fragment, the product sAPPα plays important functional roles supporting neuroprotection, cell proliferation, and neurogenesis [240].

These data assign a primary role to the P2X7R in ATP-mediated release of membrane-bound proteins which subsequently regulate a variety of cellular functions.

Lipid mediators

It has long been known that ATP stimulates synthesis and release of arachidonic acid, an intermediate of prostaglandin synthesis, created in a variety of cells from diacylglycerol and phospholipase-A_2. In the osteoblast-like cell line MC3T3-E1, extracellular ATP stimulated Ca^{2+} influx resulting in the release of arachidonic acid. This enhanced prostaglandin E2 synthesis and cell proliferation [186]. ATP or UTP equipotently stimulated release of [^{3}H]arachidonic acid from prelabeled primary cultures of human nasal epithelium and a cell line (BEAS39) derived from bronchial epithelium, presumably through the $P2Y_2R$. Receptor activation triggered cytosolic Ca^{2+} elevation and phospholipase A_2 activation. The authors suggested that exposure to the nucleotides might be of relevance for Cl^- secretion from airway epithelium

[187]. In preloaded primary cultures from rat cerebellum [^{3}H]arachidonic acid release could be equally stimulated with ATP or UTP. Ca^{2+} and protein kinase C interacted to regulate this response [188]. In primary total rat brain-derived astrocyte-enriched cell cultures, ATP induced the release of arachidonic acid. The release was mediated through phospholipase-A_2 but the subtype of P2R involved was not identified [189].

Prostaglandin E2 (PGE2) is the main prostaglandin involved in inflammation. It is produced by several cell types, including antigen-presenting cells, and it plays various roles in the regulation of the immune response but also in other cellular functions [241]. In a human alveolar epithelial cell carcinoma cell line (A549 cells), ATPγS induced production of reactive oxygen species (ROS) through P2R/PKC C/NADPH oxidase signaling. This lead to increased activity of cyclooxygenase-2 (COX-2), another enzyme involved in prostaglandin synthesis and PGE2 release. The authors suggested that inhibitors of P2Rs may be proven useful in diminishing ATP-induced lung inflammation and chronic pathology [190]. Similarly, ATPγS stimulated expression of COX-2 and PGE2 release in rat vascular smooth muscle cells [191]. Activation of the P2X7R enhanced and inhibitors of the receptor blocked the release of PGE2 in primed mouse and primary human macrophages through an inflammosome-independent mechanism. P2X7R-stimulated PGE2 release depended on intracellular Ca^{2+} increase and MAPK signaling. On the other hand, in rabbit articular chondrocytes ATP triggered the release of PGE2 via activation of $P2Y_2$Rs which recruited cytosolic phospholipase A2 by activating both p38 and ERK1/2 mitogen-activated protein kinases. The intracellular pathways acted in concert to stimulate Ca^{2+}-dependent phospholipase A2 (cPLA2), resulting in the liberation of the precursor AA, which is the rate-limiting step for the ATP-stimulated production of PGE2 [192]. In astrocytes cultured from murine brain, ATP released PGE2 via a $P2Y_1$R-mediated mechanism involving Ca^{2+} release from internal stores [56]. Similarly, in primary dissociated cultures of rat dorsal spinal cord ATP was found to stimulate release of arachidonic acid and PGE2 through the $P2Y_1$R. This depended on the transactivation of the EGFR and the phosphorylation of ERK1/2 and cPLA2 [193]. In rat vas deferens, ATP inhibited smooth muscle contraction via P2YR-coupled Ca^{2+} mobilization leading to release of PGE2 from epithelial cells which in turn hyperpolarized the smooth muscle cells, a mechanism by which the epithelium can regulate the contractility of smooth muscle cells [194]. The data suggests that nucleotide-mediated PGE2 release is a common mechanism and that the underlying receptor profiles vary between cell types.

Additional eicosanoids whose release is stimulated by ATP include prostacyclin (prostaglandin I2), thromboxane A2, and leukotriene B4. ATP-mediated release of prostacyclin, which stimulates vasodilation and inhibits platelet activation, has been demonstrated for several endothelial cell preparations. In a preparation of perfused veins from the hamster hindlimb intraluminal ATP stimulated abluminal release of prostacyclin from the venous endothelium through P2Rs [195]. ATP or UTP released prostacyclin from cultured bovine pulmonary artery endothelial cells following an increase in intracellular levels of Ca^{2+} and IP3 and activation of phosphatidylcholine-specific phospholipase A_2 [196]. Similarly, ATP increased the release of Ca^{2+} from intracellular stores and release of prostacyclin in cultured bovine adrenal medullary endothelial cells [197] and in cultured human umbilical vein endothelial cells [198].

In platelets, release of the prothrombotic thromboxane A2 was induced through activation of $P2Y_{12}$Rs [199, 200]. ATP or ADP induced the mobilization of intracellular Ca^{2+} and promoted the release of thromboxane A2 from astrocytes. This may affect the surrounding microvasculature [201]. Thromboxane B2, a metabolite of thromboxane A2, was released from primary human peritoneal macrophages and blood monocytes following ATP application and P2X7R activation [202]. Besides PGE2, P2X7R activation stimulated release of leukotriene B4, another pro-inflammatory lipid mediator, from primed mouse and primary human macrophages [202].

Sphingosine 1-phosphate (S1P) is a polar sphingolipid metabolite. By binding to cell surface G protein-coupled receptors or by eliciting intracellular actions, it functions as both extracellular and intracellular signal molecule. It is stored and released from activated platelets but can also be synthesized in a wide variety of cell types in response to extracellular stimuli. It mediates the regulation of several biological processes, including proliferation, differentiation, apoptosis, and cell motility [242]. Addition of ATP to rat platelets induced S1P release and this was greatly enhanced by thrombin. The authors suggest that an ABCA family transporter with an extracellular ATP-binding domain (rather than a P2R) mediated S1P release [203].

Nitric oxide signaling

Nitric oxide (NO), a free radical and short lived membrane permeable gaseous signal substance, is generated via nitric oxide synthase (NOS) and released in essentially every tissue. It is thus equally ubiquitous as ATP. It can have multiple cellular effects, including autocrine and paracrine functions and—at high concentrations—induce cell damage [243, 244]. There are several reports that ATP induces signaling via NO in the nervous system and in other tissues. ATP functions as an important mediator in neuron-mediated NO-regulated functions such as neurotransmitter release, synaptic dynamics and development, neuropathic pain, and blood flow [204]. In rat hippocampus slices and in isolated hippocampal neurons, ATP increased NO production through P2X7R activation. This effect was independent of glutamate-mediated

NMDA receptor activation, a pathway which also can lead to NO release [205]. Similarly, ATP, via P2XRs, induced increases in intracellular Ca^{2+} and stimulated the production of NO in neuronal cultures of the rat carotid body [206] and in cochlear spiral ganglion neurons [207]. Microinjection of the ATP analog α,β-methylene ATP into the paraventricular nucleus induced local NO production leading to glutamate release acting on postsynaptic NMDA receptors [208]. Similarly, microinjections of ATP or α,β-methylene ATP into the dorsal facial area of the cat medulla induced activation of neuronal NOS/guanylyl cyclase, causing glutamate release acting on postsynaptic glutamate receptors and eventually leading to an increase in common carotid artery blood flow [209].

Nucleotides induce NO production also in astrocytes in a Ca^{2+}-dependent manner [210, 211]. Astrocytic processes surround synaptic contacts and thus participate in neuron-glial signaling in a reciprocal manner [245]. In primary mixed cultures of hippocampal neurons and astrocytes, ATP and UTP evoked astrocytic production of NO, presumably via $P2Y_2$Rs and $P2Y_4$Rs. This suppressed the synchronized Ca^{2+} oscillations in the networked neurons by inhibiting spontaneous glutamate release. The data implied that nucleotides can trigger release of NO as a messenger molecule from astrocytes which in turn modulates synaptic strength in networked neurons [212].

Microglia shows high-intrinsic motility of its cellular extensions, rapidly reacts to lesions of the CNS, and plays a central roles in inflammatory processes. In cultured rat microglia, ATP induced production of NO [213]. In the injured mouse spinal cord white matter, NO acts as a chemoattractant for microglia. ATP enhanced the attraction of microglia to local NO sources which can influence the innate immune processes after spinal cord injury [214].

Additional examples of ATP-elicited production and release of NO include the cochlea [215] or the vascular system, where NO released from endothelium induces smooth muscle relaxation [216].

Reactive oxygen species

Reactive oxygen species (ROS) comprise a number of reactive molecules and free radicals derived from molecular oxygen such as hydrogen peroxide or the hydroxyl radical. They can be toxic but also function as signal molecules [246]. In the RAW 264.7 murine macrophage cell line, P2X7R activation contributed to the production of ROS, an effect augmented by LPS treatment. ROS in turn contributed to nucleotide receptor-mediated p38 and c-Jun N-terminal kinase activation. The study suggested that—via ROS production—P2X7Rs play a critical role in macrophage inflammatory and antimicrobial activities [217]. Similarly, rat peritoneal macrophages were found to respond to ATP with ROS production, presumably involving P2X7Rs [218]. Also, stimulation of the NR8383 rat alveolar macrophage cell line with ATP (mostly via P2X7Rs) and other extracellular nucleotides lead to transient production of high levels of ROS. This was found to stimulate the PI3-kinase pathway and subsequent Akt (protein kinase b) and ERK1/2 activation. Interestingly, ROS-dependent PI3 kinase activation was involved in the activation of caspase-1 and processing and secretion of IL-1β and IL-18, suggesting that ROS can in turn be important mediators of P2X7R-induced pro-inflammatory cytokine release [219]. In mouse skeletal muscle, ATP released by electrical stimulation induced ROS production via activation of $P2Y_1$Rs [220].

Nucleotide activates or co-activates the receptor of another messenger

Growth factors and their receptors constitute a typical example of the close relationship with nucleotide-mediated cellular control. Nucleotides can mimic growth factor signaling by activating or co-activating their receptors. In this way, nucleotides can take advantage of the repertoire of intracellular signal pathways induced through specific growth factor receptors which typically are receptor tyrosine kinases, and considerably broaden their cellular impact (Table 3). In principle, the overlap in intracellular signal pathways could be due to parallel activation of nucleotide and growth factor receptors or due to nucleotide-mediated transactivation of the growth factor receptor. Unless analyzed in detail, these two possibilities may not easily be distinguished and the primary role of the nucleotide in signal initiation may be overlooked.

Also, without receptor cross-talk nucleotides can induce the same intracellular signal cascades as growth factors in the identical cell type. Similar to growth factors, ATP exerts mitogenic effects on numerous cell types [315]. And similar to nucleotides, the growth factors epidermal growth factor (EGF) and vascular endothelial growth factors (VEGF) stimulated a variety of cellular responses, including cell proliferation and migration [316, 317]. ATP and UTP were found to drive, in the identical cell type, intracellular signal cascades and cellular responses corresponding to those of EGF. Adenosine-5 -O-beta-thiodiphosphate (ADPβS, via the $P2Y_1$R and $P2Y_{13}$R), UTP (via the $P2Y_2$R), and EGF all elicited phosphorylation of ERK1/2, CREB, AKT, and focal adhesion kinase in cultured neural progenitor cells and simulated cell proliferation and migration [318–320]. Similarly, ATPγS and NGF acted synergistically to enhance neuronal survival through increased tyrosine receptor kinase signaling [321].

The mechanisms by which the nucleotides stimulate growth factor receptor transactivation are diverse. They can involve direct phosphorylation of the growth factor receptor via activation of intracellular signal cascades or also P2R-mediated shedding of the growth factor ligand leading to

Table 3 Nucleotide activates or coactivates the receptor of another messenger

Receptor type	Nucleotide/receptor implicated	Outcome	References
Epidermal growth factor (EGF) receptor	ATP, UTP/$P2Y_1$, $P2Y_2$, $P2Y_4$	Transactivation and EGF receptor signaling in various cell types	[191, 193, 247–253]
Platelet-derived growth factor (PDGF) receptor	UTP/$P2Y_2$	Transactivation and PDGF receptor signaling in P2Y2 receptor-transfected cells	[248]
Vascular endothelial growth factor (VEGF) receptor-2	2-methyl-thio-ATP, UTP/$P2Y_1$, $P2Y_2$	Transactivation and VEGF receptor-2 signaling in endothelial cells	[248, 254–258]
NGF receptor TrkA	ATPγS/$P2Y_2$	Receptor crosstalk by physical interaction, enhancement of neuronal differentiation	[259]
Insulin-like growth factor 1 (IGF-1)	2MeSADP/$P2Y_{12}$	Increased protein kinase B phosphorylation resulting from cross-talk with IGF-1R in C6 glioma cells	[260]

growth factor receptor activation. The shed growth factor ligand may then act in an autocrine or paracrine way. Transactivation through the human UTP- (and ATP)-activated $P2Y_2R$ has been particular well investigated [254]. The $P2Y_2R$ and the EGFR were found to cooperate in upregulating CXCL8 production via the ERK1/2 pathway, thereby promoting prostate cancer cell invasion and migration [247]. Activation of the human $P2Y_2R$ by UTP induced transactivation of several growth factor receptors, including the EGF receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) in $P2Y_2R$-transfected human 1321N1 astrocytoma cells [248], or of vascular endothelial growth factor-2 receptor (VEGFR-2) in human coronary artery endothelial cells [255]. This involved Src activation via proline-rich SH3-domain-binding motifs at the C-terminus of the $P2Y_2R$ [248, 254, 256]. Activation of the $P2Y_2R$ in human salivary gland cells promoted the formation of heteromers of the EGFR (ErbB1) and of ErbB3, another member of the Erb family of growth factor receptors, resulting in the activation of both EGFR and ErbB3 [249].

EGFR ligand shedding represents an important mechanism of $P2Y_2R$-mediated EGFR transactivation. ATP- and UTP-mediated transactivation of the EGFR via $P2Y_2Rs$ (or $P2Y_4Rs$) has been observed in bovine chromaffin cells. It involved nucleotide-mediated ERK1/2 phosphorylation and metalloproteinase-dependent transactivation of the EGFR [250]. In human pulmonary mucoepidermoid NCI-H292 carcinoma cells, ATP-mediated EGFR activation via the $P2Y_2R$ involved phosphorylation of the EGFR via intracellular signal mechanisms as well as ADAM-17-promoted EGFR ligand shedding [251]. In human corneal epithelial cells, the nucleotide analog ATPγS stimulated PI3K and ERK activation in a Ca^{2+}-dependent manner and through induction of ADAM-mediated heparin-binding EGF-like growth factor (HB-EGF) ectodomain shedding and EGFR transactivation [252] The authors suggested that ATP released from damaged cells triggers P2R-mediated intracellular Ca^{2+} waves leading to activation of ADAM protein(s) in non-injured neighboring cells. The ADAM protein(s) would cleave pro-HB-EGF at the cell surface and HB-EGF would activate the EGFR in an autocrine and/or paracrine manner, activating intracellular signal pathways eventually leading to wound healing.

Additional P2Rs can be involved in EGFR transactivation. In tumoral HeLa cells and FRT epithelial cells, transactivation of the EGFR via the $P2Y_1R$ was reported [253]. In spinal cord astrocytes in primary culture, ATP stimulated the release of prostaglandin E2 via activation of the $P2Y_1R$ involving transactivation of the EGFR, an increase in intracellular Ca^{2+}, phosphorylation of ERK1/2 and stimulation of cytosolic phospholipase A2 [193]. In rat vascular smooth muscle cells, the slowly hydrolysable P2R agonist ATPγS was found to transactivate the EGFR via phosphorylation of protein kinase Cδ and Src, which led to activation of downstream components including PI3-kinase, Akt, ERK1/2 and the transcription factor Elk-1. This lead to increased expression of cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin synthesis and PGE2 release, eventually inducing increased cell motility. Since ATP, ADP, UTP, and UDP elicited similar effects, several P2R may have been involved [191].

In human coronary artery endothelial cells, UTP via the $P2Y_2R$ induced Src- and VEGFR-2-dependent activation of VE-cadherin, resulting in a multi-receptor complex to regulate the function of adherens junctions [257]. Activation of endothelial cell tubulogenesis by 2-methyl-thio-ATP (2MeSATP), presumably involving the $P2Y_1R$, was found to be mediated through VEGFR-2, suggesting that VEGFR-2 can also be activated in the absence of its agonist VEGF [258]. In addition, evidence was provided that Src family kinases regulate molecular crosstalk between the ATPγS-activated $P2Y_2R$ and the NGF receptor TrkA, resulting in enhanced neurotrophin signaling and neuronal differentiation [259]. In rat C6 glioma cells, activation of the $P2Y_{12}R$ by 2MeSATP induced cross-talk with insulin-like growth factor 1 (IGF-I) receptors which proceeded through Gβγ-mediated Ca^{2+} influx and phospholipase D2 (PLD2)-dependent activation of the Pyk2/Src pathway, eventually resulting in increased PKB phosphorylation [260].

Together, these studies show that apparent cellular effects of growth factors may be co-initiated by nucleotides or even mediated through nucleotides alone, whereby the molecular mechanisms of receptor interrelation can vary.

Nucleotide enhances release of a messenger conditioned by another signal pathway

While in the majority of cases nucleotides directly release other messenger molecules, in some cases, a conditioning stimulus is required for induction of nucleotide-mediated messenger release. This conditioning stimulus may itself initiate cellular nucleotide release or nucleotide receptor expression. Mostly, this concerns secretion of certain interleukins but it can also apply to hormone secretion (Table 4). Not in every case does the experimental protocol permit to decide whether preconditioning of the releasing cell was essential for nucleotide-mediated messenger release.

Hormones from pancreatic islets

The release of insulin, a polypeptide hormone produced by pancreatic β cells and promoting the cellular uptake of glucose from blood, has been particularly well investigated. The release of insulin from pancreatic β cells is typically initiated by an increase in extracellular glucose concentrations and a rise in intracellular Ca^{2+} concentrations. The glucose-mediated increase in exocytosis can be amplified by additional stimuli. Notably, the amplification pathway remains functionally silent as long as the intracellular Ca^{2+} concentrations have not been raised by the initial glucose stimulus. The amplification pathway is thought to serve to optimize the secretory response not only to glucose but also to non-glucose stimuli [322].

The observations that ATP releases insulin from rabbit [261] and primate pancreas [262] belong to the first examples ever of a physiological function of extracellular ATP. Since then, the mechanisms of nucleotide-mediated regulation of insulin release have been intensively studied with partially conflicting results and have recently been reviewed in detail [8, 263–265]. Notably, the impact of nucleotides and the cellular mechanisms leading to insulin release vary between species. Nucleotides can be derived from two different sources. One source is neuronal. ATP (and presumably also ADP) can be co-released with acetylcholine from parasympathetic and with noradrenaline from sympathetic neurons innervating pancreatic islets. In addition, insulin containing granules of β cells contain and release the P2R agonists ATP and ADP on glucose stimulation [266, 267]. ATP or ADP secreted by islets at low glucose concentrations can amplify insulin secretion during glucose stimulation. Nucleotide release from β cells can thus exert a positive feedback on insulin release. In

Table 4 Nucleotide enhances release of a messenger conditioned by another signal pathway

Messenger released	Nucleotide/receptor implicated	Conditioning stimulus	Outcome	References
Insulin	ATP, ADP, Ap_4A/P2R, P2X3, $P2Y_1$, $P2Y_6$, $P2Y_{13}$	Glucose	Enhancement of insulin release from pancreatic islands	[261–280]
Glucagon[??]	ATP, Ap_4A/P2, P2X4	Glucose	Enhancement of glucagon release from pancreatic islets	[276, 278, 280]
Somatostatin[??]	α,β-Methylene ADP, ATP/P2Y, $P2Y_1$	Glucose	Somatostatin release from perfused dog or rat pancreas	[281–283]
Histamine[??]	ATP, UTP/$P2Y_1$, $P2Y_2$	Anti-IgE	Enhancement of histamine release from mast cells	[284–288]
Interleukin-1β (IL-1 β)	ATP, UTP/P2X4, P2X7, P2Y	PAMP	Enhancement of IL-1β release from various types of immune cells	[155, 163, 166, 289–306]
Interleukin-1α (IL-1 α)	ATP/P2X7	PAMP	Enhancement of IL-1α release from monocytes, macrophages	[297, 307]
Interleukin-1 receptor antagonist (IL-1Ra)	ATP/P2X7	PAMP	Enhancement of IL-1Ra release from endothelial cells, macrophages	[308–310]
Interleukin-18 (IL-18)	ATP/P2X4, P2X7	PAMP	Enhancement IL-8 release from monocytes, macrophages	[163, 291, 297]
Interleukin-33 (IL-33)	ATP/$P2Y_2$	PAMP	Enhancement of IL-33 release from respiratory epithelium	[311]
Interleukin-1F6 (IL-1F6)	ATP/P2X7	PAMP	Enhancement of IL-1F6 release fom macrophages	[312]
Interferon-β	ATP/P2X7	PAMP	Enhancement of interferon-β release, macrophage	[313]
Nicotinamide phosphoribosyltransferase (Nampt)	ATP/P2X7	PAMP	Enhancement of Nampt release, monocyte	[314]

PAMP pathogen-associated molecular pattern molecule

?? Requirement of conditioning stimulus needs further clarification

rat perfused pancreas, both ATP and ADP triggered the amplification pathways of glucose-stimulated insulin release [268–270]. In isolated rat islet cells, structural analogs of ADP and ATP induced uptake of Ca^{2+} through Ca^{2+} channels in the β cell plasma membrane which in turn was accompanied by an increase in insulin secretion [271]. In other studies, extracellular ATP was shown to trigger exocytosis by mobilizing acidic Ca^{2+} stores via IP_3 receptors [272, 273]. In addition, there is evidence for the involvement of the cAMP/protein kinase A pathway in nucleotide-mediated insulin secretion. Accordingly, in rat pancreatic islets, non-degradable P2YR agonists amplified glucose-induced insulin secretion by activating adenylyl cyclase and the subsequent cAMP/protein kinase A signaling pathway [274].

In contrast to rat, ATP and ADP was reported to reduce insulin release in mice [323]. Accordingly, in $P2Y_1R$ KO mice, glucose-induced insulin release from isolated pancreatic islets was enhanced [324]. In a more recent study ADP exhibited a dual role in insulin secretion from purified mouse β cells. It stimulated insulin release through $P2Y_1Rs$, but inhibited it via $P2Y_{13}Rs$ [275]. Moreover, the $P2Y_6R$ agonist uridine 5'-O-ß-thiodiphosphate (UDPßS) enhanced both insulin and glucagon release from isolated mouse islets [276].

In human pancreatic islets, activation of P2Rs were found to stimulate insulin release [277]. In a study on human β cells, ATP signaling enhanced the sensitivity and responsiveness of β cells to glucose fluctuations [278]. Human β cells expressed ionotropic P2X3Rs and activation of these receptors through ATP co-released with insulin amplified glucose-induced insulin secretion. P2X3R activation resulted in increased intracellular Ca^{2+} concentrations, independent of intracellular Ca^{2+} stores. The authors suggested that ATP released from the human islets forms a positive autocrine feedback loop which sensitizes the secretory machinery of β cells. This would enable effective activation of insulin release despite relatively modest changes in blood glucose concentration. In this study, UTP and ADP could also increase insulin release but the appropriate P2YRs were not involved in the response to endogenously released ATP during glucose stimulation. In contrast, a recent study placed the $P2Y_1R$ at center stage in ATP-mediated insulin release from human islets [279]. The authors showed that ATP acts as a positive autocrine signal in human β cells by activating $P2Y_1Rs$, stimulating electrical activity and coupling Ca^{2+} influx to Ca^{2+} release from ER stores. These results imply that multiple ATP receptor subtypes can be expressed by human β cells and involved in autocrine enhancement of glucose release.

Taken together, these data—even though varying between animal and experimental conditions—suggest that ATP (ADP) released from nerve terminals and from insulin secretory granules modulates (mostly increases) insulin secretion by interacting with P2XRs, allowing Ca^{2+} influx, and with G protein-coupled P2YRs. The P2YRs couple to phospholipase C (PLC) and induce the generation of IP_3, which mobilizes Ca^{2+} from endoplasmic reticulum, and diacylglycerol which in turn stimulates the protein kinase C pathway. They also involve adenylate cyclase and the cAMP-protein kinase A pathway [265].

Less is known concerning the impact of nucleotides on the release of glucagon from pancreatic α cells, the hormone counteracting the actions of insulin by promoting glucose synthesis and mobilization from the liver. In mouse pancreatic α cells ATP inhibited Ca^{2+} signaling and glucagon secretion [325] whereas ATP stimulated small increases in glucagon secretion in human, monkey, and mouse islets, whereby a subset of human α cells expressed P2X4Rs [278]. In addition to mononucleotides, the dinucleoside polyphosphate diadenosine tetraphosphate (Ap_4A), which can act on a variety of nucleotide receptors [326], stimulated both glucagon and insulin release from rat pancreas perfused with varying glucose concentrations [280]. Possibly, Ap_4A is contained in glucagon-containing granules and released from islet cells similar to granules from chromaffin cells [327].

The stable ADP analog α,β-methylene ADP infused into the isolated perfused dog pancreas induced stimulation of somatostatin secretion, a hormone that, among others, inhibits secretion of insulin and glucagon [281]. Furthermore, the nucleotide analog and P2YR agonist ADPβS induced an immediate increase in somatostatin-like immunoreactivity in vivo in anesthetized fasted dogs [282]. In a subsequent study on perfused rat pancreas, the $P2Y_1R$ antagonist MRS 2179 reduced the release of somatostatin into the portal vein, suggesting an intrinsic activation of somatostatin release via ATP-activated $P2Y_1Rs$ [283]. Somatostatin may be released by nucleotides independent of a glucose stimulus.

Histamine

It has long been known that external ATP through activation of P2Rs induces exocytotic degranulation of rat mast cells and release of the biogenic amine histamine in a Ca^{2+}-dependent manner. ATP can thus increase allergic responses [284–286]. Release appeared to be species- as well as histaminocyte subtype-specific and was demonstrated in rat peritoneal, serosal, and mesenteric mast cells and mouse peritoneal mast cells. But it was absent from the guinea pig and human mast cells tested [287]. In another study, in human lung mast cells, both ATP and UTP enhanced anti-IgE induced histamine release. The study suggested that the enhancement of release is mediated through P2Rs including the $P2Y_1R$ and/or $P2Y_2R$ subtypes [288].

Interleukin-1 family

For a number of interleukins, ATP-stimulated release requires a conditioning stimulus. Nucleotide-mediated release of

interleukin-1β (IL-1β) has been particularly well documented. IL-1β, a member of the IL-1 family, is a primary proinflammatory cytokine and a key mediator of host response to pathogens and tissue injury. Monocytes, macrophages, microglia, and dendritic cells all constitute major sources of IL-1β [328]. Production and release of IL-1β are typically stimulated by pathogen-associated molecular pattern molecules (PAMPs) such as the microbial cell-wall component lipopolysaccharide (LPS) or damage-associated molecular pattern molecules (DAMPs) such as heat-shock proteins or abnormally processed extracellular matrix proteins and are mediated through signaling via Toll-like receptor (TLR) or NOD-like receptor (NLR) family pathways [329]. IL-1β is initially translated as the inactive 31-kDa leaderless secretory protein pro-IL-1β which needs to be proteolytically processed by caspase-1 activation via an inflammosome complex to generate the 17-kDa mature and releasable cytokine [330, 331]. Of note, cells capable of IL-1β production and release also express high levels of the P2X7R [332, 333]. Activation of the P2X7R induces the NLRP3/caspase-1 inflammosome signaling complexes [334] and equally drives the proteolytic maturation and secretion of IL-1β. K^+ efflux mediated through P2X7R activation leading to a drop in cytoplasmic K^+ is considered to be a major trigger for promoting inflammosome assembly [289, 328, 329, 335–337]. Recent evidence suggests that the P2X7R directly interacts with the NLRP3 inflammosome scaffold protein [338].

LPS-primed mononuclear phagocytes and other cell types massively released IL-1β following activation of the P2X7R [289, 290]. Similarly, primary human monocytes released ATP following stimulation with microbial components acting on different pathogen-sensing receptors, or stimulation with uric acid. This lead to autocrine activation of P2X7Rs and maturation and release of IL-1β. The authors suggested that stimuli acting on different pathogen-sensing receptors converge on a common pathway where ATP externalization is the first step in the cascade of events leading to inflammosome activation and IL-1β secretion [291]. Similarly, IL-1β was released through P2X7R activation from LPS-primed human monocyte-derived macrophages [292, 293] and from a mouse leukemic monocyte macrophage cell line [294]. IL-1β was also released from a LPS-primed mouse Kupffer cell line in response to activation of the P2X7R by ATP [295, 296]. Importantly, LPS-primed peritoneal mouse macrophages derived from a P2X7R-deficient mouse line failed to release IL-1β when challenged with ATP [155, 297].

While in both monocytes and macrophages activation of P2X7Rs significantly enhances the production of LPS-induced IL-1β, synthesis and release of IL-1β differ between human monocytes and macrophages. Monocytes have constitutively activated caspase-1 due to constitutive autocrine activation by ATP via the P2X7R. This leads to release of active IL-1β already after a single stimulation event with bacterial ligands such as LPS. In contrast, macrophages which do not themselves release ATP require an initial stimulus which induces IL-1β transcription and translation, and a second stimulus for caspase-1 activation with subsequent IL-1β processing and secretion. This second stimulus is again ATP [298, 299]. As compared to macrophages, the antigen-presenting dendritic cells, which represent the most efficient initiators of primary immune responses, released modest amounts of IL-1β. Stimulation of human monocyte-derived dendritic cells by extracellular ATP via the P2X7R caused release of IL-1β—via microvesicles—from which IL-1β can be released by further incubation with ATP [300]. In LPS-primed mouse bone marrow-derived dendritic cells, ATP was found to co-activate release of both IL-1β and IL-18 which was amplified through activation of the P2X4R [163]. For comparison, a study on primary murine bone marrow-derived dendritic cells and macrophages revealed that in both cell types, release of Il-1β was predominantly a P2X7R-dependent process. Interestingly, dendritic cells and macrophages displayed distinct patterns of Il-1β regulation as a consequence of cell type-specific differences in P2X7R variants [301]. In an earlier study on primary murine dendritic cells from peripheral lymphoid organs, UTP was found to stimulate—via P2YRs—the expression and secretion of an entire array of cytokines including IL-1β, IL-6, and IL-10 [166].

ATP and P2X7Rs also regulate the secretion of mature IL-1β from LPS-primed and activated immortalized microglial cells [302, 303] and microglia within dorsal horn slices, thereby contributing to enhanced nociceptive transmission and behavioral hypersensitivity [304]. In primary co-cultures of rat astrocytes and microglial cells, ATP, released from mechanically stimulated astrocytes, induced the formation and release of IL-1β in nearby microglia [305].

In addition to classical inflammatory cells, such as leucocytes, macrophages and mast cells, the periodontal ligament cells (PDLs) contribute to periodontal inflammation via production and release of cytokines and chemokines. Human PDLs were shown to release IL-1β following mechanical stress-mediated induction of IL-1β and P2X7R-mediated ATP stimulation [306].

Interleukin-1α (IL-1α), another inflammatory cytokine, has properties similar to IL-1β. It is also a leaderless secretory protein but is not cleaved by caspase-1 [235]. Similar to IL-1β it was released from mouse LPS-primed peritoneal macrophages. Release was abrogated in macrophages derived from P2X7R-deficient mice [297]. In blood monocytes from patients affected by NLRP3-mediated cryopyrin-associated periodic syndromes (CAPS), which are autoinflammatory diseases, LPS first induced the externalization of ATP. The enhanced ATP release drove the release not only of IL-1β but also of IL-1α and IL-18, through activation of the P2X7R [307].

Signaling via IL-1α or IL-1β can be regulated by the naturally occurring inhibitory interleukin-1 receptor antagonist (IL-1Ra). It competitively inhibits binding of IL-1α and IL-1β to their receptors. The balance between IL-1 and IL-1Ra is believed to determine the outcome of the overall inflammatory response [339]. Similar to IL-1β, the intracellular form of IL-1Ra has no signal peptide. Following P2X7R activation, the leaderless form of IL-1Ra was released from LPS-primed murine RAW264.7 macrophages and from human umbilical vein endothelial cells (HUVECS). The balance of IL-1s and their inhibitor may provide a means for altering the inflammatory state of the arterial vessel wall [308, 309]. In pancreatic β cells of obese patients, P2X7Rs are upregulated. Elevated glucose and non-esterified fatty acids rapidly activated P2X7Rs and IL-1Ra secretion from islets. Accordingly, P2X7-knockout mice had a lower capacity to secrete IL-1Ra. The authors suggested that the increase in P2X7 production is one mechanism which may explain how β cells adapt to the higher insulin demand in obesity [310].

The closely related interleukin-18 (IL-18) is expressed in essentially every cell of the body. It is involved in the pathogenesis of several inflammatory diseases [340]. It plays a major role in the production of interferon-γ from T cells and natural killer cells and thus has a major role in the Th1 lymphocyte response. Similar to IL-1β, IL-18 is processed by inflammosome-activated caspase-1 [341]. When stimulated with microbial components, human monocytes released not only IL-1β but also IL-18 through an ATP- and P2X7R-mediated pathway. The microbial components triggered the release of endogenous ATP. The autocrine stimulation of P2X7Rs by the released ATP then induced the cascade of events leading to maturation and secretion of IL-18 [291]. The ATP-mediated release of IL-18 from LPS-primed mouse macrophages similarly involved the P2X7R. It was abrogated in P2X7R-deficient mice [297].

Interleukin-33 (IL-33) is another potent molecule of the innate immune system. Unlike pro-IL-1β, pro-IL-33 has biological activity and IL-33 does not require proteolytic maturation by caspase-1. Among others, it has been implicated in the initiation of innate and adaptive proallergic Th2-type lymphocyte responses in the airways. It is constitutively stored in the nuclei of human airway epithelial cells. Allergen exposure lead to extracellular accumulation of ATP which induced increases in intracellular Ca^{2+} concentrations via an autocrine pathway and caused release of IL-33 through the $P2Y_2R$. ATP and purinergic signaling in the respiratory epithelium were thus considered to be critical sensors for airway exposure to airborne allergens. They may provide novel opportunities to dampen the hypersensitivity response in Th2-type airway diseases such as asthma [311].

The more recently identified leaderless and inflammatory cytokine interleukin-1F6 (IL-1F6) can be externalized by a mechanism comparable to that of IL-1β, involving LPS treatment and activation of the P2X7R in transduced mouse bone marrow macrophages [312].

Interferon-β

Interferons represent an additional group of immunostimulating and in particular, antiviral and antitumoral substances. And again, the P2X7R can be involved in its production and release. In the RAW 264.7 mouse macrophage line P2X7R agonists substantially enhanced LPS-triggered interferon-β (IFN-β) expression and release. Activation occurred at the promoter level of the IFN-β gene and was related to an increase in active interferon regulatory factor 3. This enhancement was ablated in macrophages which do not express functional P2X7Rs. P2X7R stimulation alone did not induce IFN-β expression [313].

Nicotinamide phosphoribosyltransferase

Nicotinamide phosphoribosyltransferase (Nampt) catalyzes the generation of nicotinamide mononucleotide from nicotinamide and phosphoribosyl-pyrophosphate. It is the key enzyme in the conversion of nicotinamide to NAD^+. The expression of Nampt is induced by inflammatory stimuli in cells involved in innate immunity. While Nampt functions as an essential intracellular enzyme, recent evidence suggests that Nampt is also released as an extracellular mediator, exerting cytokine-like effects. Extracellular Nampt can function as a proinflammatory cytokine and display antiapoptotic effects in macrophages and neutrophils [342]. Interestingly, extracellular ATP greatly enhanced secretion of Nampt, a leaderless protein, in LPS primed human peripheral blood monocytes, similar to the release of IL-1β. Release of Nampt was stimulated by agonists and inhibited by antagonists of the P2X7R [314].

ATP-mediated ATP release

Multiple studies revealed that ATP can stimulate its own release via P2Rs both in an autocrine and paracrine manner. This mechanism permits regenerative signal amplification via positive feedback and also ATP-mediated propagation of the ATP-induced signal (Tables 5 and 6). Cell to cell spread of Ca^{2+} signals mediated through ATP receptors has been observed early on in rat basophilic leukemia cells and mast cells [343]. The authors suggested that extracellular ATP accelerates the release of secretory granules containing additional ATP by triggering an increase in intracellular Ca^{2+} in quiescent neighboring cells or in cells which have begun to degranulate, thus amplifying the initial response. A later study showed that addition of ADP and a variety of nucleotide analogs stimulated release of ATP through P2YRs from

Table 5 Nucleotide acts as an autocrine/paracrine signal stimulating its own release and inducing cell to cell spread of a Ca^{2+} signal

Nucleotide	Tissue investigated	Substance released/receptor involved	References
ATP	Rat basophilic leukemia cells and mast cells	ATP/P2R	[343]
ADP, nucleotide analogs	Guinea pig heart endothelial cells	ATP/P2YR	[344]
ATP	Cultured human umbilical vein endothelial cells	ATP/P2R	[345]
ATP, UTP	Cultured astrocytes	ATP, UTP/$P2Y_1$, $P2Y_2$	[92–97]
ATP	Cortical astrocytes in vivo	Nucleotide/P2R	[98, 99]
ATP	Rat retinal Müller cells	ATP/P2Y	[346]
ATP	Chicken retinal pigment epithelium	ATP/P2R	[347]
ATP	Rat hypothalamic tanycytes	ATP/$P2Y_1$	[348]
ATP	Rat embryonic radial glial cells	ATP/$P2Y_1$	[349]
ATP	Microglia cultured from rat cerebral cortex	ATP/P2R	[350]
ATP	Mouse pancreatic β cells	ATP/P2YR	[351, 352]

endothelial cells isolated from guinea pig heart [344]. Similarly, ATP induced the release of ATP in cultured human umbilical vein endothelial cells resulting in maintained extracellular ATP concentrations. This implicated a self-perpetuating mechanism of ATP-induced ATP release likely to play a role in local vascular control [345].

ATP-mediated ATP release has been intensively studied in astrocytes in connection with the propagation of intercellular Ca^{2+} waves and astrocyte-neuron interplay. Since ATP can propagate astrocytic ATP release via paracrine mechanisms, ATP-mediated release of neurotransmitters may spread through neighboring assemblies of astrocytes [227, 354]. There is strong evidence from cultured astrocytes that ATP can serve as the extracellular messenger in wave propagation—even though the mechanism of ATP release has been debated. The paracrine action of ATP was elegantly demonstrated in cultured mouse cortical astrocytes. A diffusible signal was found to pass between non-contacting cells and mediate an increase in intracellular Ca^{2+} concentrations. The diffusible signal was identified as ATP [92]. Several studies support the notion of regenerative ATP release in glial Ca^{2+} wave propagation [93, 94]. Alternatively, in a study on rat cortical astrocytes, the spatial expansion of calcium waves was explained by simple diffusion of ATP, requiring neither amplification nor regeneration for spatial expansion to proceed [355]. $P2Y_1$Rs have been identified in ATP-mediated Ca^{2+} wave propagation in cultured rat dorsal spinal cord astrocytes [95] whereas in IL-1β-treated human fetal cortical astrocytes $P2Y_2$Rs [96] and in another study on rat spinal cord astrocytes, $P2Y_1$R2 and $P2Y_2$Rs [97] have been implicated. Interestingly, Ca^{2+} waves propagating via $P2Y_2$Rs were found to travel faster and further than those propagating via $P2Y_1$Rs.

In the acutely isolated rat retina Ca^{2+} waves propagated from astrocytes to Müller cells and from Müller cells to other Müller cells were primarily mediated through the release of ATP [346]. In the isolated embryonic chicken retina, ATP was found to be spontaneously released through gap junctional hemichannels from trigger cells of the retinal pigment epithelium. This initiated a P2R-mediated Ca^{2+} wave in the pigment epithelium. The diffusion of ATP to the underlying neural retina then speeded both cell division and proliferation of cells in the retinal ventricular zone [347]. As shown in acute slices of rat brain, tanycytes, special cells in the lining of the third ventricle, release ATP in response to glucose and may act as glucusensor signaling to hypothalamic nuclei involved in the control of feeding behavior. ATP elicited Ca^{2+} waves between tanycytes through $P2Y_1$Rs which could amplify the detection of glucose [348]. ATP also plays an important role in the control of neurogenesis [6]. Radial glial cells in the embryonic mammalian brain function as neural progenitor cells and produce cortical pyramidal neurons. In slices from embryonic rat brain spontaneously occurring radial glial calcium waves

Table 6 Nucleotide acts as an autocrine signal enhancing its own release and that of another messenger

Nucleotide	Messenger	Substance released/receptor involved	References
ATP	Insulin	ATP + insulin/P2	[264, 265, 279]
ATP	Co-released neurotransmitters including noradrenaline and glutamate	ATP + neurotransmitter/P2R	[49, 50, 80]
ATP	Hormones including vasopressin, luteinizing hormone, gonadotrophin-releasing hormone, prolactin	ATP + hormone/P2	[104, 109, 110, 114]
ATP, ADP, UDP	Cytokines, including IL-1β, CXCL8 (IL-8), IL-10, IL-18, or IL-33	ATP + cytokine/P2	[142, 291, 311, 353]

required intracellular IP3-mediated Ca^{2+} release, opening of connexin hemichannels and transcellular ATP signaling involving $P2Y_1$Rs [349].

The in vitro data obtained with cultured astrocytes created considerable excitement as they would allow for long-distance signaling via astrocytes in the brain, including astrocyte neuron-signaling [354]. More recent studies suggest, however, that in vivo astrocytic calcium waves occur only following a pathological insult [356]. This includes brain ischemia [357] and traumatic brain injury [98]. In vivo imaging studies on the exposed rat cortex revealed that a micromechanical impact triggered intercellular Ca^{2+} waves in cortical astrocytes which were attenuated by addition of the ATP-degrading enzyme apyrase. The data suggested that mechanical trauma to astrocytes in vivo triggers ATP-mediated Ca^{2+} waves transmitting even beyond the initial epicenter of mechanical trauma [98]. These studies imply that cultured astrocytes represent a reactive phenotype different from in situ "resting" astrocytes. In another in vivo study, astrocyte activity was imaged in the mouse hippocampus after ablation of the cortical tissue. Ca^{2+} activity could be monitored simultaneously from hundreds of astrocytes. Almost all astrocytes participated in regenerative waves which propagated from cell to cell. Spread of Ca^{2+} waves required gap junctions and ATP receptor activation [99]. Together, these studies demonstrate that ATP-induced astrocytic Ca^{2+} waves propagated by an autocrine/paracrine mechanism play an important functional role in the central nervous system, particularly after an insult and in disease.

In addition to astrocytes, microglial cells can communicate via ATP-induced ATP release. In microglia cultured from rat cerebral cortex, ATP induced P2YR-mediated release of ATP from microglial lysosomes which in turn contributed to the directional migration of remote microglia [350]. Similarly, pancreatic β cells were shown to communicate via release of ATP. β Cells are biological oscillators which respond to a glucose stimulus with rhythmic depolarization and subsequent entry of Ca^{2+} via voltage-activated channels. In addition to releasing insulin via autocrine or paracrine ATP-mediated mechanisms, β cells release ATP as a diffusible messenger to coordinate the rhythmicity of the β cells for pulsatile insulin release within an the islet in the pancreas. It has been suggested that β cells receive an initial neuronal ATP signal inducing intracellular Ca^{2+} transients which in turn can propagate this message via intermittent release of ATP to neighboring cells [351, 352].

Furthermore, ATP can simultaneously amplify both its own release and that of another messenger when ATP-releasing cells express P2 autoreceptors inducing ATP release. ATP-induced insulin release from pancreatic β cells is one example [264, 265, 279]. Similarly, ATP co-released from nerve terminals can amplify—via facilitatory presynaptic autoreceptors—its own release and that of the co-released neurotransmitter. Examples include noradrenaline [80] or glutamate [49, 50]. Additional examples for enhanced messenger release by autocrine action of ATP include the release of vasopressin [104], gonadotrophins [109, 110], prolactin [114], IL-1β [291], IL-6 [353], CXCL8 [142], IL-10, or IL-33 [311]. Signal maintenance or amplification by ATP-induced ATP release may thus be of considerably greater physiological relevance than hitherto anticipated.

Summary and outlook

This commentary highlights the impressive part ATP and other nucleotides play in stimulating the cellular release of other intercellular messenger substances. These include neurotransmitters, hormones, growth factors, a considerable variety of other proteins including enzymes, numerous cytokines, lipid mediators, nitric oxide, and reactive oxygen species. Moreover, nucleotides activate or co-activate growth factor receptors.

The literature available does not permit to draw general conclusions regarding the subtype of P2 receptor typically involved in the release of a particular messenger substance and the extracellular nucleotide concentration required. In many cases both P2XRs and P2YRs have been implicated. ATP-enhanced presynaptic release of amino acid neurotransmitters through P2XRs and the release of many cytokines through the P2X7R are an exception. Similarly, transactivation of growth factor receptors always involves P2YRs. In general, activation through P2XRs is faster and requires higher nucleotide (ATP) concentrations than activation through P2YRs. Data on nucleotide concentrations actually occurring in situ in resting or activated tissue are scarce. Using microdialysis techniques, the interstitial concentration of ATP in living rat brain (striatum) was estimated to be in the range of 30 nM [358], which comes close to the EC_{50} value of some P2YRs. Inhibiting ectonucleotidases raised the ATP concentrations to 260 nM, which would still be too low to efficiently activate P2XRs. Following release of packages of ATP by exocytosis, local extracellular concentrations can be expected to ensure both P2YR and P2XR activation. At the surface of activated human platelets, transient local ATP concentrations in the range of 15–20 μM were measured using cell surface-attached firefly luciferase. This would be sufficient to activate autocrine P2X receptors [359].

One pathophysiological sector is outstanding. Nucleotides are a common denominator for varied inflammatory insults. Among the substances released by nucleotides and involved in inflammatory events are numerous cytokines, eicosanoids, or also reactive oxygen species. Several types of nucleotide receptor where shown to be involved in their release but the contribution of the P2X7R is outstanding. Its requirement for high extracellular ATP concentrations makes it a "sensor of

danger" monitoring cell damage and the increased release of the ATP at inflammation sites [360–362]. Absence of ATP would prevent or alleviate proinflammatory mediator release whereas high ATP concentrations would ensure rapid release. This makes the P2X7R a particularly attractive target for the development of anti-inflammatory drugs. Accordingly, a large number of selective P2X7R antagonists developed by several drug companies are currently under clinical trials [363, 364].

Why is the release of so many different extracellular messenger substances stimulated by nucleotides? Releasing extracellular messengers considerably broadens both the range of the final cellular targets of nucleotides and of the signal mechanisms eventually elicited. In the case of hormone release, the initially paracrine or autocrine nucleotide-mediated signal spreads through to the entire organism. Furthermore, from a teleological point of view, a ubiquitously available mechanism for stimulating or boosting messenger release on demand would be advantageous. Nucleotides are at hand everywhere. This is particularly obvious regarding the release of inflammatory cytokines in response to infectious agents, trauma, or ischemia. In a similar way, glucose-induced insulin release is amplified on demand by nucleotide release from peripheral nerve terminals or the release of neurotransmitters can be enhanced through nucleotides released from the surrounding activated brain parenchyma. Moreover, ATP is able to induce regenerative ATP release maintaining locally enhanced messenger release.

The examples provided in this commentary encompass a considerable variety of tissues, cell types, and experimental conditions. Obviously, there are species-specific differences regarding the subtype of nucleotide receptor involved and also their cellular impact. Furthermore, the expression of nucleotide receptors may considerably vary between experimental conditions. While initiation of messenger release by nucleotides as the primary messengers has been unraveled in many contexts, it may have been overlooked in others. It can be anticipated that extracellular nucleotides will be uncovered as primary signals in additional messenger-driven functions with relevance for both understanding physiology and development of therapy.

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