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ORIGINAL ARTICLE

Physiological and pathological functions of P2X7 receptor in the spinal cord

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Abstract ATP-mediated signaling has widespread actions in the nervous system from neurotransmission to regulation of proliferation. In addition, ATP is released during injury and associated to immune and inflammatory responses. Still, the potential of therapeutic intervention of purinergic signaling during pathological states is only now beginning to be explored because of the large number of purinergic receptors subtypes involved, the complex and often overlapping pharmacology and because ATP has effects on every major cell type present in the CNS. In this review, we will focus on a subclass of purinergic-ligand-gated ion channels, the P2X7 receptor, its pattern of expression and its function in the spinal cord where it is abundantly expressed. We will discuss the mechanisms for P2X7R actions and the potential that manipulating the P2X7R signaling pathway may have for therapeutic intervention in pathological events, specifically in the spinal cord.

Keywords ATP · Purinergic signaling · P2X receptors · Neuronal injury · P2X7 antagonists

ATP as extracellular signaling pathway in the CNS

Apart from its role as the main molecule that provides cellular fuel, ATP and other nucleotides function as intercellular signaling molecules when released to the extracellular space. Although considerable gaps exist in our understanding of the pathways of intercellular purinergic signaling, it is clear that purinergic signaling represents the primary non-synaptic signaling mechanism in the normal and diseased CNS.

Purinergic receptors fall into two different categories: P2X receptors—ligand-gated ion channels that allow non-specific passage of cations (Na^+, Ca^{2+}, K^+)—and P2Y receptors—G-protein-coupled receptors that mediate intracellular calcium increases upon ATP binding [1]. So far, seven P2X receptors and eight P2Y receptors (as opposed to P1 receptors, which bind the nucleoside adenosine) have been described on the basis of their molecular structure and pharmacological profile (Table 1).

ATP signaling in brain affects not only neurons, but every other major cell types present in brain: astrocytes, microglia, oligodendrocytes, endothelial cells, and pericytes [2]. ATP-mediated signaling is widespread in the nervous system, mediating fast excitatory neurotransmission both in peripheral and central neurons (for review see [3]). ATP has also been considered to be a neuromodulatory substance because it is co-released with other neurotransmitters such as noradrenaline and GABA [4, 5]. In addition, ATP is the predominant (if not the sole) extracellular messenger that mediates calcium waves in astrocytic glial cells [6, 7], which are themselves involved in modulating neuronal activity [8–10]. ATP not only acts as a neurotransmitter, but it also stimulates cell proliferation and differentiation [11, 12]. Furthermore, while ATP-mediated activation of P2X receptors is linked to opening of a cation channel, and thereby either directly or indirectly to the increase of synaptic transmission, its related metabolite adenosine is a potent inhibitor of excitatory transmission via A1 receptor activation.

Importantly, ATP is ubiquitously present in the extracellular space after injury [13, 14]. It is therefore of

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Table 1 P2X receptors

	Affinity ATP (EC50)	Agonists	Antagonists	Inhibition by divalent cations[a]
P2x1	1 μM	BzATP> ATP = 2MeSATP>α,β-meATP	Suramin, NF023, PPADS, TNP-ATP IsoPPADS, NF449, Phenol Red, PPNDS oATP (reversible)	H+, Zn^{2+} ↓
P2x2	10 μM	ATP>ATPγS≥2MeSATP>>>αβmeATP	Suramin, NF023, PPADS RB-2, NF279	Ca^{2+} ↓ Zn^{2+}, Cu^{2+}, H+ ↑
P2x3	1 μM	BzATP≥2meSATP≥ATP≥αβmeATP	Suramin, NF023, PPADS, TNP-ATP IsoPPADS, Phenol Red, A3174	Ca^{2+}, H+ ↓ Zn^{2+} ↑
P2x4	10 μM	ATP>2meSATP>α,β-meATP Ivermectin (potentiates)	TNP-ATP (weak), BBG (weak)	H+, Cu^{2+} ↓ Zn^{2+}↑
P2x5	10 μM	ATP=2MeSATP=ATPγγS>αβmeATP	Suramin, PPADS, BBG	
P2x6	10 μM	ATP>2MeSATP>ADP		
P2x7	>100 μM	BzATP>>ATP>>UTP>>2meSATP >>αβmeATP	oATP (irreversible), BBG, KN-62[b], NF-279, PPADS[a], A-438079, A-740003	Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}, H+↓

Adapted from Bianchi et al. [107], North [15], Jacobson et al. [85], Burnstock [3]

[a] Effect of divalent cations on the current flow through the receptor ion channel. *Downward-facing arrow* Decreases, *upward-facing arrow* increases

[b] Species differences (rat vs. human)

BBG Brilliant blue G, *BzATP* 2′-and 3′-*O*-(4-benzoyl-benzoyl)-ATP, *α,β-meATP* α,β˜-methylene ATP, *2-MeSATP* 2-metylthio ATP, *ATPγS* 5′-*O*-(3-thiotriphosphate), *KN-62* 1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine, *NF023* 8,8′-(carbonylbis(imino-3,1-phenylenecarbonylimino))-bis(naphthalene-1,3,5-trisulfonic acid)-hexasodium salt, *NF449* 4,4′,4″,4‴-(carbonylbis(imino-5,1,3-benzenetriylbis (carbonylimino)))tetrakis-benzene-1,3-disulfonic acidoctasodium salt, *PPADS* pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid, *RB2* reactive blue, *oATP* periodate-oxidized ATP, *TNP-ATP* trinitrophenyl-substituted ATP

considerable interest to decipher the specific actions of ATP in pathological states and to explore the possibility that manipulating ATP-mediated signaling might help to diminish neuronal damage during disease.

The P2X7 receptor

The P2x7R is significant because it is activated by high concentrations of ATP (>100 μM) and because prolonged exposure to ATP is believed to form a much larger pore than any other P2X channel [15]. Thus, upon agonist application that lasts for several seconds, the P2x7 receptor becomes permeable to larger ions, like the large cation *N*-methy-D-glucamine (NMDG). The idea that ATP could induce the formation of a pore that allows the passage of large molecules came initially from studies in mast cells and macrophages [16] and Surprenant et al. [17] later defined the P2x7 receptor as the molecular basis for the formation of a non-selective pore that could lead to cell death upon repeated agonist applications. Thus, P2X7Rs have been traditionally associated with cells from the immune system mediating cytotoxic cell death (because of changes in transmembrane ion fluxes, swelling and vacuolation), and inflammatory responses (because of release of large molecules, including pro-inflammatory mediators like IL-1 and tumor necrosis factor) [18].

P2x7R pore formation has, in general, been linked to cytolytic cell death, the uptake of calcium and fluorescent dyes and the release of inflammatory molecules. Upon ATP application, measure of the pore formation comes usually by monitoring the uptake of ethidium and YoPro1 dyes, which become fluorescent upon nucleic acids binding [19] or the release of IL-1β [20]. Importantly, the formation of a releasing-pore does not necessarily imply the existence of a cytolytic pore. This is the case of the astrocytic P2X7R that can release excitatory neurotransmitters upon BzATP activation but does not exhibit any of the signs of necrotic and/or apoptotic cell death [21].

Despite the extensive amount of data in favor of pore formation, the relationship between the P2x7R pore and the capability to uptake and release different molecules is not as straightforward as one might think. Thus, a critical question that remains in the field is how different the channel is compared to the pore. Although originally believed that pore formation was the result of true, progressive dilation of the channel [15], there is now the hypothesis that subsequent recruitment of proteins through interactions with the C-t domain are required. Support for the involvement of downstream signaling pathways in addition to channel opening comes from the fact that some P2X7R-expressing cells fail to exhibit any other quality of pore formation [22], that there are reagents that affect BzATP induced pore formation without altering P2X7-

mediated calcium influx or IL-1β release [23], that there is requirement for second messengers like calcium and MAP kinases for the P2X7 pore [24] and that there exists alternative entry pathways for different molecules like $NMDG^+$ and Yo-Pro-1 upon P2X7R activation [25].

Some of the suggested accessory molecules that could contribute to the pore formation are pannexin [26]. Pannexins are a family of proteins structurally related to the innexins, non-mammalian proteins that form gap junctions (intercellular channels) in invertebrates [27]. Pannexins have been shown to co-immunoprecipitate with P2X7Rs and inhibitory peptides against pannexin or siRNA can diminish initial dye uptake and Il-1β processing and release-mediated by P2X7R without affecting ionic currents and slow dye uptake [28, 29].

P2x7 receptor expression and function in the spinal cord

Given how dissimilar the P2X7R seems to be from the rest of the other P2X receptor subtypes, we will next review our current knowledge of P2X7 receptors expression and function in the spinal cord, and the potential for therapeutic intervention based on selective administration of P2X7R antagonists. For a more detailed analysis of P2X7R in the rest of the central nervous system, please refer to [30].

P2X7R has been reported widely in several regions of the nervous system with post- and presynaptic actions affecting synaptic currents or neurotransmitter release, respectively (Table 2 for review, see [3, 15, 31]), although the actual functional implications of its presence are still under investigation.

P2x7R in spinal cord neurons

There has been ample debate about whether P2X7R is actually expressed in neurons because original data did not identify mRNA there [32]. However, Deuchars et al. [33] detected P2X7R mRNA in dorsal neurons and ventral motoneurons of the spinal cord using RT-PCR and in situ hybridization in slices. Other groups have later reported detectable mRNA and protein levels in neurons of several regions, including spinal cord (Table 2). At the level of protein expression, there is clear immunodetection in medulla, hippocampus, cerebellum, and cortex [33–35]. In the spinal cord, P2X7R immunoreactivity has been found associated to presynaptic neurons [33, 36, 37]. A strong immunoreactivity is also detected in motoneurons and terminals of the ventral spinal cord as opposed to cortical neurons that exhibit a much weaker signal [14]. Electrophysiological recordings from these neurons indicate the possible presence of post-synaptic terminals although additional studies combining pre- and post-synaptic markers are needed to clearly establish the exact location of the P2X7R in the ventral spinal cord.

The real identity of this anti-P2X7R reaction is still puzzling, given the controversial specificity of some of the antibodies used to recognize the P2X7R protein, although attempts have been made to confirm the identity of this P2X7R-like protein, like retrograde labeling or pharmacological and electrophysiological responses to agonists [14, 33]. Despite the fact that the antibodies available to date are able to recognize a Western band and protein corresponding to the P2X7 receptor in the submandibular gland, they are also capable of recognizing a P2X7R-like protein in neurons and astrocytes of the nervous system that is not eliminated in the P2X7−/− mice [38, 39].

Still, it is very possible that this protein corresponds to a differentially spliced protein or to a protein highly related to the p2x7R originally described in macrophages. This conclusion is supported by the pharmacological profile obtained in vivo, the ability to obtain permeability to large molecules upon activation and the blockage by the commonly used antagonists of the P2X7R [14, 33].

In an attempt to further clarify the issue, Yu et al. [40] have recently performed in situ hybridization using S^{35}-labeled probes combined with immunohistochemistry with markers that differentiate between neuronal and glial populations. In this case, widespread labeling was detected in neuronal populations including cortex, hippocampus, and spinal cord, in ependymal cells and in glial cells of the adult rat nervous system.

In the spinal cord, physiological ATP actions affect fast synaptic currents [5], glycine release at dorsal horn interneural synapses [41], release of GABA and glutamate [42, 43] and glutamate-mediated excitation in dorsal horn cells [44–46] among others. P2X2, P2X2/3, P2X3, and other heteromeric receptors of not clear composition but involving, at least, P2X2, P2X4, and P2X6, have been described mostly associated to sensory pathways [43, 47]. In contrast, P2X7R has been unequivocally identified in relation to motoneurons pathways in the ventral spinal cord [14, 33, 40].

P2x7R in glial cells

In addition to neurons, P2X7R mRNA can also be detected in astrocytes [21] and microglial cells [32]. However, it is believed that the contribution of P2X7 receptors to astrocytic physiology is negligible since astrocytic P2X7R protein levels have been more elusive to establish. On one hand, two groups have described P2X immunolabeling in hippocampal astrocytes using antibodies against the p2x7R subunits [48, 49]. Pharmacological studies suggested that p2x7R could indeed be the receptor responsible for the ATP responses observed. Two studies [21, 50] later found pharmacological profiles of an astrocytic protein that

Table 2 P2X7 in neurons and glia

Cell type	Area	Method of detection	Function	References
Neurons	Hippocampus	mRNA, ICC, Zn^{2+}, BBG, BzATP, RT-PCR	Release of GABA, inhibition of Glu currents, Presynaptic: depression of synapses	[34, 35, 37, 90, 91]
	Cortex	ICC, BzATP, BBG, OxATP, Zn, PPADS, RT-PCR		[37, 90–92]
	Cerebellum	ICC, BzATP, Zn, OxATP	Vesicular Glu release	[22, 37, 91, 93]
	Brainstem	ICC		[37]
	Hypothalamus	ICC		[37]
	Midbrain	ICC, BzATP, PPADS, KN-62	presynaptic	[94]
	DRG	mRNA, PCR		[95]
	Spinal cord (VH, DH)	mRNA, ICC, BzATP, PPADS, OxATP	Presynaptic excitatory,vesicular release	[14, 33, 36, 37]
	NMJ	ICC	Presynaptic excitatory,vesicular release	[33]
	Retina	ICC, PCR		[96, 97]
Brain astrocytes	Whole brain	mRNA, PCR, BzATP, oATP, BBG, ICC, Western	Synthesis of leukotrienes, regulation of AQP4 Responds to MS lesion	[32, 54, 98–100]
	Hippocampus	ICC, BzATP,	MAPK and chemokine activation, release of Glu, Asp, LY pore	[21, 48, 49]
	Cortex	PCR, Western, BzATP, oATP, BBG	EtBr pore, Akt phosphorylation	[50, 101]
	Fetal astrocytes (no) IL1 activated astrocyte	PCR, Western, BzATP, KN-62	NO release	[54]
	Hippocampus (no)	ICC, electrophysiology		[52, 53]
S.C. Astrocytes	Spinal Cord	BBG, BzATP	ATP release for calcium waves,	[57]
	Spinal Cord (no)	ICC		[36]
Microglia	Whole brain	ICC, mRNA, BzATP, BBG, electrophysiology, Western, PCR, k/o	Modulates microglial proliferation; IL1β secretion; TNFα release, Erk/p38 activation	[32, 61, 70, 76, 102–104]
	Hippocampus	ATP, OxATP	Microglial apoptosis	[60], [62]
	Cortex	ICC, RB2, BzATP, oATP, BBG	Infarction response, superoxide and NO release, neuronal death	[77, 105]
	Spinal cord	Western, ICC		[63, 73, 75]
Schwann cells	DRG	ICC, BzATP, oATP, mRNA	LY pore	[64, 95]
Muller cells	retina	ICC, PCR, BzATP, KN-62		[106]
	Retina (no)	ICC		[97]

NMJ Neuromuscular junction, *VH* ventral horn, *DH* dorsal horn, *S.C.* spinal cord, *EtBr* ethidium bromide, *LY* Lucifer Yellow, *MS* multiple sclerosis, *NO* nitric oxide; *(no)* indicates no evidence of localization in this cell type

exhibited Lucifer Yellow or ethidium bromide permeability in response to BzATP or high ATP activation, respectively, consistent with presence of P2X7Rs. P2x7R has also been implicated in the regulation of calcium signaling by protein kinase C in an astrocytic cell line [51]. In contrast, an evaluation of the anti-P2X7R immunoreactivity observed in hippocampal sections of the P2x7R−/− mice has shown a subcellular localization incompatible with a plasmalemmal receptor in addition to absence of signal in astrocytes [52]. This same study also found that the responses obtained after addition of BzATP were indeed the result of the catabolic action of ectonucleotidases that degraded BzATP into BzAdenosine, triggering then actions trough the A1 receptors. Jabs et al. [53] have also failed to detect P2X7R currents in hippocampal astrocytic cultures.

It is not known whether these observations can be extended to astrocytes from another area. Similarly, not enough evidence exist to support the clear differences between astrocytes from brain vs. spinal cord since most of the reports on P2X7R in astrocytes come from culture preparations that could alter the expression profile. Still, there is inclination to believe that P2X7Rs are not predominant in astrocytes in general, which seem to rely more in P2Y and other P2X receptors to mediate purinergic signaling. Actually, a recent report examining the expression of P2X7R mRNA in the nervous system failed also to detect astrocytic signal for this receptor, as opposed to all the other major glial cell types [40].

However, despite low levels of P2X7R being confirmed also in human fetal brain astrocytic cultures, Narcisse et al.

[54] have shown a significant transient increase in the levels of P2X7R mRNA and protein expression when these astrocytes were treated with the inflammatory molecule IL-1β, with concomitant YO-PRO1 permeability upon BzATP stimulation. Thus, it is possible that astrocytic purinergic signaling relies on different receptors depending on the conditions tested and that P2X7Rs in astrocytes become more prominent during pathological states.

P2X7R expressed in other glial cells could also contribute to the progression of neurodegeneration. This is particularly important when we think about putative loops of purinergic signaling between neurons and glia given the critical role astrocytes play in modulating neuronal excitability and synaptic function (reviewed in [55]). In the spinal cord, somatic ATP released by DRG neurons promotes P2X7R-mediated responses in the surrounding satellite cells that, by releasing TNFα, alter the P2X3R-mediated responses and excitability of the DRG neurons [56]. The existence of positive and negative feedback between astrocytes and neurons in the spinal cord during injury and neurodegeneration could have tremendous impact in the progression and outcome of disease. In this regard, an enhanced P2X7R immunoreactivity is observed in astrocytes from autopsied tissue of multiple sclerosis patients [54]. Using spinal cord astrocytes from P2X7R-null mice, Suadicani et al. [57] has recently found that, contrary to previous ideas, P2X7 receptors could be responsible for intercellular calcium wave amplification when cells are exposed to solutions with low concentrations of divalent cations although the absence of P2X7R did not prevent the occurrence of astrocytic calcium waves. P2x7R activation and calcium signaling has also been described in optic nerve glia [58] and among bone cells [59]. These studies support the hypothesis that P2X7R could, indeed, directly mediate ATP release during episodes of astrocytic calcium signaling. In addition, Verderio and Matteoli [60] have shown that astrocytes can also transmit ATP-mediated calcium signals to co-cultured microglial cells via the p2x7R and that these effects were exacerbated by addition of interferon-γ, an inflammatory cytokine also released during injury.

In contrast to the conflicting reports on astrocytic expression, P2X7R can be unequivocally detected in microglia of cortex and spinal cord both at the mRNA and protein level [40, 61–63] consistent with the abundance of this receptor in lymphocytes, monocytes, macrophages, and cells involved in immune responses. The functional implications of microglial P2x7R are enormous and will be discussed later in detail.

Optic nerve oligodendrocytes and DRG Schwann cells have also been described to express P2x7R, based on current and calcium responses upon BzATP application, YO-PRO-1, or LY uptake in the presence of 1 mM ATP and in situ RNA labeling that colocalizes with the oligodendrocytic marker OX-42 [40, 58, 64] although no particular function for P2x7R has yet been recognized in this cell type.

Pathological role of P2x7R in spinal cord

Purinergic signaling is often associated with pain and inflammatory responses. For instance, ATP is the major mediator of pain signaling in the spinal cord [19] and promotes the release of interleukin 1 and other pro-inflammatory cytokines during injury [20, 65].

Microglial P2x7R in neuropathic pain

P2X2, P2X4, P2X6, P2X7, and P2X12 have been prominently described in microglia. Since ATP responses in microglia come mostly associated to injury, these receptors, specifically P2X4 and P2X7, have been linked to responses associated to spinal cord damage and inflammation being primary mediators of pain sensation. Thus, microglial P2X4R is responsible for the phenomenon of tactile allodynia, hypersensitivity to a non-painful stimulus after nerve damage [19]. P2X4R expression increases in microglia but not in neurons or astrocytes after spinal nerve injury. Knock-down studies revealed suppression of tactile allodynia after the injury [66]. A role for P2X7R in the development of neuropathic pain has come from studies where alteration in the P2X7 receptor pathway also shows reduction of neuropathic pain [65]. This receptor is also overexpressed in patients with chronic pain and disruption of the receptor leads to impairment in the production of IL-1β, IL-10, and IL-6, evidencing its strong involvement in inflammatory responses [67, 65]. Importantly, systemic administration of P2X7R antagonists produced a dose-dependent reduction of neuropathic pain [68, 69]. The exact mechanism by which ATP can produce neuropathic pain hypersensitivity might involve activation of the p38/ERK/2 MAPK pathway [70, 66].

However, the uniqueness of the P2X7R as compared to other P2 receptors, with its ability to mediate formation of a large pore that allows directly or indirectly the passage of inflammatory molecules, the high ATP concentration required for its activation and its susceptibility to low calcium conditions, mostly attained under pathological conditions, has additionally pointed to this receptor as a major contributor during other diseased states. Consistent with this hypothesis an increase in P2X7R immunoreactivity has been observed in activated microglia of two transgenic models of Alzheimer's disease [71, 72] in microglia/macrophages of spinal cord undergoing multiple or amyotrophic lateral sclerosis [73], in ischemic cortical tissue [74], and in a model of kainite-induced seizures [62] suggesting

that microglial P2X7R, together with P2X4R, might be a general mediator of stress during pathological states. Besides uptake and release of inflammatory molecules, other pathways might contribute to microglial effects on neuronal excitability and disease outcome. For example, Morioka et al. [75] have recently shown that p2x7 stimulation in spinal microglia produces downregulation of glutamate transport. Bianco et al. [76] have also reported the involvement of the P2X7R in modulation of the proliferation capability of microglia adding complexity to the mechanisms by which P2X7R can be involved in the microglial response to injury.

Neuronal P2X7R in spinal cord injury

Wang et al. [14] have analyzed the direct contribution of the P2X7 receptors in the amplification of cell damage in a model of spinal cord injury. Using a bioluminescence technique in the intact, live animal, they found that the peritraumatic area after acute spinal cord injury exhibit unusually high levels of ATP release, which is accompanied by the subsequent cell death of spinal neurons highly immunoreactive for P2X7 receptors. The same study showed that application of the P2X7R antagonist OxATP diminished spinal cord damage. Further studies in this model are needed to establish the source of ATP release during the injury period, but this work highlights the importance of the P2X7 receptor and purinergic signaling as a potential target for therapeutic intervention in a number of traumatic and neurodegenerative disorders. Melani et al. [77] have also evaluated the effect of the P2 unselective antagonist Reactive Blue 2 in ischemia and found a reduction in the extent of ischemic brain damage. It is noteworthy to mention that Reactive Blue 2 promoted even more the expression of P2X7R in activated microglia.

Caution, however, must be exerted when applying purinergic antagonists for the treatment of diseased CNS conditions. oATP, an antagonist considered specific of P2X7Rs, affects pro-inflammatory responses independently from the activation of P2X7 receptors because HUVEC, HEK293 and 1321N1 cells all secrete less IL-8 without expressing P2X7Rs [78]. P2X7R knock-out studies in autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis, have also shown a significant reduction in the protein levels of IL-1 and IL-6 with a concomitant decrease in lymphocytic apoptosis which, in turn, exacerbates the susceptibility to the disorder [79]. Similarly, a change in the pro-inflammatory profile by selective ablation of proliferating microglial cells exacerbates cell death and injury in the ischemic brain [80]. Thus, whereas interleukins levels are associated to inflammation, they are also important mediators in neuroprotection of the damaged tissues. Consistent with this idea is the fact that animals with P2X7R deletion show lack of protection against ischemic injury [81], although administration of exogeneous microglia and subsequent neuroprotection to global brain ischemia has also been reported [82].

In summary, ATP is released during injury and purinergic signalling can affect every major cell type in the diseased brain. ATP released from damaged neurons could stimulate ATP-dependent astrocytic calcium waves that, in turn, could affect neuronal, microglial and endothelial responses. Furthermore, ATP released from damaged neurons functions as chemotactile cue for microglia, which in turn can initiate inflammatory responses and phagocytosis [83]. Very promising therapeutic results have recently been obtained with compounds like the P2X7R antagonist oATP, which reduces neuronal cell death and improves functional recovery in a rat model of spinal cord injury [14], and the P2X4R modulator ivermectin, which promotes motoneuron survival in a model for amyotrophic lateral sclerosis [84].

Future directions

As the number of studies trying to elucidate the function of purinergic signaling increases, it has become more and more clear that this signaling mechanism is very complex. One reason why it has been difficult to fully understand the specific function of different ATP receptors relates to the pharmacological profile that has been described for them. The available drugs do not allow complete elucidation of the role of each receptor due to their actions on several P2 receptor subtypes. For example, BzATP is considered the most specific agonist for the P2X7 receptors, but it has more potent actions on other P2X receptors [85].

In addition, the available receptor antagonists are weak, may have different sensitivities depending on the species [85], or may have other pharmacological actions, like suramin, which can antagonize G-proteins [86]. In many cases, there are no specific antibodies that would enable us to establish the expression pattern of each receptor. For example, anti-P2X7R antibodies produce an immunoreactive signal that cannot be eliminated in mice deficient for this receptor [39].

Last, some of the receptors can form homo- or heteromultimers depending on the in vitro conditions used to study their pharmacology profile. This effect seems to vary in vivo and is cell-specific [87] The presence of different subunit composition involved in channel formation can, again, alter the pharmacology of the receptors and preclude us from knowing the true subtype involved in each cell type.

Yet another level of complexity in purinergic signaling comes from the fact that extracellular ATP has a short half-life once it is released. There are abundant ectonucleotidases in the cell surface that break down ATP into ADP, AMP, and adenosine, each of which can then act on different receptor

subtypes. It is possible that ATP degradation products are responsible for some of the effects of ATP on synaptic plasticity and neuroprotection [88]. Studies on the pharmacological profile of P2 receptors may also be affected by the action of ectonucleotidases, as rapid degradation of the agonists can diminish their potency and result in a pharmacological profile that depends on agonist stability rather than the true binding affinity for the receptor [85]. In addition, the expression pattern of ectonucleotidases overlaps in vivo and it has not been easy to assign specific roles of these enzymes to each purinergic pathway [89].

Despite all these shortcomings and the debatable evidence about the specific localization of p2x7R in neurons and astrocytes of the spinal cord, it is clear that there exist ATP responses in the spinal cord mediated by P2X7-like receptors. The existence of oATP and other pharmacological compounds that can block these responses and thus diminish spinal cord damage have profound implications in the field of spinal cord regeneration. Whether these compounds are specific of a p2x7 receptor per se or mediate their effects through a combination of mechanisms or through a highly related protein species should not diminish the importance of these findings. Rather, we should be encouraged by these results to further clarify the nature of the effects and refine the plethora of reagents that could be used for reducing spinal cord damage.

In addition, more studies to try to establish the true nature of purinergic signaling in the spinal cord and, by extension, the rest of the nervous system are guaranteed. Efforts should be directed into obtaining more specific antibodies for each receptor species, into improving the specificity of ATP receptors agonists and antagonists to unequivocally differentiate between receptors, into dissecting each of the cell types involved for each specific effect via cell-type-specific knock-outs taking into account the interplay between ATP and the related nucleotides, the role of nucleotidases and the striking presence of adenosine and adenosine receptors in spinal cord injury. Deciphering the exact molecular entities that result in a functional larger pore upon P2X7R activation, the existence of more downstream effectors and what the pore exactly does is also crucial in order to design meaningful therapeutic strategies to diminish receptor function during pathological states.

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