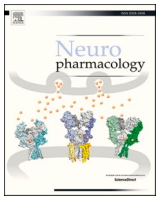
Neuropharmacology 221 (2022) 109278

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm



Rethinking purinergic concepts and updating the emerging role of P2X7 and P2X4 in amyotrophic lateral sclerosis

Cinzia Volonté [a,b,*], Susanna Amadio [b]

[a] CNR-Institute for Systems Analysis and Computer Science "Antonio Ruberti", Via Dei Taurini 19, 00185, Rome, Italy
[b] IRCCS Fondazione Santa Lucia-Cellular Neurobiology Unit, Via Del Fosso di Fiorano 65, 00143, Rome, Italy

ARTICLE INFO

Keywords:
ALS
Extracellular ATP
Motor neuron
P2X4
P2X7
Purinome

ABSTRACT

The topic of the present review regards the ubiquitous and phylogenetically most ancient prototype of intercellular signaling, the one mediated by extracellular nucleosides and nucleotides, bearing a strong influence on pathophysiological processes in the nervous system. Not by chance, purine and pyrimidine molecules are the most prevalent and ubiquitous chemical messengers in the animal and plant kingdoms, operating through a large plethora of purinergic metabolizing enzymes, P1 and P2 receptors, nucleoside and nucleotide channels and transporters. Because ectonucleotidases degrade the agonists of P2 receptors while simultaneously generate the agonists for P1 receptors, and because several agonists, or antagonists, simultaneously bind and activate, or inhibit, more than one receptor subtype, it follows that an all-inclusive "purinergic network" perspective should be better considered when looking at purinergic actions. This becomes particularly crucial during pathological conditions as for instance amyotrophic lateral sclerosis, where the contribution of purinergic signaling has been demonstrated to differ according to each target cell phenotype and stage of disease progression.

Here we will present some newly updated results about P2X7 and P2X4 as the most thoroughly investigated P2 receptors in amyotrophic lateral sclerosis, being aware that the comprehension of their actions is still in progress, and that the purinergic rationale for studying this disease must be however wide-ranging and all-inclusive.

This article is part of the Special Issue on 'Purinergic Signaling: 50 years'.

Note

A comprehensive description of ionotropic P2X, metabotropic P2Y and P1 receptors, as well as ectonucleotidases and purinergic transporters is out of the scope of the present work. Please refer to the Appendix-Glossary section for a brief description of these purinergic mediators, or to the several authoritative reviews most recently published on the topic by prominent scientists.

1. Introduction

What makes a scientist a great one is the prompt ability to change perspective, to think creatively, in other words: i) punctually understanding when things do not fit in the way we expect; ii) quickly finding out what went wrong; iii) finally elaborating a reasonable and all-inclusive explanation for the unpredictable. As such, a great scientist is i) alert, ii) knowledgeable, iii) and ingenious. Regardless of this simplistic formulation, we cannot forget the challenging complications and failures that we inevitably come across during our daily research work. With no doubt this resembles the purinergic story in its early days. Indeed, I still remember Geoff (Prof. Burnstock for those who haven't had the great honor and pleasure of knowing him) describing so vividly his initial struggle into the purinergic field, the overpowering skepticism he had to defeat in order to legitimate even the existence of a purinergic transmission, and particularly his *"significant discovery made in Australia that questioned the established doctrines and it was rejected for over 20 years*" (Burnstock, 2018). Geoff's first seminal article (Burnstock et al., 1970) and review (Burnstock, 1972) providing unambiguous evidence of ATP as the transmitter extracellularly released by non-adrenergic inhibitory nerves in the gut, represented a real astonishing breach into the contemporary scientific thinking, an abrupt "z to a" change in perspective. Now looking back, it was certainly "*against the odds*" that the extracellular ATP field would have generated more than 30 thousand publications in about 50 years of research (near 50 publications/month)

Abbreviations: ALS, amyotrophic lateral sclerosis; Fas, Fas cell surface death receptor; Hsp90, heat shock protein 90; SOD1, superoxide dismutase 1.

* Corresponding author. CNR-Institute for Systems Analysis and Computer Science "Antonio Ruberti", Via Dei Taurini 19, 00185, Rome, Italy.
E-mail address: cinzia.volonte@cnr.it (C. Volonté).

https://doi.org/10.1016/j.neuropharm.2022.109278
Received 11 July 2022; Received in revised form 27 September 2022; Accepted 29 September 2022
Available online 3 October 2022

according to a PubMed "purinergic receptor" basic query dated July 2022. Unquestionably, Geoff proved to be a great inventive scientist, a lighthouse for young and senior researchers, a fearless pioneer whom we all worship, miss and want to commemorate today through this Neuropharmacology Special Issue entitled "Purinergic Signaling: 50 years on".

2. Basic and transformative purinergic concepts

Undoubtedly, the best way to praise Geoff is to show the young generations the power of purinergic signaling (Burnstock, 2020a,b; Burnstock and Knight, 2018), and to communicate awareness and scientific interest on extracellular ATP-evoked responses and mechanisms. We already know we are succeeding on this, when we see a smiley face on our students and postdocs after a successful purinergic experiment. Another way to praise Geoff is to push the purinergic creativity to the limits, as Geoff always wanted in first person and inspired in all of us. Let us now provide some examples of basic and transformative purinergic concepts clarifying the strength of extracellular ATP signaling, from physiologic (Tang and Illes, 2017) to pathologic conditions (Huang et al., 2021), from cancer (Di Virgilio, 1995; Di Virgilio et al., 1990, 2021) to neurodegenerative diseases (Illes, 2020; Illes et al., 2019).

2.1. Positive evolutionary pressure

Evolution proceeds through trial-and-error schemes. However, it is quite difficult to unequivocally establish if and how the process of releasing ATP was maintained during evolution as a deterministic cellular mechanism, or simply as a reiterated fatalistic cellular accident. The same can be said about extracellular nucleotide-metabolizing enzymes, purinergic P1 and P2 receptors, nucleoside and nucleotide channels and transporters (from now on named "purinome" for brevity and inclusiveness, see *Glossary,* and Volonté and D'Ambrosi, 2009). Two main questions come to mind at first: i) were the purinergic mediators already present on the plasma membrane when ATP was first released into the extracellular environment? ii) Did the purinergic mediators gradually adapt from some cognate proteins to specifically and univocally bind and/or metabolize the extracellularly released purinergic ligands? No matter what the origin of purinergic signaling might have been, the emergence, propagation, and consolidation of the purinome during billions of years of evolution, and in diverse species from protozoa to humans, certainly reflects its positive evolutionary pressure and biological relevance.

Purine and pyrimidine molecules are indeed the most primitive and prevalent chemical messengers in the animal and plant kingdoms, and purinergic signaling is described not only in vertebrates, but also in lower vertebrates, invertebrates, and plants. These include the single cell green algae *Ostreococcus tauri* (the smallest free-living eukaryotes), the soil-living amoeba *Dictyostelium discoideum* (transitioning from unicellular to multicellular during developmental life cycle) and the platyhelminth *Schistosoma*. However, the purinergic receptors of these organisms share low primary sequence homology with mammalian P2X receptors (Fountain and Burnstock, 2009; Fountain, 2013). Potent pharmacological actions of nucleotides and sensitivity to purines and pyrimidines are moreover described in the plant *Arabidopsis* and in the nematode *Caenorhabditis elegans*, despite the absence of genes apparently coding for P2X receptors in these same organisms (Burnstock and Verkhratsky, 2009; Verkhratsky, 2021) . Similarly, purinergic actions have been described in *Drosophila melanogaster*, *Apis mellifera* and *Anopheles gambiae*, although the genome analysis of these insects reports no protein homologs of P2X receptors. The *Drosophila* genome instead contains some orthologues of the P2Y family, even if the molecular nature of these P2Y-like receptors is not well defined yet (Metpally and Sowdhamini, 2005). This means that novel purinergic receptors might be promptly discovered in these same organisms, and the purinergic field hasn't reached its limits yet in demonstrating its high power signaling.

2.2. The purinome cooperation network

The purinome is defined as the functional synergy existing among ectonucleotide-metabolizing enzymes, purinergic P1 receptors, P2 receptors, nucleoside/nucleotide channels and transporters, and finally purinergic ligands. It is based on a very elementary principle: purinergic molecular cooperation, i.e., the convergence, in space and time, of multiple purinergic mediators under a common final goal of providing long-lasting cellular benefit during physiological or pathological conditions, exemplified for instance by amyotrophic lateral sclerosis (ALS), as we describe in this work (Fig. 1). As such, protein cooperation is an efficient biological strategy to guarantee and optimize cellular responses to extracellular/intracellular modifications, a process far beyond mere protein interaction. Several experimental results and computational models indeed substantiate the fact that proteins have a general natural propensity to cooperate. The network-connectivity and the network-oscillations of the brain are for instance among the most sophisticated examples of molecular cooperation and higher processes cooperation (Krupnik et al., 2021; Tan et al., 2021). Within the purinome, the cooperation network allows the cell to sense the extracellular environment, and to mediate toward a final biological outcome all the responses generated by the multiple inputs instantaneously generated by the diverse types (and concentrations) of purines/pyrimidines released and/or metabolized outside the cell. By considering how fluctuating the extracellular environment can be, the final goal of purinergic cooperation is always to "choose, stabilize and reinforce" the most appropriate biological response. This will occur by activating at each time a different, but specific, network of purinergic molecular responders, among the several nucleotide-metabolizing enzymes, P1, P2 receptors, nucleoside and nucleotide channels and transporters that are concurrently present on the plasma membrane. The selection of these networks of integration and cooperation is the condition necessary for generating purines/pyrimidines input correlation, purinergic receptor-signal transduction coupling, molecular fine tuning, large-scale synchrony, but also functional complexity (Volonté et al., 2006, 2008a).

A critical issue to be considered at this point is that the larger-scale selectivity that originates within different combinations of purinergic cooperation networks is substituting the concept of receptor selectivity, thus becoming a parameter to explain not only the diversity, but also the specificity in the outcome functions. If a biological action is participated by a unique network of purinergic mediators, i.e., by a specific purinome, we might say that each biological function possesses its own purinergic signature. As an example, the P2 antagonist Reactive Blue 2 (also known as Basilen blue by an outdated nomenclature, registry number 12236-82-7) was reported to be one of the few antagonists that, coherently with its inhibition of extracellular ATP binding, can prevent Ca^{2+} uptake, neurotransmitter release and, most importantly, glutamate-evoked excitotoxicity in cerebellar (Volonté and Merlo, 1996), cortical and hippocampal neurons (Volonté et al., 1999). Reactive Blue 2 inhibits P2X1,2,3,5 and P2Y4,6 receptor subtypes, thus indicating that receptor selectivity fails in explaining its potent protective effect against glutamate. Conversely, the simultaneous antagonistic action of Reactive Blue 2 on a combination of different P2 subtypes is perhaps the requisite for its efficacy, at least in this specific case. Ironically, Reactive Blue 2 might be effective against glutamate because of nonselective P2 antagonism, when very selective structural receptor ligands have instead failed under the same conditions.

This is a striking example of how nonselective purinergic drugs can become a fruitful turnaround perspective, a powerful means not only for elucidating biological functions, but also for ameliorating pathological mechanisms and, most importantly, providing unexpected therapeutic solutions, perhaps also for ALS, as we will address later on in this work. Of note, polypharmacology remains one of the unmet needs and major challenges in drug discovery and development. Not by chance, this

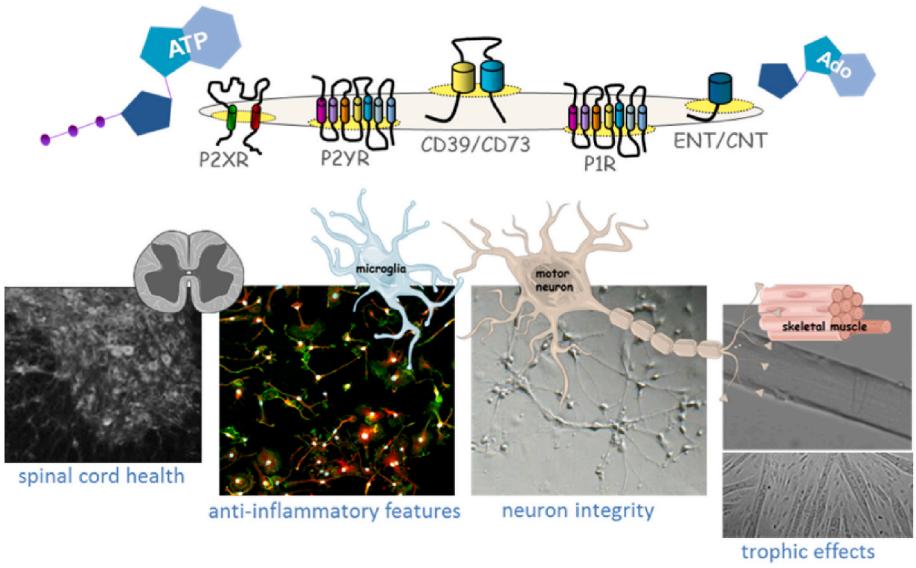


Fig. 1. The purinome network in the ALS context
Ionotropic P2XR, metabotropic P2YR receptors, ectonucleotide metabolizing enzymes (CD39/CD73), metabotropic P1R receptors, nucleoside/nucleotide channels and transporters (ENT/CNT), and purinergic ligands ATP and Adenosine (Ado) can overall participate in ALS disease.

might become particularly interesting in ALS, since only two drugs are approved by FDA up today, Riluzole/Rilutek® and Edavarone/Radicut®, however with only a modest beneficial effect on some specific subgroups of patients. Moreover and regrettably, the vast majority of therapeutics tested preclinically or in clinical trials were unsuccessful in patients, likely due to general difficulties in reaching the target motor neurons, to the genetic heterogeneity of the disease, and finally to the multifactorial nature of ALS molecular mechanisms. Thus, instead of designing always more subtype-specific purinergic agonists and antagonists, and narrowing down their receptor cross reactivity, shouldn't we take a step back and now rethink about the potential biological utility and power of non-specific purinergic ligands with larger spectrum of target?

2.3. From cooperation to molecular compartmentalization and redundancy

If we now consider the purinome not as a simple summation of elementary protein units (nucleotide-metabolizing enzymes, P1/P2 receptors, nucleoside/nucleotide channels and transporters), but as the functional cooperation existing among these units, we must contemplate the role played by the purinergic ligands as well. In this view, the fundamental elements of cooperation, i.e., macromolecule's coupling and information processing exerted by purinergic proteins must be complemented by the coupling among P2 and P1 receptor ligands, exemplifying diffusional information transfer. This occurs particularly when the same enzymatic hydrolysis (for instance by ectonucleotidases) degrades the agonists of P2 receptors, but simultaneously generates the agonists for P1 receptors, or when the same agonist (or antagonist) binds to more than one receptor subtype, although within different affinity properties. Ligand's coupling thus becomes a crucial step of purinergic cooperation, and the purinome acts both by processing information through macromolecules' coupling and by transferring information through ligand's coupling. Again, in this way, the concept of purinergic "receptor selectivity" is widening its range of action and evolving into a concept of "receptor network selectivity". The all-inclusive summation of purinergic enzymatic and receptor binding events thus triggers a specific and unique purinergic signature and biological response within a given cell.

Since multiple purinergic mediators are simultaneously localized and activated on the plasma membrane, a simple combinatorial calculation has demonstrated that for instance combinations of P2 receptors instead of single P2 receptors can contribute to a bigger number of potential cellular functions. In detail, by combinatorial calculation we can determine that seven P2X and eight P2Y receptors will be able to participate into hundreds, instead of dozens, distinct functions (Volonté et al., 2006). The combinatorial receptor model can be a way for amplifying functional diversification, and not just for justifying the purinergic receptor redundancy as a mechanism for securing house-keeping functions. While purinergic receptors might have been generated as redundant proteins by gene duplication during evolution, it remains however unclear how they might have acquired new interaction specificities to establish novel paralogous signaling pathways. As an example, extracellular ATP can become toxic for motor neurons and cortical neurons in ALS, by activation of at least a P2X7 receptor astrocyte-mediated mechanism, or by activation of the P2X7 receptor by Hsp90 inhibition (Gandelman et al., 2013; Strayer et al., 2019). Moreover, ATP can induce apoptotic and necrotic features in cerebellar, striatal, and hippocampal neurons by inducing P2Y4 expression, in addition to P2X7 (Amadio et al., 2002). Similarly, UDP exerts cytostatic and cytotoxic actions in human neuroblastoma cells by overexpressing P2Y6, but not P2X7 or P2Y4 (Apolloni et al., 2010). Conversely, in PNS sympathetic-like neurons, extracellular ATP can sustain trophic actions, survival, and promote neurite regeneration after nerve growth factor deprivation, with upregulation of P2X2 and P2X4 (D'Ambrosi et al., 2000; 2001). However, increased P2X4 immunoreactivity is selectively associated with degenerating motor neurons in ALS rat spinal cord, cerebral cortex, brainstem, locus coeruleus reticular formation, and Purkinje cells of cerebellar cortex (Casanovas et al., 2008). Moreover, neuritogenic effects are exerted in neuroblastoma cells by extracellular UTP, which can conversely induce cell death by prolonged activation of P2Y4 (Cavaliere et al., 2005). Additionally in the CNS, extracellular ATP and ADP promote oligodendrocyte migration, differentiation, and myelination with a dual regulatory role sustained at least by P2Y1, P2X4, and P2X7 (Agresti et al., 2005a,b; Domercq and Matute, 2019). Finally, depending on the specific cellular phenotype and array of purinergic receptors simultaneously co-expressed, the P2Y12 subtype can characterize either the myelinating status of mature oligodendrocytes (Amadio et al., 2006), or the ramified nature of surveilling microglia under resting conditions that are lost for instance during ALS (Amadio et al., 2014).

By retrospectively analyzing these results, we can sustain that

combinatorial cooperation of just a few receptor subtypes might allow more diversified functions. But more questions come to mind. Are there specific networks of cooperation beneath these functions? How and which purinergic cooperation network would be selected at each time?

A mechanism for predisposing proper molecular components to cooperate within a confined space, and increasing the efficacy and variety of functional processes, is compartmentalization. Protein compartments can amplify and/or diversify the protein cooperation process and related functions. In doing so, compartmentalization should not sustain a stochastic distribution of protein; rather, it promotes a specific selection culminating into a clear advantage for the cell. This can apply to protein networks in general, and to the purinergic family as well.

Molecular compartmentalization can occur at the plasma membrane within microdomains, and the biomolecular cooperation network generated in this way strictly depends on either the extracellular and intracellular hydrophilic, or the intramembrane lipophilic environments, which directly contribute to the type, magnitude, and direction of the signals that are propagated. These signals are "vertical" when directed towards the extra and intracellular sides of the cell, or "horizontal" when restricted to the plasma membrane (Agnati et al., 2005). Protein compartmentalization at the plasma membrane often occurs within specialized sub-membrane microdomains named lipid rafts. They are highly enriched in cholesterol and sphingolipids and form the liquid-ordered phase of the lipid bilayer. Lipid rafts are crucial for many functions in neurons, including neurotrophic factor signaling, adhesion, axon guidance, vesicular trafficking and axonal versus dendritic sorting (Grassi et al., 2020). Lipid rafts and membrane/lipid rafts scaffolding proteins play a beneficial role also in ALS, by protecting spinal motor neurons, preserving neuromuscular function, and extending longevity in a familial mouse model of ALS (Wang et al., 2022). While the ionotropic P2X3 (Vacca et al., 2004) and the metabotropic P2Y12 (Bodin et al., 2003) were the very first purinergic receptors that were identified within lipid rafts, all P2/P1 subtypes and ectonucleotidases are now reported to associate into these specialized submembrane microdomains (Lasley, 2011; D' Ambrosi and Volonté, 2013; Murrell-Lagnado, 2017). Additional experimental work by FRET, BRET, EM analysis has moreover demonstrated that not only P2X and P2Y subtypes, but also A1 and A2 receptors, associate in these microdomains into hetero-oligomeric complexes (Jiang et al., 2003; Nakata et al., 2005; Ecke et al., 2008; Compan et al., 2012; Schonenbach et al., 2016). However, hybrid ionotropic-metabotropic associations are still to be demonstrated.

It is evident that recruiting various combinations of purinergic receptors to associate and cooperate within a single compartment (a cell, a chunk, or a lipid raft molecular correlate) to fulfill multiple heterogeneous functions responds to the energy-saving principle of minimal effort with maximal efficacy (Volonté et al., 2008b). The next challenging issue is now to understand how to compose and regulate the dynamic architecture of these purinergic building blocks within a membrane microdomain. We trust that more sophisticated experimental models, tools, and technologies will soon identify novel and now unpredictable purinergic associations to still open new frontiers in the purinergic field.

3. Unpredicted purinergic matters

Milestone scientific discoveries are often sustained by unpredictable thinking at the intersection of chance, knowledge, and intuition. This applies to the purinergic field as well, as we mentioned above about the "incipit" of the purinergic saga 50 years ago with that "*significant discovery made in Australia that questioned the established doctrines and it was rejected for over 20 years*". Surely astonishing, against the odds, "*accepted with explosive worldwide developments …. changing the face of neuropharmacology and leading to novel therapeutic developments*" (Burnstock, 2018). Now we present a case of unpredicted complex behavior of purinergic receptors linked to a pathological condition of the nervous system, ALS, that indeed requires an open-minded perspective to be explained.

3.1. P2X7 in amyotrophic lateral sclerosis

When dissecting the role of any receptor, chiefly purinergic receptors that are ubiquitously expressed on each cell phenotype and tissue, it's important to reinforce that their functional specification is temporary correlated to the microenvironment in which they reside. We now briefly introduce ALS, with the purpose of exemplifying this concept.

ALS is a rare (affecting 1 in 50,000 individuals worldwide), multifactorial, neurodegenerative-neuroinflammatory disease whose incidence is expected to escalate by 30–50% by 2030, and whose pathogenesis and treatment options are still elusive and inadequate today (Brown et al., 2021; Goutman et al., 2022a,b). ALS has both genetic (about 10% of total cases) and sporadic/environmental (about 90% of cases) etiology, with *C9ORF72*, *SOD1*, *TDP-43*, and *FUS* representing the top-mutated genes. ALS was originally classified as a motor neuron disease, but cardiovascular, cognitive-behavioral, and psychiatric symptoms are lately emerging as the most common comorbidities, thus explaining the actual need of reclassifying ALS as a broader-spectrum disorder (Zucchi et al., 2019; Diekmann et al., 2020; Xu et al., 2022). The main feature of ALS is that motor neurons gradually and irreversibly lose their synaptic integrity and axonal innervation of the target muscle cells. This evolves into muscle atrophy, paralysis, and early death usually from respiratory failure. In addition to motor neurons as the prime target of the disease, sensory nerves (Tao et al., 2018; Vaughan et al., 2018), Purkinje neurons (Tan et al., 2016), skeletal muscles (Pikatza-Menoio et al., 2021), glia (Filipi et al., 2020), mast cells (Trias et al., 2018), and immune cells (Beers and Appel, 2019; McCombe et al., 2020) are also considered strategic cell mediators and targets of the disease. From a purinergic point of view, this non-cell autonomous mechanism of ALS finds an important functional correlation with the purinome cooperation network, since purinergic ligands released from one cell type can transfer either detrimental or beneficial information to a different ALS-targeted phenotype, and then selectively activate cell-specific arrays of purinergic receptors and propagate the disease. Therefore, several different cell phenotypes have begun to be investigated in order to provide an accurate view of purinergic signaling during the ALS pathogenic process (Sluyter et al., 2017; Calzaferri et al., 2020; Volonté et al., 2022).

Regarding P2X7, comparative analysis performed in various tissues from ALS patients has indeed demonstrated: an augmented expression of P2X7 protein in microglia/macrophages from spinal cord (Yiangou et al., 2006); a constant expressionin motor cortex at early disease stage (Van Weehaeghe et al., 2020); a down-regulation in peripheral blood mononuclear cells (Liu et al., 2016). In rodent models of ALS, P2X7 was confirmed to be upregulated in the spinal cord of superoxide dismutase 1 (SOD1)-G93A rats (Casanovas et al., 2008) and mice (Apolloni et al., 2016), and in sciatic nerves from symptomatic ALS mice (Volonté et al., 2016).

Likely related to the receptor distribution and level of expression, *in vitro* studies have next shown that agonist-dependent activation of P2X7 in ALS primary microglia further aggravates pro-inflammatory responses and triggers indirect toxicity towards motor neuron-like cells (D'Ambrosi et al., 2009; Apolloni et al., 2013a; Parisi et al., 2013, 2016). Likewise, repetitive agonist stimulation of P2X7 in cultured ALS astrocytes causes indirect damage to motor neurons (Gandelman et al., 2010). However, in primary cultures of motor neurons purified from spinal cord, low doses of P2X7 agonists (ATP up to 0,1 mM, or 2′(3′)-O-(4-Benzoylbenzoyl)-ATP in the 0,1–100 μM rage) cause only a moderate toxic effect (27% and 31% cell death, respectively), which is moreover peroxynitrite-, caspase-, and Fas-dependent (Gandelman et al., 2013). In explaining these results, we have to keep in mind that a biological response as cell death might be contributed and made more effective (or counteracted on the other hand) by the presence of a cell-specific purinome cooperation network. The larger-scale ligand

selectivity that originates from considering the purinergic network then advances the concept of receptor ligand selectivity, to the point that broad ranging and less selective agonists/antagonists simultaneously targeting various receptor subtypes could be more efficacious in inducing/combatting ALS.

In motor neuron-like hybrid SOD1-G93A NSC-34 cells (Cashman et al., 1992) constitutively expressing P2X7, aggregated SOD1-G93A protein has been recently shown to be rapidly released into the extracellular space either constitutively or after P2X7 activation, and then re-up-taken by naïve NSC-34 cells or microglia cell lines to induce endoplasmic reticulum stress and tumor necrosis factor α release, respectively mediating neurodegeneration- and neuroinflammation-associated ALS events (Bartlett et al., 2022). Interestingly, the very specific P2X7 antagonist AZ10606120 completely blocks the ATP-induced SOD1 release from NSC-34 cells. Collectively, these results provide further support to the prion-like propagation of SOD1 in ALS (Bartlett et al., 2022), and moreover add substantial evidence to the therapeutic benefits already demonstrated by P2X7 antagonism in ALS mice.

When studying the role of P2X7 in ALS, we have to consider also the P2X7 receptor signaling complex (Kim et al., 2001). The heat shock protein 90 (Hsp90), a pro-survival chaperone needed for proper protein folding, is an integral regulatory component of the P2X7 receptor complex, with the function of antagonizing the ligand-induced receptor activation by decreasing the affinity for ATP and promoting a beneficial effect (Adinolfi et al., 2003; Migita et al., 2016). Hsp90 is endogenously nitrated in spinal cord motor neurons of ALS patients (Chatterjee et al., 2022). Interestingly, nitration of Hsp90 is toxic to motor neurons through a pathological gain-of-function exerted by P2X7 activation and decreased mitochondrial activity (Chatterjee et al., 2022). In addition, inhibition of Hsp90 in motor neurons by geldanamycin leads to ligand independent activation of P2X7 and motor neuron death by apoptosis. Downstream of Hsp90 inhibition, the P2X7 stimulation activates phosphatase and tensin homolog protein (PTEN), which in turn suppresses the pro-survival phosphatidylinositol 3 kinase/protein kinase B pathway, leading to Fas-dependent motor neuron apoptosis. Pharmacological inhibition and down-regulation of the P2X7 receptor even in the absence of extracellular ATP prevents motor neuron apoptosis triggered by Hsp90 inhibition (Strayer et al., 2019). Finally, motor neuron death by intracellular transfer of nitrated Hsp90 (inhibiting intrinsic ATPase activity) requires P2X7 activation, thus suggesting a tight P2X7-Hsp90 interplay (Franco et al., 2013).

In vivo studies in SOD1-G93A mice have shown that pharmacological inhibition of P2X7 by several antagonists and particularly Brilliant Blue G, A804598, JNJ-47965567, or AXX71 (Fig. 2) (within different degrees of efficacy strictly correlated to the different protocols of administration) rescues spinal cord motor neurons from death, also in part improving selected disease features (Table 1) (Cervetto et al., 2013; Apolloni et al., 2014, 2021; Fabbrizio et al., 2017; Sluyter et al., 2017; Bartlett et al., 2017; Ly et al., 2020; Ruiz-Ruiz et al., 2020). However, differently from what expected from the above results, systemic acute administration of the P2X7 agonist 2′(3′)-O-(4-Benzoylbenzoyl)-ATP exerts beneficial actions in skeletal muscles of SOD1-G93A mice, by enhancing metabolism, improving denervation, and promoting new fibers myogenesis (Fabbrizio et al., 2020). Furthermore, chronic intramuscular administration of 2′(3′)-O-(4-Benzoylbenzoyl)-ATP in ALS mice further ameliorates motor performance by augmenting the number of satellite cells and the muscle pro-regenerative activity of infiltrating macrophages (Fabbrizio et al., 2021) (Table 1).

Once more unpredictably respect to the beneficial results obtained by the pharmacological inhibition of P2X7 *in vivo*, genetic ablation of P2X7 in SOD1-G93A ALS mice anticipates the insurgence and accelerates the progression of the disease (Apolloni et al., 2013b).

While apparently contradictory and unexpected, these results that blockade, as well as activation, of P2X7 may improve disease progression clearly illustrate the complexity of purinergic signaling in a multifarious neurodegenerative disease such as ALS. These findings do not reduce, but instead update and expand our knowledge of P2X7 in ALS, with the consequence of strongly encouraging a thoughtful and perhaps all-inclusive purinergic rethinking of the role played by a single receptor subtype in the disease.

If we now wish to correlate P2X7 for instance to the autophagic flux of ALS microglia, once more we must cope with multifaceted and unpredicted results. The short-stimulation of P2X7 augments the autophagy substrate marker microtubule-associated protein 1 light chain 3 (LC3)-II via the mTOR pathway and decreases the autophagosome cargo protein sequestosome 1 (SQSTM1/p62) level, concurrently with up regulation of M2 anti-inflammatory mediators. Conversely, a persistent stimulation of P2X7 impairs the autophagic flux with consequent increase of SQSTM1/p62, and polarization of microglia toward the M1 pro-inflammatory state (Fabbrizio et al., 2017). These notions extend to the process of autophagy the twofold role now well recognized for P2X7 in the ALS context (Volonté et al., 2020).

In synthesis, we can conclude that P2X7 is downregulated in ALS peripheral immune cells perhaps for preventing cytotoxicity and ameliorating inflammation, events that are both detrimentally sustained by P2X7 activation (Hanley et al., 2012). Similarly, in CNS compartments, the receptor must be antagonized for preventing astrocyte-, microglia-dependent, as well as direct toxicity to motor neurons. However, the receptor must be activated in peripheral tissue and particularly symptomatic muscles, clearly corroborating the previously unpredicted beneficial role of P2X7 also demonstrated by genetic ablation.

Overall, we have highlighted the complexity of P2X7 in ALS and the importance of different cellular clues in driving motor neuron loss, muscular impairment, paralysis and finally death. Recent findings indicate that novel P2X7 antagonists are under study for the treatment of an extensive variety of disorders (Pevarello et al., 2017; Andrejew et al., 2020; Mishra et al., 2021). Added to the general challenge for better ability to diagnose, prevent or treat ALS, the potential success of these ligands in clinical trials is however hindered by the existence of P2X7 genetic variations within patient populations (McHugh et al., 2012; Caseley et al., 2014). Although future research will focus on alternative

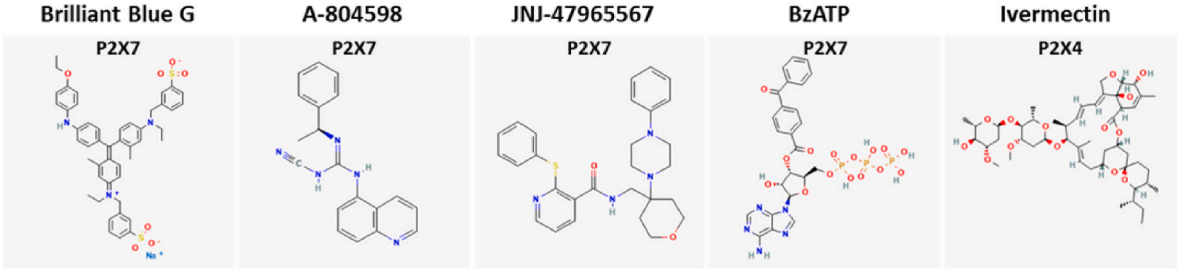


Fig. 2. Commercially available P2X7 and P2X4 ligands tested on SOD1-G93A ALS mouse model
The 2D chemical structures of commercially available P2X7 and P2X4 ligands tested *in vivo* on SOD1-G93A ALS mice are retrieved from https://pubchem.ncbi.nlm.nih.gov/.

Table 1
Biological *in vivo* effects of P2X7 and P2X4 ligands on ALS mice.

DRUG	Molecular formula	EC50	ALS-related action	Ref
BBG **45.5 mg/kg i.p.** **3/w, P90,** ♂♀	$C_{47}H_{48}N_3NaO_7S_2$	Rat = 10.1 nM Human = 265 nM	↑ motor skills ♂♀ ↓ body weight loss ♂	Cervetto et al. (2013)
BBG **250 mg/kg i.p.** **3/w, P100,** ♂♀	“	“	delays onset ♀♂ ↑ motor skills ♀♂ ↑ motor neuron survival	Apolloni et al. (2014)
BBG **45.5 mg/kg i.p.** **3/w, P62,** ♂♀	“	“	↓ body weight loss ♀ ↑ life span ♀	Bartlett et al. (2017)
A-804598 **30 mg/kg i.p.** **5/w, P100,** ♂♀	$C_{19}H_{17}N_5$	Mouse = 9 nM Rat = 11 nM Human = 10 nM	modulates autophagy ♂♀ no effect on disease onset, motor skills, life span ♂♀	Fabbrizio et al. (2017)
JNJ-47965567 **30 mg/kg i.p.** **3/w, P100,** ♂♀	$C_{28}H_{32}N_4O_2S$	Mouse = 7.5 nM Rat = 7.2 nM Human = 8.3 nM	no effect on clinical score, weight loss, motor skills, life span ♂♀	Ly et al. (2020)
JNJ-47965567 **30 mg/kg i.p.** **4/w, P60,** ♀	“	“	delays onset, ↓ body weight loss ↑ motor skills no effect on life span	Ruiz-Ruiz et al. (2020)
AXX71 **30 mg/kg i.p.** **5/w, P100,** ♀	not disclosed	Mouse = 210 nM Human = 14 nM	delays onset ↑ short term motor skills no effect on motor neuron survival	Apolloni et al. (2021)
BzATP **1–10 mg/kg i.m.** **2/w, P84,** ♂♀	$C_{24}H_{24}N_5O_{15}P_3$	Mouse = 285 μM Rat = 3.6 μM	↑ myofibers innervation ♂♀ ↑satellite cell proliferation-differentiation ↑ motor skills, delays onset ♀	Fabbrizio et al. (2020), (2021)
Ivermectin **4.8–12 mg/l oral** **7/w, P50,** ♂♀	C48H74O14	Mouse = 257 nM	↑ life span ♂♀ ↑ motor neuron survival	Andries et al. (2007)

Doses (mg/kg), route (i.p. = intraperitoneal, i.m. = intramuscular), frequency (times/week), and day (P = post-natal day) of administration are reported, together with the gender (♂♀) where the trials were performed and the effects most evident. Antagonists are in black; agonist or allosteric modulator is in purple. BBG: Brilliant Blu G.

approaches circumventing also this limitation, with no doubts the duality of P2X7 in ALS remains a critical feature and a true challenge in our ability to ensure a P2X7-targeted strategy with significant therapeutic value against the disease.

Most of all, in the search for ALS therapies we cannot neglect the role simultaneously played by the additional components of the purinome, besides P2X7. In this regard, we have described above that the concept of purinergic selectivity is lately updating into receptor network selectivity, and receptor ligand selectivity into network ligand selectivity, especially due to the elevated grade of interconnected binding reactions concurrently occurring in the highly fluctuating extracellular purinergic environment. It follows that a better prospect to identify purinergic-dependent therapeutic strategies will have to take into consideration not just the single P2X7, but the summation of the purinergic events concomitantly taking place during the pathologic circumstances of ALS. Although further challenging, this approach is likely to play a more comprehensive and valuable role in advancing our goal: the aim of the present review is to convey optimism in this direction.

3.2. P2X4 in amyotrophic lateral sclerosis

P2X7 and P2X4 receptor genes are in proximity on chromosome 12 in humans, and their overlapping expression is documented in several cell phenotypes and tissues in many different organisms. Moreover, structural and functional interactions between these P2X subtypes have been unequivocally demonstrated to the point of proposing that P2X4 and P2X7 can associate to form heteromeric receptors, thus greatly increasing the diversity of ligand selectivity, receptor currents, and functional responses mediated by these two subtypes, although their overexpression does not apparently result in a novel electrophysiological-discriminable P2X receptor phenotype (Guo et al., 2007; Craigie et al., 2013; Schneider et al., 2017; Trang et al., 2020; Kanellopoulos et al., 2021). Whether P2X7 and P2X4 indeed behave as heterotrimers or interact as homotrimers, in any case it implies a further step in the complexity of the purinome signaling system. This may account for novel and unexpected pharmacological properties and for the fine-tuning of the pathophysiological processes also sustained singularly by these receptors. This acquires a clear relevance particularly in ALS.

Regarding P2X4, recent progress has witnessed the involvement of this subtype not only in an increasing variety of diseases (Stokes et al., 2017), but also in neurological disorders with associated motor symptoms (Oliveira-Giacomelli., 2018). For instance in ALS, the P2X4 is the only other P2X subtype with an apparently recognized role, in addition to P2X7. However, differently from P2X7, P2X4 shows no difference of expression in peripheral mononuclear blood cells of ALS patients compared to healthy individuals (Liu et al., 2016). The receptor is instead upregulated in sciatic nerves (Volonté et al., 2016) and in spinal cord microglia (Bertin et al., 2022) from SOD1-G93A mice during the symptomatic phase of the disease. Both total and membrane-bound P2X4 protein is augmented also in immortalized and primary SOD1-G93A microglia (D'Ambrosi et al., 2009). Moreover, strong P2X4 immunoreactivity is selectively associated with degenerating motor neurons in SOD1-G93A rat spinal cord, cerebral cortex, brainstem, locus coeruleus reticular formation, and Purkinje cells of cerebellar cortex (Casanovas et al., 2008). While degenerating P2X4-positive neurons do not show common apoptotic features as chromatin condensation or caspase 3 activation, they elicit other signs of aberration such as recruitment of microglial cells with neuronophagic activity. To explain these changes, abnormal trafficking and proteolytic processing of P2X4 was suggested (Casanovas et al., 2008). Importantly, ivermectin (Fig. 2) known to specifically augment ion currents through P2X4 channel and allosterically modulate the receptor (Khakh et al., 1999), extends the life span of SOD1-G93A mice of about 10%, by boosting P2X4-mediated neuroprotection against excitotoxicity in motor neurons *in vivo*, and *in vitro* against AMPA receptor-mediated excitotoxicity (Andries et al., 2007) (Table 1). Further research has demonstrated that misfolded mutant SOD1 expression and accumulation in degenerating motor neurons of SOD1-G93A rats are recognized by antibodies against the P2X4 (Hernández et al., 2010). Finally, very recent data have shown that ALS-related misfolded SOD1 and TDP-43 mutant proteins impair the endocytosis mechanism of P2X4 internalization leading to a significant increase in P2X4 plasma membrane density and signaling, particularly

in peripheral macrophages (Bertin et al., 2022). By assessing the impact that membrane overexpression or absence of P2X4 might have on ALS, Bertin and colleagues have finally demonstrated that P2X4 is instrumental for motor symptoms, disease progression and survival of SOD1 mice, also highlighting the receptor as potential early disease biomarker and therapeutic target (Bertin et al., 2022).

Hallmarks of ALS are motor neuron degeneration, neuroinflammation and muscle atrophy, and both P2X7 and P2X4 are present in these districts and have a certain distinctive role in these actions, as we have described here. However, a direct and exhaustive comparative analysis of their single involvement in ALS is not available yet, and the potential direct interaction between P2X4 and P2X7 has neither been investigated in the ALS context, although representing a very appealing matter.

While P2X7 and P2X4 are the only P2X receptors that were thoroughly analyzed in the ALS context and, most importantly, in preclinical testing, also metabotropic purinergic receptors, particularly P2Y6, P2Y12, adenosine A2A, other than ectonucleotidases (CD39, CD39L1) and transporters, have been preliminarily described to participate to the ALS pathomechanism (for a recent review of their role, see Volonté et al., 2022). However, their involvement still needs further and meticulous investigation, together with a more comprehensive purinergic rationale for studying the disease.

4. Conclusions

Before an idea becomes a novel trend, it must be accepted by the scientific community. However, the scientific debate on new topics and original developments of old concepts must necessarily be open-minded.

The leitmotif of the present work has regarded the ubiquitous and phylogenetically most ancient prototype of intercellular signaling, the one mediated by extracellular nucleosides and nucleotides, and particularly, we have referred about the involvement of P2X7 and P2X4 subtypes in ALS. We have presented general examples of how the purinergic signaling, from down to modern days, has required several turnaround thinking and changes in perspective, before being accepted as a legitimate and trendy scientific field. Moreover, we have elaborated on challenges and provided suggestions to address limitations. We have focused our analysis on the spectrum of possible variants among the pathophysiological effects triggered by purinergic receptor ligands in neuronal contexts and in ALS. We have highlighted some interrelated features among these variants and justified that very often the purinergic functional responses must be retrospectively categorized and explained. We have emphasized that the concept of purinergic "receptor selectivity" should be exceeded to evolve into a criterion of "network selectivity". Of note, instead of distinct and single purinergic elements, we must recognize that the full repertoire, or network, of purinergic mediators should be always considered when decoding how the purinergic machinery sustains, optimizes, and reinforces a plethora of fundamental cellular functions. In other words, we indicate that a more holistic view of purinergic signaling better describes any biological outcome, and in particular a disease such as ALS.

At last, we are convinced that the time has come, and the purinergic field has finally opened its breach, despite the strong skepticism encountered by the purinergic theory in its early days. The purinergic challenges and ambitions are now demonstrated to be worthwhile and important. There is now also considerable enthusiasm about the prospect that purinergic mechanisms will improve our knowledge of pathophysiological processes, will contribute to the identification/efficacy/safety of therapeutic solutions, and will prove exciting potential applications in the early detection, screening, diagnosis, prognosis of diseases, and precision medicine. Only by maintaining a keen process of insightful questions, constructive criticism, and open-minded thinking, we will further promote purinergic concepts, identify a purinergic polypharmacology and novel treatment options.

In the end, we hope to have convinced the reader that the continuous success of the entire purinergic field will depend on how seriously our "*changes in perspective*" will be taken, and how sturdily we will be able to push the purinergic creativity "*to the limits and against the odds*" in the next 50 years.

5. Challenging issues for purinergic innovation

- To understand how purinergic mediators are selected and gathered within cellular membranes to cooperate into specific and unique functions.
- To identify the purinome signature distinctive of diseased conditions and particularly of ALS.
- To introduce a targeted purinergic polypharmacology to respond to the evolving concept of receptor network selectivity.

Funding

This work has not received dedicated financial support from funding agencies in the public, commercial, or not-for-profit sectors. Publications fees for this paper and the described recent studies from the authors' laboratory are supported by FATALSDRUG project from National Research Council Progetti di Ricerca@CNR to CV.

CRediT authorship contribution statement

Cinzia Volonté: Conceptualization, Writing – original draft, editing, Table/figures preparation, Funding acquisition. **Susanna Amadio:** Critical review & editing, The corresponding author is responsible for ensuring that the descriptions are accurate and agreed by all authors.

Declaration of competing interest

None.

Data availability

No data was used for the research described in the article.

Acknowledgements

We are most grateful to students and postdocs in our lab for having enthusiastically and critically followed our "z to a" map on the never-ending road to purinergic knowledge. We thank and are in depth with colleagues for having supported and encouraged our purinergic discussions and commitment. Last, but not least, thank you Geoff, for having always instilled in us your overwhelming purinergic passion, and having sustained our sometimes "weird" purinergic ideas, no matter what.

We do apologize to those colleagues and scientists whose findings were not mentioned in this work due to space constraints or oversight.

APPENDIX

Glossary (993 words)

Ectonucleotidases, or ectonucleotide-metabolizing enzymes, are expressed at the plasma membrane with an extracellularly oriented catalytic site. They can hydrolyze nucleoside 5′-tri-, 5′-di-, and 5′-monophosphates, hence giving rise to multiple break-down products (ADP, AMP, UDP, UMP, adenosine, etc.) from extracellular ATP and the other nucleotides. These extracellularly generated nucleotides/nucleosides then serve as ligands for the distinct cell surface receptors belonging to the P1, P2X and P2Y family. There are four major families of ectonucleotidases, namely the ectonucleoside triphosphate diphosphohydrolases (E-NTPDase, hydrolyzing the extracellular nucleoside tri- and

di-phosphates, but not mono-phosphates); moreover the ectonucleotide pyrophosphatase/phosphodiesterase (E-NPP) that hydrolyzes pyrophosphate 5′-monodiester bonds in ATP and dinucleoside polyphosphates; the ecto-5′-nucleotidase also called CD73, which hydrolyzes only nucleosides monophosphates; finally, the alkaline phosphatase (AP) non-specific phosphomonoesterase, which releases inorganic phosphate from nucleoside 5′-tri, -di, and -monophosphates. These enzymes possess a very wide, but partially overlapping tissue distribution.

P1 receptors refer to adenosine receptors by their natural ligand adenosine, and are G-protein coupled receptors comprising four different subtypes (A1, A2A, A2B and A3), each encoded by a different gene (ADORA1-2A-2B-3). A1 and A3 receptors are Gi/o protein-coupled receptors with consequent decreased cAMP production, while A2A and A2B receptors are coupled to Gs protein with resulting increased cAMP production. Each receptor subtype elicits different cellular functions ranging from regulation of myocardial oxygen consumption and coronary blood flow, anti-inflammatory effects, neurotransmitter release in the CNS, and inflammation and immune responses in the periphery.

P2X receptors, in turn subclassified as P2X1-7 subtypes, define the third major family of ionotropic receptors and are non-selective cation channels that mediate fast responses solely to ATP. They are expressed as 379 to 595 amino acid proteins, having tertiary structure with two transmembrane domains, intracellular N- and C-terminus, and post-translational glycosylation and phosphorylation modifications. P2X receptors can be regulated allosterically by extracellular protons, divalent cations, and metals. Histidine residues mediate pH regulation and form metal binding sites. The transmembrane domains are less conserved, while the mostly conserved extracellular loop contains disulfide bridges forming the structural constraints for coupling the ATP-binding site to the ion pore. Desensitization occurs for all P2X receptors, lasting for milliseconds (fast desensitization: P2X1, P2X3 receptors), or 100–1000 times more slowly (slow desensitization: P2X2, P2X4, P2X5, P2X6 and P2X7 receptors). A direct influx of extracellular Ca^{2+} (or Na^{+} and/or K^{+}) through the receptor channel itself is common to all P2X subtypes. P2X receptors can occur as stable monomers, or omo- and hetero-multimeric assemblies (mostly trimers or hexamers). As P1 receptors, they also mediate different physiological and pathological functions.

P2Y receptors, in particular classified as P2Y1, P2Y2, P2Y4, P2Y6, P2Y11, P2Y12, P2Y13 and P2Y14 subtypes, respond to ATP, ADP, UTP, UDP, and UDP-glucose with receptor subtype selectivity, and typically mediate slow responses to nucleotides. They are present as 308 to 379 amino acid proteins, with a mass of 41–53 KDa after glycosylation, and possess tertiary structures with seven transmembrane domains, an extracellular N-terminus, and an intracellular C-terminus. P2Y receptors couple to functionally distinct G proteins: with some exceptions, P2Y1, P2Y2, P2Y4 and P2Y6 are positively coupled to PLC, via Gq/11 proteins, with the generation of IP3 and subsequent mobilization of intracellular calcium, while P2Y12, P2Y13 and P2Y14 are negatively coupled to adenylyl cyclase via Gi proteins. Being coupled to second messenger systems and/or G protein-mediated ionic conductance, the cellular response time of P2Y receptors is longer (less than 100 ms) than that of P2X subtypes. Activation of P2Y receptors is often associated with activation of mitogen activated protein kinases, protein kinase C, and PI3–K. As P1 and P2X receptors, they are ubiquitously distributed in the body to perform a wide range of functions.

Purinergic release, ATP release was originally demonstrated from sensory neurons and was instrumental for developing the new concept of purinergic signaling. Today, the release of ATP particularly as a neurotransmitter or cotransmitter has become well established. The extracellular release of ATP and in general of purine/pyrimidine molecules is never constant, but susceptible to changing over time. It is the result of local extracellular release from secretory cells via conventional vesicular exocytosis, non-exocytotic pathways including connexin-43, pannexin-1, or from damaged or dying cells by passive lytic mechanisms. It can occur by electro-diffusional transport through channels (for instance calcium homeostasis modulator-1), by facilitated diffusion via nucleotide-specific transporters (concentrative and equilibrative types, SNARE complex), and finally dissociation-association reactions. In the nervous system, neurons, astrocytes, and microglia can release ATP.

Purinergic selectivity/specificity, from Latin *seligere* meaning to choose, selectivity is defined as the ability of a ligand, either agonist or antagonist, to bind to a proper purinergic receptor subtype unequivocally and exclusively, while in the presence of several other receptor subtypes expected to be expressed on the same cellular membrane. It is the degree of interference of a ligand with other receptor subtypes belonging to the same receptor family. Specificity is referred to the ability of an agonist/antagonist to interfere with a particular action. A drug of complete specificity of action might decrease or increase a specific function (of a given gene, protein, or cell type), but it must do either one, not both.

Purinome, within a general reference to a large collection of a few thousand distinct proteins expressed by the genome that simply bind purines (Haystead, 2006; Murray and Bussiere, 2009), the term purinome was specifically applied to the purinergic field as a whole (Volonté and D'Ambrosi, 2009), with the precise intent of representing a particular example of molecular cooperation, the purinergic cooperation, and illustrating the functional synergy existing among ectonucleotide-metabolizing enzymes, purinergic P1 receptors, P2 receptors, nucleoside/nucleotide channels and transporters, and finally purinergic ligands. This array of different proteins and molecules are altogether responsible for triggering and transducing the biological effects of extracellular purine and pyrimidine ligands inside the cells. In this way, the purinome is an operational definition that links the microenvironment in which ectonucleotidases, P1, P2 receptors, nucleoside and nucleotide channels and transporters are embedded, to the biological effects directly triggered by extracellular purine and pyrimidine ligands.

References

Adinolfi, E., Kim, M., Young, M.T., Di Virgilio, F., Surprenant, A., 2003. Tyrosine phosphorylation of HSP90 within the P2X7 receptor complex negatively regulates P2X7 receptors. J. Biol. Chem. 278 (39), 37344–37351. https://doi.org/10.1074/jbc.M301508200.

Agnati, L.F., Tarakanov, A.O., Ferré, S., Fuxe, K., Guidolin, D., 2005. Receptor-receptor interactions, receptor mosaics, and basic principles of molecular network organization: possible implications for drug development. J. Mol. Neurosci. 26 (2–3), 193–208. https://doi.org/10.1385/JMN:26:2-3:193.

Agresti, C., Meomartini, M.E., Amadio, S., Ambrosini, E., Volonté, C., Aloisi, F., Visentin, S., 2005a. ATP regulates oligodendrocyte progenitor migration, proliferation, and differentiation: involvement of metabotropic P2 receptors. Brain Res. Brain Res. Rev. 48 (2), 157–165. https://doi.org/10.1016/j.brainresrev.2004.12.005.

Agresti, C., Meomartini, M.E., Amadio, S., Ambrosini, E., Serafini, B., Franchini, L., Volonté, C., Aloisi, F., Visentin, S., 2005b. Metabotropic P2 receptor activation regulates oligodendrocyte progenitor migration and development. Glia 50 (2), 132–144. https://doi.org/10.1002/glia.20160.

Amadio, S., D'Ambrosi, N., Cavaliere, F., Murra, B., Sancesario, G., Bernardi, G., Burnstock, G., Volonté, C., 2002. P2 receptor modulation and cytotoxic function in cultured CNS neurons. Neuropharmacology 42 (4), 489–501. https://doi.org/10.1016/s0028-3908(01)00197-6.

Amadio, S., Tramini, G., Martorana, A., Viscomi, M.T., Sancesario, G., Bernardi, G., Volonté, C., 2006. Oligodendrocytes express P2Y12 metabotropic receptor in adult rat brain. Neurosciences 141 (3), 1171–1180. https://doi.org/10.1016/j.neuroscience.2006.05.058.

Amadio, S., Parisi, C., Montilli, C., Carrubba, A.S., Apolloni, S., Volonté, C., 2014. P2Y (12) receptor on the verge of a neuroinflammatory breakdown. Mediat. Inflamm., 975849 https://doi.org/10.1155/2014/975849, 2014.

Andrejew, R., Oliveira-Giacomelli, Á., Ribeiro, D.E., Glaser, T., Arnaud-Sampaio, V.F., Lameu, C., Ulrich, H., 2020. The P2X7 receptor: central hub of brain diseases. Front. Mol. Neurosci. 13, 124. https://doi.org/10.3389/fnmol.2020.00124.

Andries, M., Van Damme, P., Robberecht, W., Van Den Bosch, L., 2007. Ivermectin inhibits AMPA receptor-mediated excitotoxicity in cultured motor neurons and extends the life span of a transgenic mouse model of amyotrophic lateral sclerosis. Neurobiol. Dis. 25 (1), 8–16. https://doi.org/10.1016/j.nbd.2006.08.018.

Apolloni, S., Finocchi, P., D'Agnano, I., Alloisio, S., Nobile, M., D'Ambrosi, N., Volonté, C., 2010. UDP exerts cytostatic and cytotoxic actions in human neuroblastoma SH-SY5Y cells over-expressing P2Y6 receptor. Neurochem. Int. 56 (5), 670–678. https://doi.org/10.1016/j.neuint.2010.02.003.

Apolloni, S., Parisi, C., Pesaresi, M.G., Rossi, S., Carrì, M.T., Cozzolino, M., Volonté, C., D'Ambrosi, N., 2013a. The NADPH oxidase pathway is dysregulated by the P2X7

receptor in the SOD1-G93A microglia model of amyotrophic lateral sclerosis. J. Immunol. 190 (10), 5187–5195. https://doi.org/10.4049/jimmunol.1203262.
Apolloni, S., Amadio, S., Montilli, C., Volonté, C., D'Ambrosi, N., 2013b. Ablation of P2X7 receptor exacerbates gliosis and motoneuron death in the SOD1-G93A mouse model of amyotrophic lateral sclerosis. Hum. Mol. Genet. 22 (20), 4102–4116. https://doi.org/10.1093/hmg/ddt259.
Apolloni, S., Amadio, S., Parisi, C., Matteucci, A., Potenza, R.L., Armida, M., Popoli, P., D'Ambrosi, N., Volonté, C., 2014. Spinal cord pathology is ameliorated by P2X7 antagonism in a SOD1-mutant mouse model of amyotrophic lateral sclerosis. Dis. Model Mech. 7 (9), 1101–1109. https://doi.org/10.1242/dmm.017038.
Apolloni, S., Fabbrizio, P., Parisi, C., Amadio, S., Volonté, C., 2016. Clemastine confers neuroprotection and induces an anti-inflammatory phenotype in SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Mol. Neurobiol. 53 (1), 518–531. https://doi.org/10.1007/s12035-014-9019-8.
Apolloni, S., Fabbrizio, P., Amadio, S., Napoli, G., Freschi, M., Sironi, F., Pevarello, P., Tarroni, P., Liberati, C., Bendotti, C., Volonté, C., 2021. Novel P2X7 antagonist ameliorates the early phase of ALS disease and decreases inflammation and autophagy in SOD1-G93A mouse model. Int. J. Mol. Sci. 22 (19), 10649 https://doi.org/10.3390/ijms221910649.
Bartlett, R., Sluyter, V., Watson, D., Sluyter, R., Yerbury, J.J., 2017. P2X7 antagonism using Brilliant Blue G reduces body weight loss and prolongs survival in female SOD1G93A amyotrophic lateral sclerosis mice. PeerJ 5, e3064. https://doi.org/10.7717/peerj.3064.
Bartlett, R., Ly, D., Cashman, N.R., Sluyter, R., Yerbury, J.J., 2022. P2X7 receptor activation mediates superoxide dismutase 1 (SOD1) release from murine NSC-34 motor neurons. Purinergic Signal. https://doi.org/10.1007/s11302-022-09863-5. Apr 28. Epub ahead of print.
Beers, D.R., Appel, S.H., 2019. Immune dysregulation in amyotrophic lateral sclerosis: mechanisms and emerging therapies. Lancet Neurol. 18 (2), 211–220. https://doi.org/10.1016/s1474-4422(18)30394-6.
Bertin, E., Martinez, A., Fayoux, A., Carvalho, K., Carracedo, S., Fernagut, P.O., Koch-Nolte, F., Blum, D., Bertrand, S.S., Boué-Grabot, E., 2022. Increased surface P2X4 receptors by mutant SOD1 proteins contribute to ALS pathogenesis in SOD1-G93A mice. Cell. Mol. Life Sci. 79 (8), 431. https://doi.org/10.1007/s00018-022-04461-5.
Bodin, S., Viala, C., Ragab, A., Payrastre, B.A., 2003. Critical role of lipid rafts in the organization of a key FcgammaRIIa-mediated signaling pathway in human platelets. Thromb. Haemostasis 89 (2), 318–330. https://doi.org/10.1055/s-0037-1613449.
Brown, C.A., Lally, C., Kupelian, V., Flanders, W.D., 2021. Estimated prevalence and incidence of amyotrophic lateral sclerosis and SOD1 and C9orf72 genetic variants. Neuroepidemiology 55 (5), 342–353. https://doi.org/10.1159/000516752.
Burnstock, G., Campbell, G., Satchell, D., Smythe, A., 1970. Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. Br. J. Pharmacol. 40 (4), 668–688. https://doi.org/10.1111/j.1476-5381.1970.tb10646.x.
Burnstock, G., 1972. Purinergic nerves. Pharmacol. Rev. 24 (3), 509–581.
Burnstock, G., Verkhratsky, A., 2009. Evolutionary origins of the purinergic signalling system. Acta Physiol. 195 (4), 415–447. https://doi.org/10.1111/j.1748-1716.2009.01957.x.
Burnstock, G., 2018. Against the Odds, an Autobiography. The Lavenham press Ltd, Lavenham, Suffolk, UK.
Burnstock, G., Knight, G.E., 2018. The potential of P2X7 receptors as a therapeutic target, including inflammation and tumour progression. Purinergic Signal. 14 (1), 1–18. https://doi.org/10.1007/s11302-017-9593-0.
Burnstock, G., 2020a. Introduction to purinergic signaling. Methods Mol. Biol. 2041, 1–15. https://doi.org/10.1007/978-1-4939-9717-6_1.
Burnstock, G., 2020b. Introduction to purinergic signalling in the brain. Adv. Exp. Med. Biol. 1202, 1–12. https://doi.org/10.1007/978-3-030-30651-9_1.
Calzaferri, F., Ruiz-Ruiz, C., de Diego, A.M.G., de Pascual, R., Méndez-López, I., Cano-Abad, M.F., Maneu, V., de Los Ríos, C., Gandía, L., García, A.G., 2020. The purinergic P2X7 receptor as a potential drug target to combat neuroinflammation in neurodegenerative diseases. Med. Res. Rev. 40 (6), 2427–2465. https://doi.org/10.1002/med.21710.
Casanovas, A., Hernández, S., Tarabal, O., Rosselló, J., Esquerda, J.E., 2008. Strong P2X4 purinergic receptor-like immunoreactivity is selectively associated with degenerating neurons in transgenic rodent models of amyotrophic lateral sclerosis. J. Comp. Neurol. 506 (1), 75–92. https://doi.org/10.1002/cne.21527.
Caseley, E.A., Muench, S.P., Roger, S., Mao, H.J., Baldwin, S.A., Jiang, L.H., 2014. Non-synonymous single nucleotide polymorphisms in the P2X receptor genes: association with diseases, impact on receptor functions and potential use as diagnosis biomarkers. Int. J. Mol. Sci. 15 (8), 13344–13371. https://doi.org/10.3390/ijms150813344.
Cashman, N.R., Durham, H.D., Blusztajn, J.K., Oda, K., Tabira, T., Shaw, I.T., Dahrouge, S., Antel, J.P., 1992. Neuroblastoma x spinal cord (NSC) hybrid cell lines resemble developing motor neurons. Dev. Dynam. 194 (3), 209–221. https://doi.org/10.1002/aja.1001940306.
Cavaliere, F., Nestola, V., Amadio, S., D'Ambrosi, N., Angelini, D.F., Sancesario, G., Bernardi, G., Volonté, C., 2005. The metabotropic P2Y4 receptor participates in the commitment to differentiation and cell death of human neuroblastoma SH-SY5Y cells. Neurobiol. Dis. 18 (1), 100–109. https://doi.org/10.1016/j.nbd.2004.09.001.
Chatterjee, T., Marean-Reardon, C., Franco, M., Estevez, A., 2022. Structural changes induced in Hsp90 by nitration lead to a pathological gain-of-function. Faseb. J. 36 (S1) https://doi.org/10.1096/fasebj.2022.36.S1.R5745.
Cervetto, C., Frattaroli, D., Maura, G., Marcoli, M., 2013. Motor neuron dysfunction in a mouse model of ALS: gender-dependent effect of P2X7 antagonism. Toxicology 311 (1–2), 69–77. https://doi.org/10.1016/j.tox.2013.04.004.
Compan, V., Ulmann, L., Stelmashenko, O., Chemin, J., Chaumont, S., Rassendren, F., 2012. P2X2 and P2X5 subunits define a new heteromeric receptor with P2X7-like properties. J. Neurosci. 32 (12), 4284–4296. https://doi.org/10.1523/JNEUROSCI.6332-11.2012.
Craigie, E., Birch, R.E., Unwin, R.J., Wildman, S.S., 2013. The relationship between P2X4 and P2X7: a physiologically important interaction? Front. Physiol. 4, 216. https://doi.org/10.3389/fphys.2013.00216.
D' Ambrosi, N., Volonté, C., 2013. Metabotropic purinergic receptors in lipid membrane microdomains. Curr. Med. Chem. 20 (1), 56–63. https://doi.org/10.2174/0929867311302010007.
D'Ambrosi, N., Cavaliere, F., Merlo, D., Milazzo, L., Mercanti, D., Volonté, C., 2000. Antagonists of P2 receptor prevent NGF-dependent neuritogenesis in PC12 cells. Neuropharmacology 39 (6), 1083–1094. https://doi.org/10.1016/s0028-3908(99)00213-0.
D'Ambrosi, N., Murra, B., Cavaliere, F., Amadio, S., Bernardi, G., Burnstock, G., Volonté, C., 2001. Interaction between ATP and nerve growth factor signalling in the survival and neuritic outgrowth from PC12 cells. Neurosciences 108 (3), 527–534. https://doi.org/10.1016/s0306-4522(01)00431-6.
D'Ambrosi, N., Finocchi, P., Apolloni, S., Cozzolino, M., Ferri, A., Padovano, V., Pietrini, G., Carrì, M.T., Volonté, C., 2009. The proinflammatory action of microglial P2 receptors is enhanced in SOD1 models for amyotrophic lateral sclerosis. J. Immunol. 183 (7), 4648–4656. https://doi.org/10.4049/jimmunol.0901212.
Diekmann, K., Kuzma-Kozakiewicz, M., Piotrkiewicz, M., Gromicho, M., Grosskreutz, J., Andersen, P.M., de Carvalho, M., Uysal, H., Osmanovic, A., Schreiber-Katz, O., Petri, S., Körner, S., 2020. Impact of comorbidities and co-medication on disease onset and progression in a large German ALS patient group. J. Neurol. 267 (7), 2130–2141. https://doi.org/10.1007/s00415-020-09799-z.
Di Virgilio, F., Vultaggio-Poma, V., Sarti, A.C., 2021. P2X receptors in cancer growth and progression. Biochem. Pharmacol. 187, 114350 https://doi.org/10.1016/j.bcp.2020.114350.
Di Virgilio, F., 1995. The P2Z purinoceptor: an intriguing role in immunity, inflammation and cell death. Immunol. Today 16 (11), 524–528. https://doi.org/10.1016/0167-5699(95)80045-X.
Di Virgilio, F., Pizzo, P., Zanovello, P., Bronte, V., Collavo, D., 1990. Extracellular ATP as a possible mediator of cell-mediated cytotoxicity. Immunol. Today 11 (8), 274–277. https://doi.org/10.1016/0167-5699(90)90111-l.
Domercq, M., Matute, C., 2019. Targeting P2X4 and P2X7 receptors in multiple sclerosis. Curr. Opin. Pharmacol. 47, 119–125. https://doi.org/10.1016/j.coph.2019.03.010.
Ecke, D., Hanck, T., Tulapurkar, M.E., Schäfer, R., Kassack, M., Stricker, R., Reiser, G., 2008. Hetero-oligomerization of the P2Y11 receptor with the P2Y1 receptor controls the internalization and ligand selectivity of the P2Y11 receptor. Biochem. J. 409 (1), 107–116. https://doi.org/10.1042/BJ20070671.
Fabbrizio, P., Amadio, S., Apolloni, S., Volonté, C., 2017. P2X7 receptor activation modulates autophagy in SOD1-G93A mouse microglia. Front. Cell. Neurosci. 11, 249. https://doi.org/10.3389/fncel.2017.00249.
Fabbrizio, P., Apolloni, S., Bianchi, A., Salvatori, I., Valle, C., Lanzuolo, C., Bendotti, C., Nardo, G., Volonté, C., 2020. P2X7 activation enhances skeletal muscle metabolism and regeneration in SOD1G93A mouse model of amyotrophic lateral sclerosis. Brain Pathol. 30 (2), 272–282. https://doi.org/10.1111/bpa.12774.
Fabbrizio, P., D'Agostino, J., Margotta, C., Mella, G., Panini, N., Pasetto, L., Sammali, E., Raggi, F., Sorarù, G., Bonetto, V., Bendotti, C., Nardo, G., 2021. Contingent intramuscular boosting of P2XR7 axis improves motor function in transgenic ALS mice. Cell. Mol. Life Sci. 79 (1), 7. https://doi.org/10.1007/s00018-021-04070-8.
Filipi, T., Hermanova, Z., Tureckova, J., Vanatko, O., Anderova, A.M., 2020. Glial cells-the strategic targets in amyotrophic lateral sclerosis treatment. J. Clin. Med. 9 (1), 261. https://doi.org/10.3390/jcm9010261.
Fountain, S.J., Burnstock, G., 2009. An evolutionary history of P2X receptors. Purinergic Signal. 5 (3), 269–272. https://doi.org/10.1007/s11302-008-9127-x.
Fountain, S.J., 2013. Primitive ATP-activated P2X receptors: discovery, function and pharmacology. Front. Cell. Neurosci. 7, 247. https://doi.org/10.3389/fncel.2013.00247.
Franco, M.C., Ye, Y., Refakis, C.A., Feldman, J.L., Stokes, A.L., Basso, M., Melero Fernández de Mera, R.M., Sparrow, N.A., Calingasan, N.Y., Kiaei, M., Rhoads, T.W., Ma, T.C., Grumet, M., Barnes, S., Beal, M.F., Beckman, J.S., Mehl, R., Estévez, A.G., 2013. Nitration of Hsp90 induces cell death. Proc. Natl. Acad. Sci. U.S.A. 110 (12), E1102–E1111. https://doi.org/10.1073/pnas.1215177110.
Gandelman, M., Peluffo, H., Beckman, J.S., Cassina, P., Barbeito, L., 2010. Extracellular ATP and the P2X7 receptor in astrocyte-mediated motor neuron death: implications for amyotrophic lateral sclerosis. J. Neuroinflammation 7, 33. https://doi.org/10.1186/1742-2094-7-33.
Gandelman, M., Levy, M., Cassina, P., Barbeito, L., Beckman, J.S., 2013. P2X7 receptor-induced death of motor neurons by a peroxynitrite/FAS-dependent pathway. J. Neurochem. 126 (3), 382–388. https://doi.org/10.1111/jnc.12286.
Goutman, S.A., Hardiman, O., Al-Chalabi, A., Chió, A., Savelieff, M.G., Kiernan, M.C., Feldman, E.L., 2022a. Emerging insights into the complex genetics and pathophysiology of amyotrophic lateral sclerosis. Lancet Neurol. 21 (5), 465–479. https://doi.org/10.1016/S1474-4422(21)00414-2.
Goutman, S.A., Hardiman, O., Al-Chalabi, A., Chió, A., Savelieff, M.G., Kiernan, M.C., Feldman, E.L., 2022b. Recent advances in the diagnosis and prognosis of amyotrophic lateral sclerosis. Lancet Neurol. 21 (5), 480–493. https://doi.org/10.1016/S1474-4422(21)00465-8.
Grassi, S., Giussani, P., Mauri, L., Prioni, S., Sonnino, S., Prinetti, A., 2020. Lipid rafts and neurodegeneration: structural and functional roles in physiologic aging and neurodegenerative diseases. J. Lipid Res. 61 (5), 636–654. https://doi.org/10.1194/jlr.TR119000427.

Guo, C., Masin, M., Qureshi, O.S., Murrell-Lagnado, R.D., 2007. Evidence for functional P2X4/P2X7 heteromeric receptors. Mol. Pharmacol. 72 (6), 1447–1456. https://doi.org/10.1124/mol.107.035980.

Hanley, P.J., Kronlage, M., Kirschning, C., Del Rey, A., Di Virgilio, F., Leipziger, J., Chessell, I.P., Sargin, S., Filippov, M.A., Lindemann, O., Mohr, S., Königs, V., Schillers, H., Bähler, M., Schwab, A., 2012. Transient P2X7 receptor activation triggers macrophage death independent of Toll-like receptors 2 and 4, caspase-1, and pannexin-1 proteins. J. Biol. Chem. 287 (13), 10650–10663. https://doi.org/10.1074/jbc.M111.332676.

Haystead, T.A., 2006. The purinome, a complex mix of drug and toxicity targets. Curr. Top. Med. Chem. 6 (11), 1117–1127. https://doi.org/10.2174/156802606777812059.

Hernández, S., Casanovas, A., Piedrafita, L., Tarabal, O., Esquerda, J.E., 2010. Neurotoxic species of misfolded SOD1G93A recognized by antibodies against the P2X4 subunit of the ATP receptor accumulate in damaged neurons of transgenic animal models of amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 69 (2), 176–187. https://doi.org/10.1097/NEN.0b013e3181cd3e33.

Huang, Z., Xie, N., Illes, P., Di Virgilio, F., Ulrich, H., Semyanov, A., Verkhratsky, A., Sperlagh, B., Yu, S.G., Huang, C., Tang, Y., 2021. From purines to purinergic signalling: molecular functions and human diseases. Signal Transduct. Targeted Ther. 6 (1), 162. https://doi.org/10.1038/s41392-021-00553-z.

Illes, P., 2020. P2X7 receptors amplify CNS damage in neurodegenerative diseases. Int. J. Mol. Sci. 21 (17), 5996. https://doi.org/10.3390/ijms21175996.

Illes, P., Rubini, P., Huang, L., Tang, Y., 2019. The P2X7 receptor: a new therapeutic target in Alzheimer's disease. Expert Opin. Ther. Targets 23 (3), 165–176. https://doi.org/10.1080/14728222.2019.1575811.

Jiang, L.H., Kim, M., Spelta, V., Bo, X., Surprenant, A., North, R.A., 2003. Subunit arrangement in P2X receptors. J. Neurosci. 23 (26), 8903–8910. https://doi.org/10.1523/JNEUROSCI.23-26-08903.2003.

Kanellopoulos, J.M., Almeida-da-Silva, C.L.C., Rüütel Boudinot, S., Ojcius, D.M., 2021. Structural and functional features of the P2X4 receptor: an immunological perspective. Front. Immunol. 12, 645834 https://doi.org/10.3389/fimmu.2021.645834.

Khakh, B.S., Proctor, W.R., Dunwiddie, T.V., Labarca, C., Lester, H.A., 1999. Allosteric control of gating and kinetics at P2X(4) receptor channels. J. Neurosci. 19 (17), 7289–7299. https://doi.org/10.1523/JNEUROSCI.19-17-07289.1999.

Kim, M., Jiang, L.H., Wilson, H.L., North, R.A., Surprenant, A., 2001. Proteomic and functional evidence for a P2X7 receptor signalling complex. EMBO J. 20 (22), 6347–6358. https://doi.org/10.1093/emboj/20.22.6347.

Krupnik, R., Yovel, Y., Assaf, Y., 2021. Inner hemispheric and interhemispheric connectivity balance in the human brain. J. Neurosci. 41 (40), 8351–8361. https://doi.org/10.1523/JNEUROSCI.1074-21.2021.

Lasley, R.D., 2011. Adenosine receptors and membrane microdomains. Biochim. Biophys. Acta 1808 (5), 1284–1289. https://doi.org/10.1016/j.bbamem.2010.09.019.

Liu, J., Prell, T., Stubendorff, B., Keiner, S., Ringer, T., Gunkel, A., Tadic, V., Goldhammer, N., Malci, A., Witte, O.W., Grosskreutz, J., 2016. Down-regulation of purinergic P2X7 receptor expression and intracellular calcium dysregulation in peripheral blood mononuclear cells of patients with amyotrophic lateral sclerosis. Neurosci. Lett. 630, 77–83. https://doi.org/10.1016/j.neulet.2016.07.039.

Ly, D., Dongol, A., Cuthbertson, P., Guy, T.V., Geraghty, N.J., Sophocleous, R.A., Sin, L., Turner, B.J., Watson, D., Yerbury, J.J., Sluyter, R., 2020. The P2X7 receptor antagonist JNJ-47965567 administered thrice weekly from disease onset does not alter progression of amyotrophic lateral sclerosis in SOD1G93A mice. Purinergic Signal. 16 (1), 109–122. https://doi.org/10.1007/s11302-020-09692-4.

McCombe, P.A., Lee, J.D., Woodruff, T.M., Henderson, R.D., 2020. The peripheral immune system and amyotrophic lateral sclerosis. Front. Neurol. 11, 279. https://doi.org/10.3389/fneur.2020.00279.

McHugh, S.M., Roman, S., Davis, B., Koch, A., Pickett, A.M., Richardson, J.C., Miller, S. R., Wetten, S., Cox, C.J., Karpe, F., Todd, J.A., Bullmore, E.T., 2012. Effects of genetic variation in the P2RX7 gene on pharmacodynamics of a P2X(7) receptor antagonist: a prospective genotyping approach. Br. J. Clin. Pharmacol. 74 (2), 376–380. https://doi.org/10.1111/j.1365-2125.2012.04200.x.

Metpally, R.P., Sowdhamini, R., 2005. Cross genome phylogenetic analysis of human and Drosophila G protein-coupled receptors: application to functional annotation of orphan receptors. BMC Genom. 6, 106. https://doi.org/10.1186/1471-2164-6-106.

Migita, K., Ozaki, T., Shimoyama, S., Yamada, J., Nikaido, Y., Furukawa, T., Shiba, Y., Egan, T.M., Ueno, S., 2016. HSP90 regulation of P2X7 receptor function requires an intact cytoplasmic C-terminus. Mol. Pharmacol. 90 (2), 116–126. https://doi.org/10.1124/mol.115.102988.

Mishra, A., Behura, A., Kumar, A., Naik, L., Swain, A., Das, M., Sarangi, S.S., Dokania, P., Dirisala, V.R., Bhutia, S.K., Mishra, A., Singh, R., Dhiman, R., 2021. P2X7 receptor in multifaceted cellular signalling and its relevance as a potential therapeutic target in different diseases. Eur. J. Pharmacol. 906, 174235 https://doi.org/10.1016/j.ejphar.2021.174235.

Murrell-Lagnado, R.D., 2017. Regulation of P2X purinergic receptor signaling by cholesterol. Curr. Top. Membr. 80, 211–232. https://doi.org/10.1016/bs.ctm.2017.05.004.

Murray, J.M., Bussiere, D.E., 2009. Targeting the purinome. Methods Mol. Biol. 575, 47–92. https://doi.org/10.1007/978-1-60761-274-2_3.

Nakata, H., Yoshioka, K., Kamiya, T., Tsuga, H., Oyanagi, K., 2005. Functions of heteromeric association between adenosine and P2Y receptors. J. Mol. Neurosci. 26 (2–3), 233–238. https://doi.org/10.1385/JMN:26:2-3:233.

Oliveira-Giacomelli, Á., Naaldijk, Y., Sardá-Arroyo, L., Gonçalves, M.C.B., Corrêa-Velloso, J., Pillat, M.M., de Souza, H.D.N., Ulrich, H., 2018. Purinergic receptors in neurological diseases with motor symptoms: targets for therapy. Front. Pharmacol. 9, 325. https://doi.org/10.3389/fphar.2018.00325.

Parisi, C., Arisi, I., D'Ambrosi, N., Storti, A.E., Brandi, R., D'Onofrio, M., Volonté, C., 2013. Dysregulated microRNAs in amyotrophic lateral sclerosis microglia modulate genes linked to neuroinflammation. Cell Death Dis. 4 (12), e959. https://doi.org/10.1038/cddis.2013.491.

Parisi, C., Napoli, G., Amadio, S., Spalloni, A., Apolloni, S., Longone, P., Volonté, C., 2016. MicroRNA-125b regulates microglia activation and motor neuron death in ALS. Cell Death Differ. 23 (3), 531–541. https://doi.org/10.1038/cdd.2015.153.

Pevarello, P., Bovolenta, S., Tarroni, P., Za, L., Severi, E., Torino, D., Vitalone, R., 2017. P2X7 antagonists for CNS indications: recent patent disclosures. Pharm. Pat. Anal. 6 (2), 61–76. https://doi.org/10.4155/ppa-2016-0044.

Pikatza-Menoio, O., Elicegui, A., Bengoetxea, X., Naldaiz-Gastesi, N., López de Munain, A., Gerenu, G., Gil-Bea, F.J., Alonso-Martín, S., 2021. The skeletal muscle emerges as a new disease target in amyotrophic lateral sclerosis. J. Personalized Med. 11 (7), 671. https://doi.org/10.3390/jpm11070671.

Ruiz-Ruiz, C., García-Magro, N., Negredo, P., Avendaño, C., Bhattacharya, A., Ceusters, M., García, A.G., 2020. Chronic administration of P2X7 receptor antagonist JNJ-47965567 delays disease onset and progression, and improves motor performance in ALS SOD1G93A female mice. Dis. Model Mech. 13 (10), dmm045732 https://doi.org/10.1242/dmm.045732.

Schneider, M., Prudic, K., Pippel, A., Klapperstück, M., Braam, U., Müller, C.E., Schmalzing, G., Markwardt, F., 2017. Interaction of purinergic P2X4 and P2X7 receptor subunits. Front. Pharmacol. 8, 860. https://doi.org/10.3389/fphar.2017.00860.

Schonenbach, N.S., Rieth, M.D., Han, S., O'Malley, M.A., 2016. Adenosine A2a receptors form distinct oligomers in protein detergent complexes. FEBS Lett. 590 (18), 3295–3306. https://doi.org/10.1002/1873-3468.12367.

Sluyter, R., Bartlett, R., Ly, D., Yerbury, J.J., 2017. P2X7 receptor antagonism in amyotrophic lateral sclerosis. Neural Regen. Res. 12 (5), 749–750. https://doi.org/10.4103/1673-5374.206643.

Stokes, L., Layhadi, J.A., Bibic, L., Dhuna, K., Fountain, S.J., 2017. P2X4 receptor function in the nervous system and current breakthroughs in pharmacology. Front. Pharmacol. 8, 291. https://doi.org/10.3389/fphar.2017.00291.

Strayer, A.L., Dennys-Rivers, C.N., Ricart, K.C., Bae, N., Beckman, J.S., Franco, M.C., Estevez, A.G., 2019. Ligand-independent activation of the P2X7 receptor by Hsp90 inhibition stimulates motor neuron apoptosis. Exp. Biol. Med. 244 (11), 901–914. https://doi.org/10.1177/1535370219853798.

Tan, L.L., Oswald, M.J., Kuner, R., 2021. Neurobiology of brain oscillations in acute and chronic pain. Trends Neurosci. 44 (8), 629–642. https://doi.org/10.1016/j.tins.2021.05.003.

Tan, R.H., Kril, J.J., McGinley, C., Hassani, M., Masuda-Suzukake, M., Hasegawa, M., Mito, R., Kiernan, M.C., Halliday, G.M., 2016. Cerebellar neuronal loss in amyotrophic lateral sclerosis cases with ATXN2 intermediate repeat expansions. Ann. Neurol. 79 (2), 295–305. https://doi.org/10.1002/ana.24565.

Tang, Y., Illes, P., 2017. Regulation of adult neural progenitor cell functions by purinergic signaling. Glia 65 (2), 213–230. https://doi.org/10.1002/glia.23056.

Tao, Q.Q., Wei, Q., Wu, Z.Y., 2018. Sensory nerve disturbance in amyotrophic lateral sclerosis. Life Sci. 203, 242–245. https://doi.org/10.1016/j.lfs.2018.04.052.

Trang, M., Schmalzing, G., Müller, C.E., Markwardt, F., 2020. Dissection of P2X4 and P2X7 receptor current components in BV-2 microglia. Int. J. Mol. Sci. 21 (22), 8489. https://doi.org/10.3390/ijms21228489.

Trias, E., King, P.H., Si, Y., Kwon, Y., Varela, V., Ibarburu, S., Kovacs, M., Moura, I.C., Beckman, J.S., Hermine, O., Barbeito, L., 2018. Mast cells and neutrophils mediate peripheral motor pathway degeneration in ALS. JCI Insight. 3 (19), e123249 https://doi.org/10.1172/jci.insight.123249.

Vacca, F., Amadio, S., Sancesario, G., Bernardi, G., Volonté, C., 2004. P2X3 receptor localizes into lipid rafts in neuronal cells. J. Neurosci. Res. 76 (5), 653–661. https://doi.org/10.1002/jnr.20069.

Van Weehaeghe, D., Van Schoor, E., De Vocht, J., Koole, M., Attili, B., Celen, S., Declercq, L., Thal, D.R., Van Damme, P., Bormans, G., Van Laere, K., 2020. TSPO versus P2X7 as a target for neuroinflammation: an in vitro and in vivo study. J. Nucl. Med. 61 (4), 604–607. https://doi.org/10.2967/jnumed.119.231985.

Vaughan, S.K., Sutherland, N.M., Zhang, S., Hatzipetros, T., Vieira, F., Valdez, G., 2018. The ALS-inducing factors, TDP43A315T and SOD1G93A, directly affect and sensitize sensory neurons to stress. Sci. Rep. 8 (1), 16582 https://doi.org/10.1038/s41598-018-34510-8.

Verkhratsky, A., 2021. Early evolutionary history (from bacteria to hemichordata) of the omnipresent purinergic signalling: a tribute to Geoff Burnstock inquisitive mind. Biochem. Pharmacol. 187, 114261 https://doi.org/10.1016/j.bcp.2020.114261.

Volonté, C., Merlo, D., 1996. Selected P2 purinoceptor modulators prevent glutamate-evoked cytotoxicity in cultured cerebellar granule neurons. J. Neurosci. Res. 45 (2), 183–193. https://doi.org/10.1002/(SICI)1097-4547(19960715)45:2<183::AID-JNR10>3.0.CO;2-6.

Volonté, C., Ciotti, M.T., D'Ambrosi, N., Lockhart, B., Spedding, M., 1999. Neuroprotective effects of modulators of P2 receptors in primary culture of CNS neurones. Neuropharmacology 38 (9), 1335–1342. https://doi.org/10.1016/s0028-3908(99)00034-9.

Volonté, C., Amadio, S., D'Ambrosi, N., Colpi, M., Burnstock, G., 2006. P2 receptor web: complexity and fine-tuning. Pharmacol. Ther. 112 (1), 264–280. https://doi.org/10.1016/j.pharmthera.2005.04.012.

Volonté, C., D'Ambrosi, N., Amadio, S., 2008a. Protein cooperation: from neurons to networks. Prog. Neurobiol. 86 (2), 61–71. https://doi.org/10.1016/j.pneurobio.2008.07.001.

Volonté, C., Amadio, S., D'Ambrosi, N., 2008b. Receptor webs: can the chunking theory tell us more about it? Brain Res. Rev. 59 (1), 1–8. https://doi.org/10.1016/j.brainresrev.2008b.04.004.

Volonté, C., D'Ambrosi, N., 2009. Membrane compartments and purinergic signaling: the purinome, a complex interplay among ligands, degrading enzymes, receptors and transporters. FEBS J. 276 (2), 318–329. https://doi.org/10.1111/j.1742-4658.2008.06793.x.

Volonté, C., Apolloni, S., Parisi, C., Amadio, S., 2016. Purinergic contribution to amyotrophic lateral sclerosis. Neuropharmacology 104, 180–193. https://doi.org/10.1016/j.neuropharm.2015.10.026.

Volonté, C., Amadio, S., Liguori, F., Fabbrizio, P., 2020. Duality of P2X7 receptor in amyotrophic lateral sclerosis. Front. Pharmacol. 11, 1148. https://doi.org/10.3389/fphar.2020.01148.

Volonté, C., Yerbury, J.J., Sluyter, R., 2022. A step in the ALS direction: lessons from the purinome. In: Ulrich, H., Illes, P., Glaser, T. (Eds.), Purinergic Signaling in Neurodevelopment, Neuroinflammation and Neurodegeneration". Springer Nature Book (in press).

Wang, S., Ichinomiya, T., Savchenko, P., Wang, D., Sawada, A., Li, X., Duong, T., Li, W., Bonds, J.A., Kim, E.J., Miyanohara, A., Roth, D.M., Patel, H.H., Patel, P.M., Tadokoro, T., Marsala, M., Head, B.P., 2022. Subpial delivery of adeno-associated virus 9-synapsin-caveolin-1 (AAV9-SynCav1) preserves motor neuron and neuromuscular junction morphology, motor function, delays disease onset, and extends survival in hSOD1G93A mice. Theranostics 12 (12), 5389–5403. https://doi.org/10.7150/thno.72614.

Xu, K., Ji, H., Hu, N., 2022. Cardiovascular comorbidities in amyotrophic lateral sclerosis: a systematic review. J. Clin. Neurosci. 96, 43–49. https://doi.org/10.1016/j.jocn.2021.12.021.

Yiangou, Y., Facer, P., Durrenberger, P., Chessell, I.P., Naylor, A., Bountra, C., Banati, R. R., Anand, P., 2006. COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord. BMC Neurol. 6, 12. https://doi.org/10.1186/1471-2377-6-12.

Zucchi, E., Ticozzi, N., Mandrioli, J., 2019. Psychiatric symptoms in amyotrophic lateral sclerosis: beyond a motor neuron disorder. Front. Neurosci. 13, 175. https://doi.org/10.3389/fnins.2019.00175.