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P2Y PURINERGIC RECEPTORS:

NEW TARGETS FOR ANALGESIC AND ANTIMIGRAINE DRUGS

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Running Title

Involvement of G protein-coupled P2Y receptors in pain transmission

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Non-standard abbreviations:

AR-C126313, 5-(7-chloro-4*H*-1-thia-3-aza-benzo[*f*]-4-yl)-3-methyl-6-thioxo-piperadin-2-one; AR-C67085, [[[[(2R,3S,4R,5R)-5-(6-amino-2-propylsulfanylpurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl]-dichloromethyl]phosphonic acid; **AR-C69931MX,** [dichloro-[[[(2R,3S,4R,5R)-3,4-dihydroxy-5-[6-(2-methylsulfanylethylamino)-2-(3,3,3-trifluoropropylsulfanyl)purin-9-yl]oxolan-2-yl]methoxy-hydroxyphosphoryl]oxy-hydroxyphosphoryl]methyl]phosphonic acid; **AZD6140,** 3-[7-[[2-(3,4-difluorophenyl)cyclopropyl]amino]-5-

propylsulfanyltriazolo[5,4-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)cyclopentane-1,2-diol; **BDNF,** brain-derived neurotrophic factor; **BK,** bradykinin; **CFA,** Complete Freund's adjuvant; **CGRP,** calcitonin gene-related peptide; **DRG,** dorsal root ganglia; **FHM1,** familial hemiplegic migraine type 1; **INS37217,** P(1)-(uridine 5')-P(4)- (2'-deoxycytidine 5')tetraphosphate, tetrasodium salt; **INS48823,** {[(3aR,4R,6R,6aR)-2-benzyl-6-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-tetrahydro-2H-furo[3,4-d][1,3]dioxol-4-yl]methoxy}({[({[(2S,3R,4S,5S)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl]methoxy}(hydroxy)phosphoryl)oxy]-(hydroxy)phosphoryl}oxy)phosphinic acid; **MRS2179,** [2-[(hydroxy-oxidophosphoryl)oxymethyl]-5-(6-methylaminopurin-9-yl)oxolan-3-yl] hydrogen phosphate; **MRS2211,** [(2Z)-2-[(2-chloro-5-nitrophenyl)hydrazinylidene]-4-formyl-6-methyl-5-oxopyridin-3-yl]methyl dihydrogen phosphate; **MRS2365,** (N)-methanocarba-2MeSADP; **MRS2395,** 2,2-Dimethyl-propionic acid 3-(2-chloro-6-methylaminopurin-9-yl)-2-(2,2-dimethyl-propionyloxymethyl)-propyl ester; **MRS2500,** [(1R,2S,5S)-4-(2-iodo-6-methylaminopurin-9-yl)-1-(phosphonooxymethyl)-2-bicyclo[3.1.0]hexanyl] dihydrogen phosphate; **MRS2578, 1**,4-Di[3-(3-isothiocyanatophenyl)thioureido]butane; **MRS2690,** Diphosphoric acid 1-α-D-glucopyranosyl ester 2-[(4'-methylthio)uridin-5''-yl] ester disodium salt; **MRS2693,** [({[(2R,3S,4R,5R)-3,4-dihydroxy-5-(6-iodo-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)oxolan-2-yl]methoxy}(hydroxy)phosphoryl)oxy]phosphonic acid; **MRS2698,** [(2R,3S,4R,5R)-4-amino-3-hydroxy-5-(4-oxo-2-sulfanylidenepyrimidin-1-yl)oxolan-2-yl]methyl (hydroxy-phosphonooxyphosphoryl) hydrogen phosphate; **MRS2768,** uridine-5'-tetraphosphate δ-phenyl ester; **MRS2802,** α,β-difluoromethylene-UDP; **NF340,** 4-({3-[({5-[(3,7-disulfonaphthalen-1-yl)carbamoyl]-2-methylphenyl}carbamoyl)amino]-4-methylbenzene}amido)naphthalene-2,6-disulfonic acid; **NF546,** 4,4-(Carbonylbis(imino-3,1-phenylene-carbonylimino-3,1-(4-methyl-phenylene)-carbonylimino))-bis(1,3-xylene- α , α '-

diphosphonic Acid) Tetrasodium Salt; **NGF,** nerve growth factor; **NSAIDs,** non-steroidal anti-inflammatory drugs; **PSB-0474,** 3-phenacylUDP; **PSB-0739,** 1-amino-4-[4-phenylamino-3-sulfophenylamino]-9,10-dioxo-9,10-dihydroanthracene-2- sulfonate; **PSB-716,** 1-amino-4-(2-methoxyphenyl)-2-sulfoanthraquinone; **TG,** trigeminal ganglia; **$TRPV_1$**, transient receptor potential vanilloid 1; **VGCCs,** voltage-gated calcium channels.

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Abstract

Millions of individuals worldwide suffer from acute and, more severely, chronic pain conditions (e.g., neuropathic pain, and migraine). The latter bear tremendous personal, familial, and social costs, since sufferers and their relatives undergo a complete turnaround of their lives with the search of relief from pain becoming their pivotal thought. Sadly, to date no effective pharmacological approaches are available which can alleviate chronic pain significantly or in the long run in all patients. The current central strategy for the development of new and effective painkillers lies in the hypothesis that cellular and/or molecular players in nociception must exists that are not targeted by “classical” analgesics, and therefore

researchers have put tremendous efforts into the in-depth analysis of the pathways leading to pain development and maintenance over time. In this complex scenario, two outsiders are now taking the center stage: glial cells in sensory ganglia and in the central nervous system, thanks to their ability to communicate with neurons and to modulate their firing, and the purinergic system. Extracellular purine and pyrimidine nucleotides are involved in the physiology of virtually every body district, and their extracellular concentrations massively increase under pathological situations, suggesting that they might represent potential targets for the modulation of disease-associated symptoms, like pain. Here, we provide an overview of the present knowledge of the role of nucleotides in nociception, with a particular emphasis on G protein-coupled P2Y receptors and their involvement in the communication between first- and second-order neurons in sensory nerve pathways and surrounding glial cells.

Keywords: nucleotides, P2 receptors, satellite glial cells, microglia, migraine, algogens.

ARTICLE OUTLINE

1. Introduction

According to the International Association for the Study of Pain (IASP; http://www.iasp-pain.org/), pain is defined as *"an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage"*. Chronic pain (like headache, migraine, cancer or neuropathic pain) persists over time as a consequence of a number of pathologies and diseases, and sometimes represents the pathology itself. Regardless the cause, chronic pain has the power to dramatically affect the patients' quality of life with enormous associated personal, social, and economic costs. It is estimated that, in the U.S. alone, more than 100 million people suffer from chronic pain, with an estimated cost from $560 billion to $635 billion, which combines the medical costs of pain care and the economic costs related to disability days and lost wages and productivity [1]. Moreover, despite the large number of marketed analgesic and painkiller drugs, many sufferers are (or slowly become) insensitive to the currently available pharmacological approaches [2], and to date no adequate therapies are available for some common types of chronic pain, such as neuropathic pain or migraine.

Based on this scenario, research has focused on the in-depth understanding of causal mechanisms of pain generation and maintenance, in order to identify new promising cellular or molecular players, which can be exploited as pharmacological targets for innovative therapies. In this commentary, we focus our attention on one of these possible yet-to-be fully understood targets: the purinergic system, and in particular P2 nucleotide receptors. The role of nucleotides as neurotransmitters in the associative areas of the central nervous system

(CNS), where painful signals from the periphery are integrated and modulated, is currently virtually unknown. Conversely, at the periphery and in the spinal cord, nucleotides have emerged as relatively new players in pain development with the discovery that they can activate specific membrane receptors and that their extracellular concentrations rise to the micromolar range at any site of injury, tissue damage, or inflammation. We will briefly review already available data on the role of neuronal ionic P2X receptors, and we will then concentrate on G protein-coupled P2Y purinergic receptors in sensory nerve pathways.

2. Classification of P2 receptors

Adenosine-5'-triphosphate (ATP) has long been recognized as an intracellular energy supplier, but its acceptance as an extracellular signaling molecule has taken a considerably long period of time [3]. In 1972 Burnstock proposed a new role for ATP as a neurotransmitter in non-adrenergic, non-cholinergic nerves in the gut and bladder [4]. In the following years ATP metabolites derived from its enzymatic hydrolysis (such as nucleotide adenosine-5'-diphosphate, ADP, and nucleoside adenosine, Ado, as well as other extracellular nucleotides like the uridine-5'-triphosphate,UTP, uridine-5'-diphosphate,UDP, and sugar nucleotides), also were proposed as transmitters not only in sensory, but also in motor nerves and in CNS neurons [5,6], thanks to the discovery and cloning of specific receptors (see below). This new role of ATP, as a part of the complex extracellular network involved in cell-to-cell communication, has been established not only for fast neurotransmission, but also for a wide range of long-lasting biological processes, including release of cytokines, neurotransmitters and hormones, cell proliferation, differentiation and apoptosis in tissues as diverse as the skin, skeletal muscle, bone, nervous and immune system [5,6]. The first classification of purinergic receptors dates back to 1978, when Burnstock proposed criteria to differentiate these

receptors based on their natural ligands: the P1 receptors activated by Ado and antagonized by methylxanthines, and the P2 receptors, responding to nucleotides. To date, P2 receptors are further subdivided into two families: the ionotropic P2X receptors and the metabotropic P2Y receptors. P2X receptors are ligand-activated cationic channels, specifically activated by ATP, while P2Y receptors are activated by purine or pyrimidine nucleotides, or by sugar-nucleotides, and couple to intracellular second-messenger systems through heteromeric G proteins [3]. In vertebrates, seven genes encode for P2X receptor subunits, which are 40-50% identical in their amino acid sequence. As of today, seven homomeric channels (P2X1-7) have been identified, but functional expression studies have also highlighted the existence of heteromeric receptors (e.g., P2X1/5 and P2X2/3) with subtly different pharmacology with respect to the homomeric counterparts [7]. P2Y receptors belong to the superfamily of G protein-coupled receptors (GPCR). Eight P2Y members ($P2Y_{1,2,4,6,11,12,13,14}$) have been cloned in mammals [3]. From a pharmacological point of view, P2Y receptors can be broadly subdivided in four groups based on their responsiveness to nucleotides: i) adenine nucleotide-preferring receptors, mainly responding to ADP and ATP (i.e., human and rodent $P2Y_{1,12,13}$, and human $P2Y_{11}$); ii) uracil nucleotide-preferring receptors, including the human $P2Y_4$ and $P2Y_6$ responding to UTP UDP, respectively; iii) receptors of mixed selectivity (i.e., human and rodent $P2Y_2$, and rodent $P2Y_4$ and, possibly, $P2Y_{11}$); and iv) the $P2Y_{14}$ receptor, responding to both UDP and sugar nucleotides (mainly UDP-glucose and UDP-galactose) with no differences among species [3]. The $P2Y_{1,2,4,6,11}$ subtypes are coupled to G_q/G_{11} with subsequent activation of the $PLC/IP_3/Ca^{2+}$ signaling pathway, whereas $P2Y_{12,13,14}$ inhibit adenylyl cyclase and cAMP generation through G_i/G_o [3]. The $P2Y_2$ subtype can couple to G_i as well, leading to the modulation of cell migration, at least in recombinant systems. The $P2Y_{11}$ subtype has the unique property of also coupling to G_s, leading to the generation of cAMP. Activation of G proteins by P2Y receptors leads to a large variety of biological effects,

due to the subsequent engagement of different effectors, including MAP kinases, Rho kinase, phospholipase A2, nitric oxide, transactivation of growth factor receptors, and many others [5]. A list of agonists, antagonists, and main tissue distribution for the various P2Y receptor subtypes is provided in Table 1. The chemical structures of the synthetic agonists and antagonists listed in Table 1 can be found in Figures 1 and 2, respectively.

3. Extracellular nucleotides and pain transmission

The first hints on a possible algogenic role for extracellular nucleotides date back to the 1960s, when a painful response was generated by the injection of ATP in the human blister base preparation [8] and ATP-induced pain and bronchospasm were demonstrated to be sensitive to aspirin [9].

In the 1980s, accumulating evidence led investigators to hypothesize the involvement of ATP in migraine [10], and in pain pathways in the spinal cord [11]. At the beginning of 1990s, the rapid achievements in cloning, characterization, and evaluation of tissue distribution of the various P2 receptor subtypes (mainly of ionotropic P2X receptors) led to a progressive exponential growth in the number of published papers clearly confirming that ATP acts as an important neurotransmitter at sensory nerve pathways. Focusing on ionotropic P2X receptors was the most logical initial approach, due to their neuronal expression, their fast responses, and their ability to influence the intracellular ion concentrations, typical characteristics shared with other nociceptors. More recently, the contribution of metabotropic P2Y receptors to pain transmission has started to be recognized as well (Table 2). We will now briefly summarize what is currently known about the pro-algogenic role of the most studied P2 receptor, the P2X3 subtype, while emerging data on P2Y receptors will be discussed in the subsequent paragraphs.

3.1 Role of neuronal P2X receptors in nociception

The P2X3 subtype has become a central player in nociception, and consequently a potential pharmacological target for new anti-algogenic drugs, since its cloning and initial characterization. It was originally cloned from dorsal root ganglia (DRG) neurons [12], and it was later found to be highly expressed not only in small/medium diameter sensory neurons in DRGs and in trigeminal ganglia (TG), but also in neurons forming the subepithelial sensory nerve plexus in the walls of tubes (e.g., ureter, vagina, salivary and bile ducts, gut) and sacs (e.g., urinary and gall bladders, lung), as well as in the tongue and tooth pulp [13]. The regulation of gut functions by extracellular ATP is intriguing, since P2X3 receptors are localized not only on high-threshold sensory fibers mediating pain sensation, but also on low-threshold sensory fibers controlling peristalsis [14]. Thus, moderate wall distension generates a sufficient extracellular ATP concentration to only activate peristalsis. Conversely, prolonged or more intense distension (e.g., due to stone transit in the ureter or bile duct) evokes massive ATP release from epithelial cells, which in turn activates P2X3 receptors on underlying sensory fibers with the generation of tremendous colic pain, [14]. Accordingly, P2X3 receptor antagonists could significantly reduce visceral pain.

A complex pattern of modulation of P2X3 receptor functions by known algogenic substances, such as NGF, BDNF and CGRP (which are released within the ganglion and contribute to the generation and modulation of painful sensations) through phosphorylation/dephosphorylation of specific serine and threonine residues in the P2X3 receptor sequence has been demonstrated in TG sensory neurons. As a final outcome, recovery from P2X3 receptor desensitization is accelerated, and P2X3 activity is consequently increased [15,16,17]. Moreover, an increased expression of P2X3 receptors in TG neurons

was observed upon algogen application in vitro [15,18], or following induction of trigeminal-associated pain in vivo (Fig. 1) [19,20]. Collectively, these data suggest that an extracellular milieu of pro-algogenic compounds profoundly modifies the amplitude and duration of P2X3 receptor-mediated neuronal responses, which in turn contributes to the initiation and/or maintenance of TG pain, such as during a migraine attack. A role for P2X3 receptors in migraine is further supported by the recent observations that P2X3 receptor segregation to lipid rafts is significantly increased in TG neurons in a genetic mouse model of migraine, the CACNA1A knock-in (KI) mice [21]. As a consequence, the increased amplitude of P2X3-mediated calcium responses and slower desensitization of the receptor are observed in KI mice than in wild-type (WT) mice [22]. The CACNA1A KI mice bear a gain-of-function mutation of the α subunit of the $Ca_v2.1$ neuronal calcium channel, which is typically detected in families suffering from familial hemiplegic migraine type 1 (FHM1), a severe and genetic form of migraine accompanied by hemiparesis [23]. KI mice show a higher sensitivity to the generation of Cortical Spreading Depression (CSD, the prodromic sign of migraine pain) and also a higher rate of release of pro-algogenic substances (such as CGRP) within the TG [24,25]. Taken together, the biochemical changes observed in purinergic transmission both at the central and the peripheral levels in KI mice could account for the higher susceptibility to migraine triggers in these animals (and, possibly, FHM1 patients) (see also below, Section 5.2).

It is also worth mentioning that the interest in P2X3 receptors in nociception is further increased by their possible involvement in the mechanism of action of known analgesic treatments. For example, it has been recently shown that the NSAID drug, naproxen, inhibits P2X3 activation in trigeminal ganglia [26]. Indeed, bioactive molecules (i.e., tetramethylpyrazine, sodium ferulate, and puerarine) contained in analgesic remedies utilized

in the traditional Chinese medicine also are known to inhibit P2X3-mediated transmission in primary afferent neurons [27].

The distinctive localization at sites where pain sensations are generated and processed has rendered the P2X3 receptor subtype as the most studied purinoceptor in nociception, although up to now the massive preclinical efforts in vitro and in vivo to understand its function and modulation have not led to a satisfactory clinical outcome, mostly due to the lack of selective antagonists with such a chemical structure that could be exploited in human studies. With the recent discovery of innovative chemical scaffolds for P2X3 antagonists, the first Phase 2 "proof-of-concept" studies are currently underway for inflammatory, visceral, and neuropathic pain patients [28].

P2X subtypes other than the P2X3 (e.g., the P2X4 subtype) are expressed by TG neurons [29], but their role in pain transmission has not yet been established.

4. Modulation of pain transmission by P2Y receptors in sensory neurons

In comparison to the key role played by neuronal P2X receptor subtypes (see Section 3), the specific contribution of P2Y receptors to physiological and pathological pain has been less understood. This has been mainly due to their complex pharmacology and to the long-standing lack of selective ligands for different P2Y receptor subtypes, an issue that has only recently started to be resolved (Table 1; Figures 1 and 2). Messenger RNAs for $P2Y_{1,2,4,6,12,13,14}$ subtypes have been found in DRG neurons [30,31,32], suggesting that these receptors may play an ancillary role in peripheral somatosensory transmission [33,34]. P2Y receptors are located not only in the cell body, but also in peripheral and central terminals [35,36], where their activity is presumably integrated within the complex molecular network associated with the transmission of nociceptive signals to the CNS.

Functional $P2Y_{1,2,4}$ receptors are also expressed in subsets of TG sensory neurons [30, 37], but to date their role in the modulation of trigeminal pain has not been properly investigated.

At the moment, there is limited evidence to suggest that $P2Y_4$ and $P2Y_6$ receptors play a role in nociception, and therefore most of the scientific interest has been focused predominantly on $P2Y_1$ and $P2Y_2$ receptors subtypes.

From a functional point of view, the preferential $P2Y_1$-agonist ADP (but also $P2Y_{12,13}$; see below) was found to inhibit the N-type voltage-gated calcium channels (VGCC) in rat DRG neurons. The outcome of VGCC inhibition is a decrease in sensory transmitter release from DR terminals in the spinal cord, which in turn diminishes transmission in ascending spinal nerve (pain) pathways [38]. P2Y receptor expression in sensory nerve terminals could control ATP actions on algogenic P2X subtypes, as well. In fact, $P2Y_1$ and P2X3 receptors are often coexpressed in the same DRG neurons [30], and functional studies have shown that $P2Y_1$ receptor activation leads to P2X3 receptor inhibition [39].

Collectively, these data seem to point to anti-algogenic effects of ADP under some conditions; nonetheless, recent data suggest an opposite effect for $P2Y_1$ receptor subtype in pain induction. Instead, inhibition of VGCCs was later suggested to be mediated by the ADP-activated $P2Y_{12}$ subtype rather than by $P2Y_1$ receptors [32]. Moreover, $P2Y_1$ receptors were reported to mediate hyperalgesia [32], possibly through the sensitization of $TRPV_1$ channels [40]. These collective findings suggest that, at least in DRG neurons, different classes of ADP-activated P2Y receptors act oppositely in modulating painful sensations, with the G_q-coupled $P2Y_1$ subtype exerting pro-algogenic effects and the G_i-coupled $P2Y_{12,13}$ subtypes playing an analgesic role, although the real scenario is still far from being fully understood (see below).

Also activation of $P2Y_2$ receptors can excite DRG neurons [41], but again the underlying mechanism remains obscure. This receptor subtype can sensitize $TRPV_1$ channels in mouse DRGs [33,42], and modulate pro-algogenic neuropeptide release. In fact, the $P2Y_{2,4}$ preferential agonist, UTP, can induce the release of CGRP from rat DRG neurons [43]. Other studies have failed to confirm a direct release of CGRP by UTP, but instead have suggested that UTP can enhance acid buffer solution- and capsaicin-evoked neuropeptide release [44,45]. Additionally, UTP has been reported to activate capsaicin-sensitive cutaneous C-fibers [46], supporting a role for UTP as endogenous nociceptive messenger. Moreover, after CFA-induced inflammation, $P2Y_2^{-/-}$ mice failed to develop thermal hyperalgesia, with no difference in the extent of mechanical allodynia with respect to WT mice [42]. These results demonstrate that neuronal $P2Y_2$ receptor subtype is predominantly involved in thermal nociceptive transmission.

Nucleotides were also suggested to enhance nociception by modulating voltage-gated Na^+ channels in DRG neurons [47], although the involvement of specific P2Y receptor subtypes is unclear. In addition, P2Y receptors control the function of several neuronal K^+ channels, including K_V7 [48], Kir3.1/3.2 [49], and $K_{Ca}2$ channels [50].

It has been shown that ATP, ADP, and UTP (but not UDP) caused cell depolarization when applied to DRG neurons under current- and voltage-clamp, and enhanced the action potential discharge rate during current injections. The $P2Y_2$ receptor-preferring agonist 2-thio-UTP was equipotent to UTP in eliciting these effects, whereas the selective $P2Y_1$ receptor antagonist MRS2179 largely attenuated the excitatory effects of ADP, but left those of 2-thio-UTP unaltered. Application of either ADP or 2-thio-UTP inhibited K-currents through K_V7 channels, and, in parallel, facilitated cation-current through $TRPV_1$ channels, suggesting that the excitatory effects of these nucleotides were mediated by either $P2Y_1$ or $P2Y_2$ receptor subtypes which acted downstream through a common signaling pathway [51].

Together with their already-mentioned ability to modulate VGCCs (see above), these observations reveal an increasingly important role for P2Y receptors in DRG neurons in controlling ionic currents, which has profound implications for neuronal firing and sensitivity to noxious stimuli.

As previously mentioned for the P2X3 receptor subtype (see Section 3), P2Y receptor-mediated signaling is characterized by significant plasticity upon chronic painful conditions, and this feature could be exploited in analgesic therapy (Figure 3; see below, Section 6).

$P2Y_1$ mRNA is upregulated in rat DRG cells after peripheral axotomy of the sciatic nerve [52], and again after CFA injection in the mouse hindpaw, whereas $P2Y_{2,4,6}$ mRNAs are downregulated [42]. Conversely, both $P2Y_1$ and $P2Y_2$ mRNAs are upregulated in mouse L2/L3 DRG cells at 14 and 21 days after nerve transection [53]. The upregulation of $P2Y_1$ mRNA in mouse DRGs appears to be necessary for heat sensitization of cutaneous polymodal nociceptors in response to CFA injection in the skin [54]. Although the evaluation of the mRNA expression levels has been performed on total ganglia, and a contribution of glial P2Y receptors to detected increases cannot be excluded (see also below), these results indicate that neuronal P2Y receptors are likely to contribute to the plasticity of pain signalling upon chronic or inflammatory conditions, and could represent interesting targets for the development of new analgesics.

Finally, it is known that dorsal horn spinal cord neurons at least express $P2Y_{1,2,4}$ receptor subtypes [31,55,56], although a full characterization and cellular localization of the various receptor subtypes has not yet been performed. Interesting results have been obtained following the intrathecal administration of uracil nucleotides in vivo. Here, UTP and UDP produced significant anti-allodynic effects following partial ligation of the sciatic nerve, and elevated the mechanical nociceptive threshold as well as prolonging the thermal nociceptive

latency in uninjured rats, thus suggesting an antinociceptive role for the $P2Y_2$, $P2Y_4$ and, possibly, $P2Y_6$ receptor subtypes – for which UTP and/or UDP are agonists [57].

5. New emerging players in nociception: glial cells and purinergic transmission

Far from acting passively as a scaffold or as metabolic suppliers for the neuronal network, glial cells have now taken center stage in neurotransmission, thanks to their ability to react to either injury or metabolic dysfunction and to influence neuronal firing. As detailed below, their contribution to nociception is now firmly acknowledged, both in the periphery and in the CNS (with a primary role played here by microglia and astrocytes) [58] . Within sensory ganglia, the somata of sensory neurons are surrounded and wrapped by a network of satellite glial cells (SGCs), which continually monitor the extracellular milieu and exchange information with one another and with neurons as well, so profoundly affecting neuronal firing and, ultimately, the transmission of painful sensations [59,60]. Thus, targeting glial cells with specific pharmacological compounds has recently emerged as an exciting opportunity for the development of new and effective painkillers.

Unveiling the whole molecular network involved in neuron-to-glia communication is mandatory to the identification of promising glial targets for analgesia, although the large number of currently recognized transmitters (including cytokines, growth factors, neurotransmitters and others) has sometimes frustrated researchers' efforts in this endeavor. The purinergic system has emerged as one of the key players in cell-to-cell communication in many tissues and organs, and this is particularly true in the case of pain pathways. As already mentioned, purinoceptors are widely expressed in all the cell types involved in nociception (Table 2), and large amounts of purines and pyrimidines are released during physiological neurotransmission, and, more so, following tissue damage. Nucleotide release can be further

increased under inflammatory conditions or in the presence of pro-algogenic factors, such as growth factors and cytokines [61]. Moreover, a somatic release of ATP has been demonstrated within sensory ganglia, which can further enhance the exchange of information between neurons and glial cells, ultimately leading to the modulation of painful sensations [62].

5.1 Spinal cord microglia and astrocytes

Spinal cord microglia quickly react to injury (including peripheral nerve damage), by first acquiring a phagocytic phenotype and then releasing large amounts of inflammatory mediators [63]. This pro-inflammatory extracellular milieu can dramatically influence synaptic activity and neuronal firing of second-order neurons in the spinal cord dorsal horn, and ultimately contribute to the generation and maintenance of chronic neuropathic pain [63]. Several damage-released mediators are involved in microglia activation, with extracellular ATP being one of the most important. The first and best studied purinergic receptor in microglia activation is the ionotropic P2X4 subtype, whose upregulation and activation in ipsilateral activated microglia following the chronic constriction injury (CCI) of the sciatic nerve turns out to be necessary for the development of tactile allodynia (Figure 4) [64]. Activation of the P2X4 receptor leads eventually to the activation of p38 and release of BDNF [65,66]. Up-regulation of the P2X4 receptor in spinal cord microglia was later demonstrated to require the CCL21 chemokine, which is expressed only in damaged neurons [67], and turned out to be necessary for the development of nerve injury-induced tactile allodynia and, to a lesser extent, peripheral inflammation [68]. It is worth mentioning that the prolonged activation of the P2X4 receptor opens a non-cytolytic pore that allows the efflux of large inflammatory and immune mediators from activated microglia, so contributing to

neuropathic pain [69]. Modulation by a phospholipase C-coupled pathway of the P2X4 pore-opening phenomenon in microglia tantalizingly suggests a possible cross-talk between G protein-coupled (possibly purinergic) receptors and the P2X4 subtype.

In fact, P2Y receptors may be directly involved in spinal cord microglia activation under painful conditions. Messenger RNAs for the $P2Y_{2,6,12,13,14}$ subtypes have been detected in primary microglia [70]. The UDP-sensitive $P2Y_6$ receptor subtype appears to be directly involved in the modulation of microglia phagocytosis [71], whereas a prominent role has been proposed for the $P2Y_{12}$ subtype in the development of allodynia following nerve injury. In the latter case, an ipsilateral and time-dependent up-regulation of microglial $P2Y_{12}$ was observed following nerve ligation, whereas the genetic ablation of $P2Y_{12}$ receptor expression or administration of $P2Y_{12}$ selective antagonists (i.e., intrathecal AR-C69931MX or oral Clopidogrel) prevented the development of tactile allodynia [72]. Moreover, intrathecal infusion of the $P2Y_{12}$ agonist 2-(methylthio)-ADP in naïve rats mimicked nerve injury-associated p38 activation and pain behavior [73]. It was also demonstrated that $P2Y_{12}$ receptors regulate microglial process extension and chemotaxis towards ATP by acting through integrin β1 [74], thus further confirming a pivotal role for the $P2Y_{12}$ receptor subtype in controlling microglial functions, and the development of neuropathic pain.

However, the complexity of the purinergic modulation has just started to emerge for microglial cells involved in pain development. In fact, not only mRNA for $P2Y_{12}$, but also for $P2Y_{6,13,14}$ are up-regulated in microglia in the ipsilateral spinal cord following nerve injury, and a mixture of $P2Y_6$, $P2Y_{12}$ and $P2Y_{13}$ selective antagonists showed a longer suppressive effect on pain behavior than did single treatments alone [75].

Astrocytes, as well as microglia, are directly involved in pain transmission in the spinal cord, thanks to their ability to modify and modulate neuronal firing and to secrete pro- and anti-inflammatory and algogenic molecules; accordingly, they are now recognized as

therapeutic targets for chronic pathological pain, by virtue of their delayed activation with respect to rapidly-reacting microglia [58]. Cortical brain astrocytes express the full complement of P2Y and P2X receptors, with significant changes in the expression level following traumatic or ischemic events [76]. The purinergic system is directly involved in modulating reactive astrogliosis and astrocyte secretion of cytokines and chemokines in the brain cortex [76]. Thus, although the role of purinergic receptors in the modulation of spinal cord astrocytic functions following traumatic events and in nociception has not been directly addressed. However, the available evidence suggests that targeting astrocytic P2 receptors might represent a new option for the treatment of chronic painful conditions (see also below).

5.2 Satellite glial cells in sensory ganglia

As already mentioned, SGCs wrap around the cell bodies of primary neurons in sensory ganglia, forming a morphological and functional unit [59]. The key role of SGCs in the development and maintenance of chronic pain has been demonstrated by their increased expression and release of IL-1β [77], TNFα [62], as well as by the increased gap junction-mediated cell coupling [78,79] following nerve injury. Like other glial cells, SGCs also respond to nerve injury by up-regulating the expression of glial fibrillary acidic protein (GFAP) and undergoing division in response to chronic pain [77, 80]. Altogether, these changes lead to increased excitability of both primary afferents and CNS neurons, and the development of hyperalgesia and allodynia [81]. Indeed, inhibiting the expression of one of the major gap junctions subunits expressed by glial cells (i.e., Connexin 43) [82] by RNA interference in the TG resulted in a strong reduction of pain behavior in a model of facial neuropathic pain [83].

Trigeminal satellite glial cells express functional $P2Y_{1,2,4,6,13}$ receptor subtypes under

basal conditions [37,84], whereas glial P2Y expression in DRG cells has been only evaluated at the mRNA level in rodents [31]. Those P2 purinergic receptors expressed by SGCs participate primarily in bidirectional neuron-glial communication [60], thanks to the somatic release of ATP within the sensory ganglion [62]. Released ATP may activate P2X7 receptors in SGCs, leading to the release of TNFα, which in turn can potentiate P2X3-mediated ATP currents in neurons (see also below) [62].

In recent years a close interplay between the purinergic system and other classical pain transducing signals within the TG has been elucidated, starting from the observation that chronic exposure of TG cultures to the algogenic mediator bradykinin (BK) induced the upregulation of P2Y-mediated calcium signaling in SGCs [37]. BK did not act directly on glial cells, but rather activated neuronal BK receptors, and, subsequently, caused the release of CGRP, which, in turn, stimulated the ERK1/2 MAP kinase signaling pathway in surrounding SGCs and increased P2Y receptor-mediated intracellular calcium responses. SGCs ultimately release a wide range of pro- and anti-algogenic cytokines and chemokines, which likely further contribute to communication with neurons and modulation of pain transmission (Figure 3) [24]. Pharmacological studies have revealed that those specific glial purinoceptors potentiated by CGRP are the $P2Y_1$ and $P2Y_2$ subtypes (Figure 3) [Ceruti et al., manuscript in preparation], although their exact role in pain modulation has not yet been evaluated in vivo. Interestingly, the anti-migraine drug, sumatriptan, inhibited BK-mediated CGRP release from TG neurons and P2Y receptor potentiation on SGCs, suggesting that modulation of the neuron-to-SGC communication network, which involves CGRP and P2Y receptors, is itself involved in the anti-migraine effect of triptans [24]. Additionally, this network of interaction between neurons and SGCs is overactivated in CACNA1A KI mice (see Section 3) [23,24], providing additional clues on a possible role for extracellular nucleotides in the initiation and maintenance of migraine pain. These data clearly indicate that

the complex interplay between TG neurons and SCGs could become even more important under pathological conditions, when massive amounts of algogens are released within the TG, and highly active glial P2Y receptors may significantly contribute to pain transduction, thus representing a novel target for the pharmacological modulation of migraine pain.

The complexity and plasticity of the purinergic control of neuron-to-SGC communication within the TG were further demonstrated by bidirectional calcium signaling between neurons and SGCs (which is diminished by the non-selective P2 antagonist suramin), and a switch from P2Y to P2X receptor subtypes under pathological conditions. In fact, the amplitude of Ca-signaling among SGCs and between SGC-to-neuron was increased following induction of submandibular inflammation by CFA injection, whereas the amplitude of Ca-signaling from neuron-to-SGC was reduced [60]. Moreover, the intracellular calcium response to ATP was mediated by P2Y receptors in control tissues, whereas the response was predominantly due to ionotropic P2X receptors after CFA-induced inflammation, suggesting that inflammatory conditions not only induce a large increase in the sensitivity of SGCs to ATP, but also a switch in ATP signaling through P2Y receptors to P2X receptors [85].

The role of the $P2Y_{12}$ receptor subtype within sensory ganglia is noteworthy for its possible clinical implications (see Section 6). Despite the detection of $P2Y_{12}$ mRNA in total mouse TG and of functional protein on a limited percentage of SGCs in culture [37], surprisingly no $P2Y_{12}$ immunostaining was detected on TG sections- a finding which suggested that the receptor protein is not expressed within the ganglion, but in the boundary area with the trigeminal nerve [80]. Interestingly, a recent study demonstrates the involvement of glial $P2Y_{12}$ receptor subtype in trigeminal neuropathic pain. $P2Y_{12}$ expression appeared in TG in GFAP-positive cells, but not in neurons after induction of lingual neuropathic pain. More importantly, administration of the $P2Y_{12}$ antagonist MRS2395 significantly decreased the number of TG neurons encircled with activated (GFAP-positive) SGCs, and reverted the

lowered threshold for the head-withdrawal reflex in response to mechanical and heat stimulation of the tongue [86]. Thus, data show that activation of TG SGCs following induction of neuropathic pain leads to the expression of $P2Y_{12}$ receptor, which is causally involved in the enhancement of TG neuron activity and nocifensive reflex behavior, contributing to the development of neuropathic pain.

Concerning the expression and function of P2Y receptors in SGCs from DRGs, in situ hybridization histochemistry (ISHH) has detected mRNA expression only for the $P2Y_{12}$ and $P2Y_{14}$ subtypes [31]. Later on, immunofluorescence studies showed a detectable, yet low, level of $P2Y_1$ expression [87]. Although no data are available, it is tempting to speculate that, among the various ganglion cell populations, the same molecular interplay involving the purinergic system and other known pro-algogenic mediators takes play in DRGs as well as TG, and could therefore lead to the development and maintenance of neuropathic pain.

As mentioned above [85], ionotropic P2X receptors may also contribute to neuron-to-SGC exchange of information within sensory ganglia, with the P2X7 receptor subtype possibly playing a major role. P2X7 receptor is highly expressed in immune cells, microglial cells, and astrocytes, as well as SGCs in the DRG and TG [62,85,87]. P2X7 represents a special member of the P2X ionic receptor family, since under specific conditions (e.g., repeated ATP stimulation or high extracellular ATP concentrations) it can open a membrane pore, permeable to solutes up to 900 Da and which cannot be done by any other member of the P2X receptor subfamily [7], so allowing the flux of bioactive molecules between the extracellular and intra-cellular compartments.

The role of P2X7 in the modulation of nociception is controversial. In support of an involvement, P2X7-KO mice showed a complete inhibition of chronic inflammatory and neuropathic pain, likely due to the lack of P2X7 receptor expression on inflammatory and immune cells [88]. In line with this pre-clinical observation, a genetic variance leading to

impaired pore formation has been linked to lesser susceptibility to chronic pain not only in rodents, but, more interestingly, in two cohorts of human patients with chronic pain (i.e., mastectomy- and osteoarthritis-related pain) [89]. Although no analysis of P2X7 receptor expression was provided at the cellular level, these observations push forward the P2X7 receptor as a possible target for the development of new effective agents against chronic pain, but also alert clinicians to the need for patient genotyping when anti-P2X7 strategies are studied, since patients carrying the defective haplotype would not benefit from this therapeutic approach.

However, conflicting results have been reported for P2X7 at the DRG level. An initial paper demonstrated that ATP released from neuronal somata activated P2X7 receptors on surrounding SGCs, which resulted in the release of TNFα and potentiation of neuronal P2X3 receptor function [62]. Although no in vivo data were provided, a pro-algogenic role for DRG glial P2X7 receptors was hypothesized, in accordance with the above-mentioned papers. However, the same group later showed an unexpected ability of P2X7 receptors in SCGs to inhibit the function of neuronal P2X3 receptor in DRGs [87], through the involvement of neuronal $P2Y_1$ receptors; the functional outcome of this effect is a reduction of inflammatory pain in rats. Whether these opposite effects exerted by glial P2X7 receptors are due to the specific pain paradigm utilized is not known, but once again conflicting data advocate caution when trying to draw general conclusions on the pro- or anti-algogenic role of a specific purinergic receptor subtype, an issue which further complicates research in this field.

6. Progress towards therapeutic interventions

Based on the experimental evidence presented, it has become increasingly apparent that the purinergic system significantly contributes to nociceptive signaling. Purinergic

receptors are therefore particularly attractive as targets for the development of innovative pharmacological entities against pain. However, the complexity of the system poses, several limitations, or potential pitfalls, in the translation to the clinic. The first difficulty is intrinsic to the study of nociception, not only by the existence of several different types of pain (e.g., visceral, neuropathic, acute, inflammatory etc.), but by the use of various in vivo models for the pre-clinical study of these types of pain. It has proven to be extremely difficult to draw generalized conclusions based on in vitro or pre-clinical in vivo data, apart from one specific type of pain (visceral pain) where the P2X3 subtype plays a prominent role [28]. Moreover, the large number of P2 receptor subtypes involved (to which P1 receptors responding to adenosine should be added also) [3], and long-standing lack of selective ligands to clearly identify the roles of specific P2 receptor subtypes in preclinical studies has complicated the identification of specific purinergic targets. To overcome this long-standing absence of selective ligands, many important results have been obtained using genetically modified animals, but a key issue to be addressed is the real translational potential of data obtained in knock-out animals. In fact, by selectively lacking one (or more) protein or receptor, knock-out mice have undoubtedly significantly contributed to a better understanding of the molecular pathways controlling pain transmission. Nevertheless, when trying to identify new molecular druggable targets, the data can be puzzling. Concerning the purinergic system, total inhibition of pain and development of allodynia following nerve injury has been observed in P2X4-KO, and also in $P2Y_{12}$-KO mice [68,72] (see above for details), indicating that the blockade of one or both of these receptors is sufficient to relieve neuropathic pain. So, based on these results what is the best purinergic target for drug development in pain in humans? Is it possible to think of a non-selective multi-target agent acting on different purinergic receptor subtypes simultaneously for neuropathic pain?

In the search of new pharmacological entities to relieve pain, it has become increasingly clear that focusing on one single molecular target probably does not represent the best option. Promising in vitro and preclinical observations on drugs acting selectively on a single target have disappointingly turned out to be impossible to reproduce in vivo. As a consequence, many potential analgesic drug candidates have been abandoned at early stage in preclinical trials because of their poor nociceptive activity (e.g., neurokinin 1 receptor antagonist) [90]. To further confirm this issue, the "old" drugs currently utilized in CNS therapeutics (and in some kinds of pain as well) do not act selectively on one single target, but rather on multiple molecular targets [90]. Thus, for example if a new molecule acting on the P2X4 and $P2Y_{12}$ receptors could be designed and synthesized, its use in neuropathic pain might lead to better results than drugs designed to be more selective for one or other P2 receptor subtype. As far as the $P2Y_{12}$ subtype is concerned, selective antagonists are already available in the clinic (e.g., Clopidogrel, and more recently, Prasugrel and Ticagrelor), and have been used as anti-platelet agents for some time [91]. This anti-platelet effect, which can be foreseen for $P2Y_1$ selective antagonists as well, although to a lesser extent, would of course represent potential side effects for an analgesic. In addition, the complexity and widespread distribution of purinergic receptors throughout the body suggests the possible development of additional adverse effects when targeting specific receptor subtypes (for example on peristalsis and immune functions). The synthesis and development of effective multitarget drugs can help overcoming this issue, due to their lower affinity at single receptor subtypes with respect to compounds selectively targeting only one specific receptor. It is also tempting to speculate that targeting specific P2 receptor subtypes could also reduce the development of side effects thanks to the wider therapeutic range under painful conditions. In fact, P2 receptors are up-regulated and sensitized along the pain signaling pathways (see

above), and likely respond to lower ligand concentrations than receptors expressed in non-affected body districts.

Finally, all the various cell types involved in the pain pathways from the periphery to the CNS also express numerous purinergic receptors (Table 2), whose numbers are dynamically regulated by induction of pain (Figures 3 and 4). Focusing on G protein-coupled P2Y receptors could represent an interesting option in one sense, since these receptors are expressed at both the neuronal and glial level (in contrast to P2X receptors which are mostly neuronal), and, therefore, antagonists acting on common subtypes (for example, the $P2Y_1$ and $P2Y_2$ receptors in sensory ganglia; Figure 3) could modulate pain transmission at multiple levels, especially if the target receptor is upregulated by painful conditions. In this case, a different kind of multitarget approach can be hypothesized so that the same target (for example, one or more P2Y purinergic receptors) is inhibited (or activated depending upon its pro- or anti-algogenic activity) at different cellular and tissue sites at the same time (e.g., on glial cells, neurons, in the periphery or in the spinal cord).

7. Conclusions

Available evidence indicates that targeting the purinergic system represents an innovative therapeutic option in the fight against acute and chronic pain states, due to its important role in the modulation of cell-to-cell communication at multiple sites along the pain signaling pathways from the periphery to the CNS. More work is needed, however, to fully understand the real pro- or anti-algogenic effects induced by the various receptor subtypes involved, in particular glial P2Y receptors. Moreover, a closer collaboration among experts in pharmacology, biochemistry, molecular biology, and pharmaceutical chemistry is mandatory to clearly identify the most promising druggable targets, which could be successfully utilized

in preclinical and clinical studies to accelerate the process towards new therapeutic options for the huge amount of pain patients worldwide.

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FIGURE LEGENDS

Figure 1. Chemical structures of selective agonists at the various P2Y receptor subtypes. IUPAC chemical names can be found in the list of Abbreviations.

Figure 2. Chemical structures of selective antagonists at the various P2Y receptor subtypes. IUPAC chemical names can be found in the list of Abbreviations.

Figure 3. Expression of various subtypes of ionotropic P2X and G protein-coupled P2Y nucleotide receptors in sensory ganglia (DRG and TG) and their plasticity upon painful conditions. Sensory neurons or surrounding satellite glial cells (SGCs) express a number of P2X and P2Y receptor subtypes under basal conditions. Upon induction of chronic pain, the expression levels of several P2 receptors are significantly elevated (as evaluated either at the mRNA or protein level), as shown in the right panel by the increased line weight. Indeed, the $P2Y_{12}$ receptor subtype is expressed by SGCs only under painful conditions. As detailed in the text, conflicting results have been published for some receptor subtypes (e.g., neuronal $P2Y_{2,4,6}$ receptors were found down-regulated in rat DRGs) [42]. Purinergic receptors are involved in the cross-communication between neurons and SGCs, in close collaboration with other known pro-algogenic mediators that are released in the intercellular space by either cell types (see box). Moreover, they can also either positively or negatively modulate the function of other nociceptors, such as TRPV1 or sodium, potassium, and calcium channels (see text for details).

Figure 4. Expression of various subtypes of ionotropic P2X and G protein-coupled P2Y nucleotide receptors in the dorsal horn spinal cord and their plasticity upon painful

conditions. A number of P2X and P2Y receptor subtypes are expressed by spinal cord microglia, and modulate microglia activation and reaction to traumatic events. Only sporadic reports on the expression of some P2 receptor subtypes by spinal neurons or astrocytes are currently available (see text for details). P2X3 purinergic receptors are expressed at the central terminals of DRGs sensory neurons innervating the spinal cord tissue. Nerve injury or inflammatory conditions induce the up-regulation of microglia purinergic receptors (as evaluated either at the mRNA or at the protein level; right panel, increased line weight), which promote microgliosis, and the consequent release of pro-inflammatory and pro-algogenic substances. The same process can be foreseen for astrocyte purinoceptors as well.

Subtype	Natural agonist(s)	Synthetic agonist(s)	Selective antagonist(s)	Main tissue distribution
$P2Y_1$	ADP	2-MeSADP ADP-β-S MRS2365	MRS2500 MRS2179	Epithelial and endothelial cells, platelets, immune cells, osteoclasts, brain
$P2Y_2$	UTP/ATP	MRS2698 MRS2768 UTP-γ-S INS37217	PSB-716 AR-C126313	Immune cells, epithelial and endothelial cells, kidney tubules, osteoblasts
$P2Y_4$	UTP/ATP	2'-azido-2'-deoxyUTP	Not yet available	Endothelial and epithelial cells, placenta, spleen, thymus
$P2Y_6$	UDP	PSB-0474 UDP-β-S INS48823 MRS2693	MRS2578	Airway and intestinal epithelial cells, placenta, T cells, thymus, microglia (activated)
$P2Y_{11}$	ATP	NF546 AR-C67085 ATP-γ-S	NF340	Spleen, intestine and granulocytes
$P2Y_{12}$	ADP	2-MeSADP ADP-β-S	AZD6140 (Ticagrelor) AR-C69931MX (Cangrelor) PSB-0739 MRS2395	Platelets, glial cells
$P2Y_{13}$	ADP	2-MeSADP	MRS2211	Spleen, brain, lymph nodes, bone marrow, erythrocytes
$P2Y_{14}$	UDP/ UDP-glucose/ UDP-galactose	MRS2690 MRS2802	UDP may also act as antagonist Non-nucleotide antagonists have been reported	Placenta, adipose tissue, stomach, intestine, discrete brain regions, mast cells

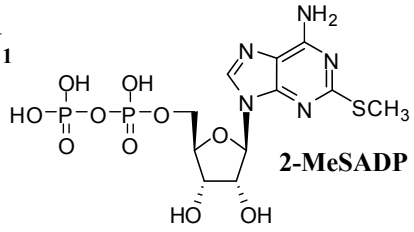
Table 1: list of natural and some synthetic agonists, selective antagonists and main tissue distribution of P2Y purinergic receptors.

		RECEPTOR EXPRESSION	FUNCTIONAL RECEPTORS
Neurons	DRG	P2X3 $P2Y_{1,2,4,6,12,13,14}$	P2X3 $P2Y_{1,2,12,13}$
	TG	P2X3, P2X4 $P2Y_{1,2,4,6}$	P2X3 $P2Y_{1,2,4}$
	Dorsal horn spinal cord	$P2Y_{1,2,4,6}$	$P2Y_{1,2,4,(6)}$
Satellite glial cells	DRG	P2X7 $P2Y_{1,12,14}$	P2X7 No functional studies on P2Y receptors
	TG	P2X7 $P2Y_{1,2,4,6,13,14}$	P2X7 $P2Y_{1,2,4,6,13,14}$ ($P2Y_{12}$ under painful conditions)
Astrocytes	Dorsal horn spinal cord[1]	P2X7 $P2Y_1$ (others?)	P2X7 $P2Y_1$ (others?)
Microglia	Dorsal horn spinal cord	P2X4, P2X7 $P2Y_{2,6,12,13,14}$	P2X4, P2X7 $P2Y_{6,12,13,14}$
Immune system cells		P2X7 $P2Y_{1,2,4,6,12,14}$	P2X7 $P2Y_{1,2,4,6,14}$

Table 2: expression of purinergic P2X and P2Y receptor and demonstration of their functionality in the various cell types involved in pain transmission. The expression data could refer to either mRNA or protein evaluation. See text for details.

[1] No systematic evaluation of P2 receptor expression has been performed in spinal cord astrocytes. However, cortical brain astrocytes express a large range of P2X and P2Y receptors. See text for details.

P2Y$_1$



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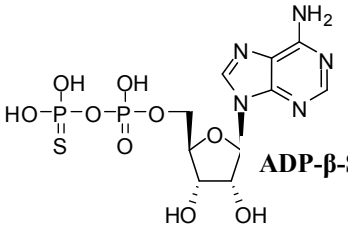
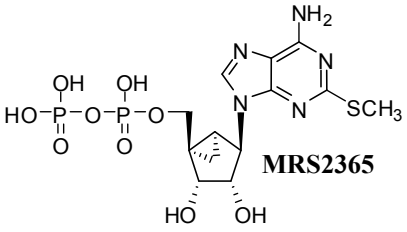
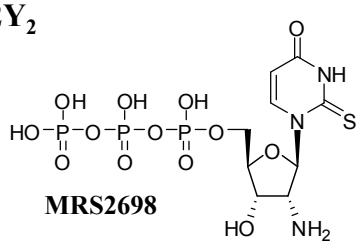


ADP-β-S



MRS2365

P2Y$_2$



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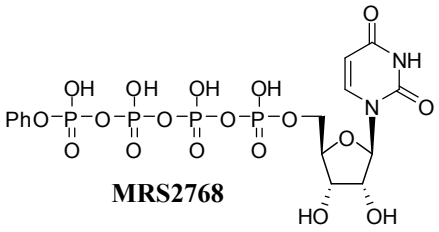

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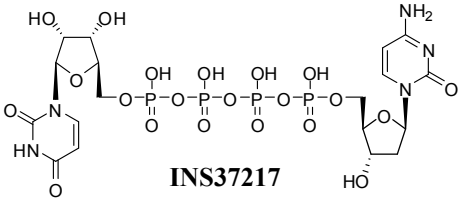

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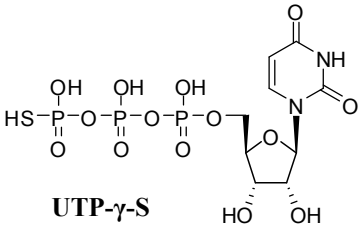
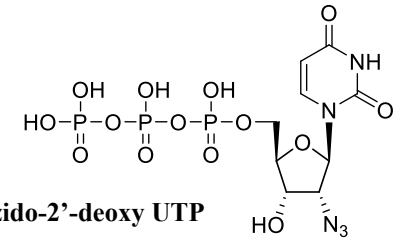


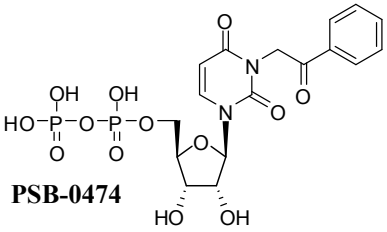
UTP-γ-S

P2Y$_4$



2'-azido-2'-deoxy UTP

P2Y$_6$



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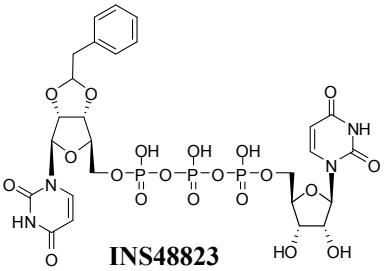

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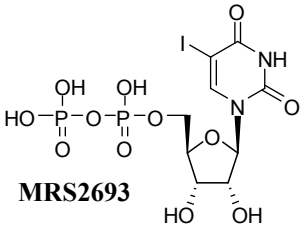

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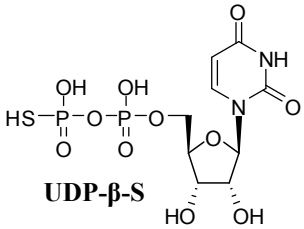

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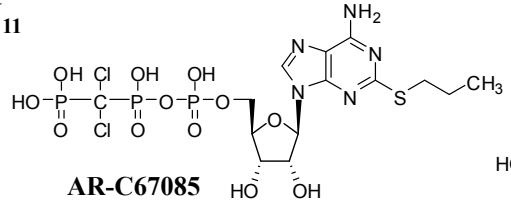
P2Y$_{11}$



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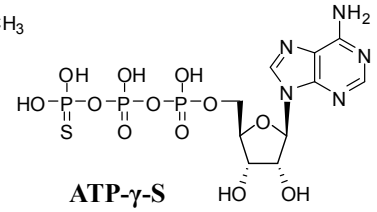
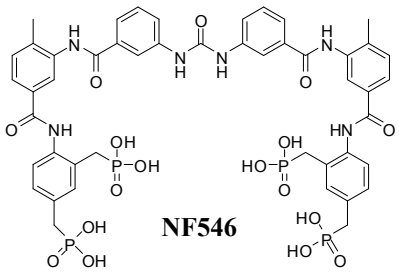
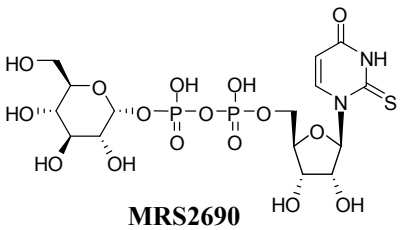


ATP-γ-S



NF546

P2Y$_{14}$



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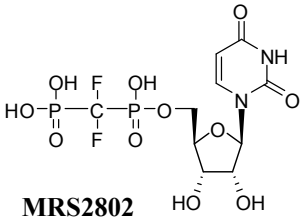
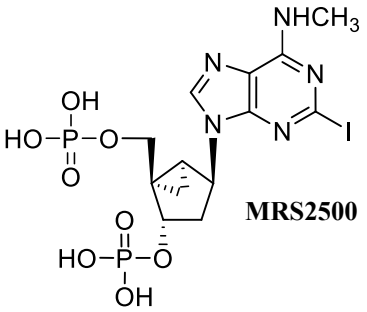


MRS2802

P2Y$_1$



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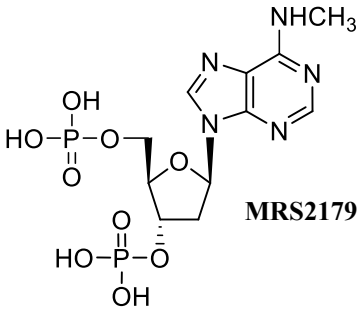

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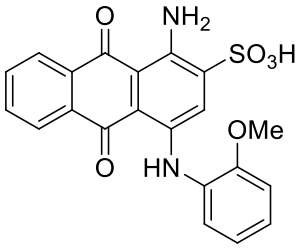
P2Y$_2$



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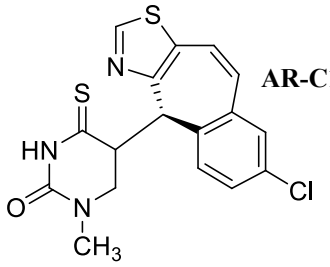

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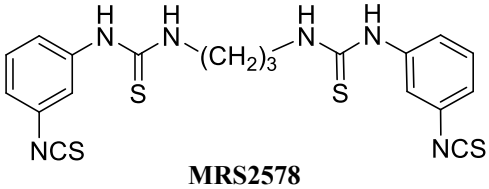

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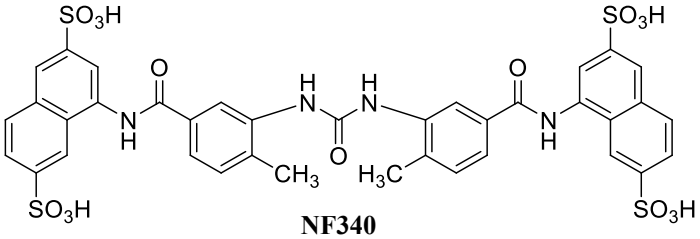
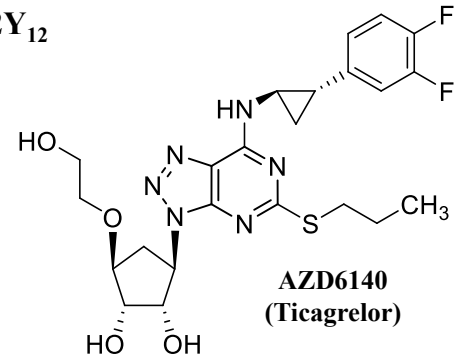
P2Y$_{11}$



NF340

P2Y$_{12}$



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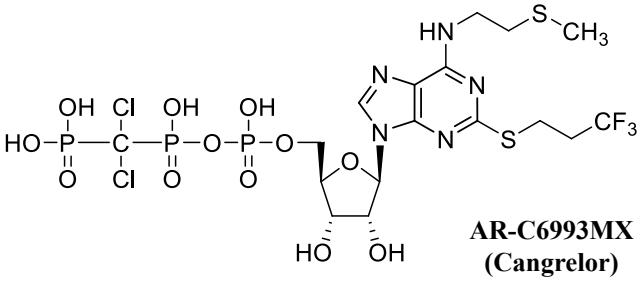
(Ticagrelor)



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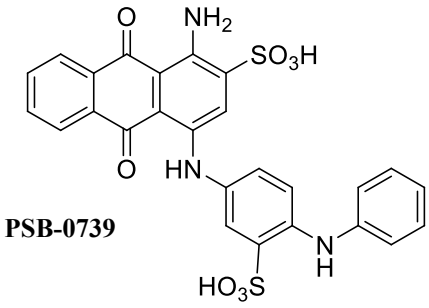
(Cangrelor)



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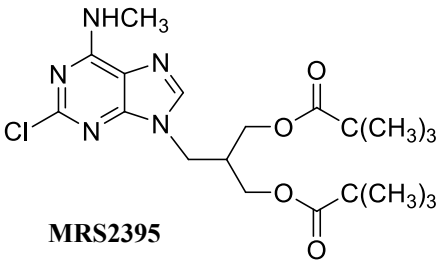

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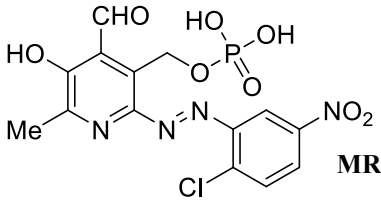

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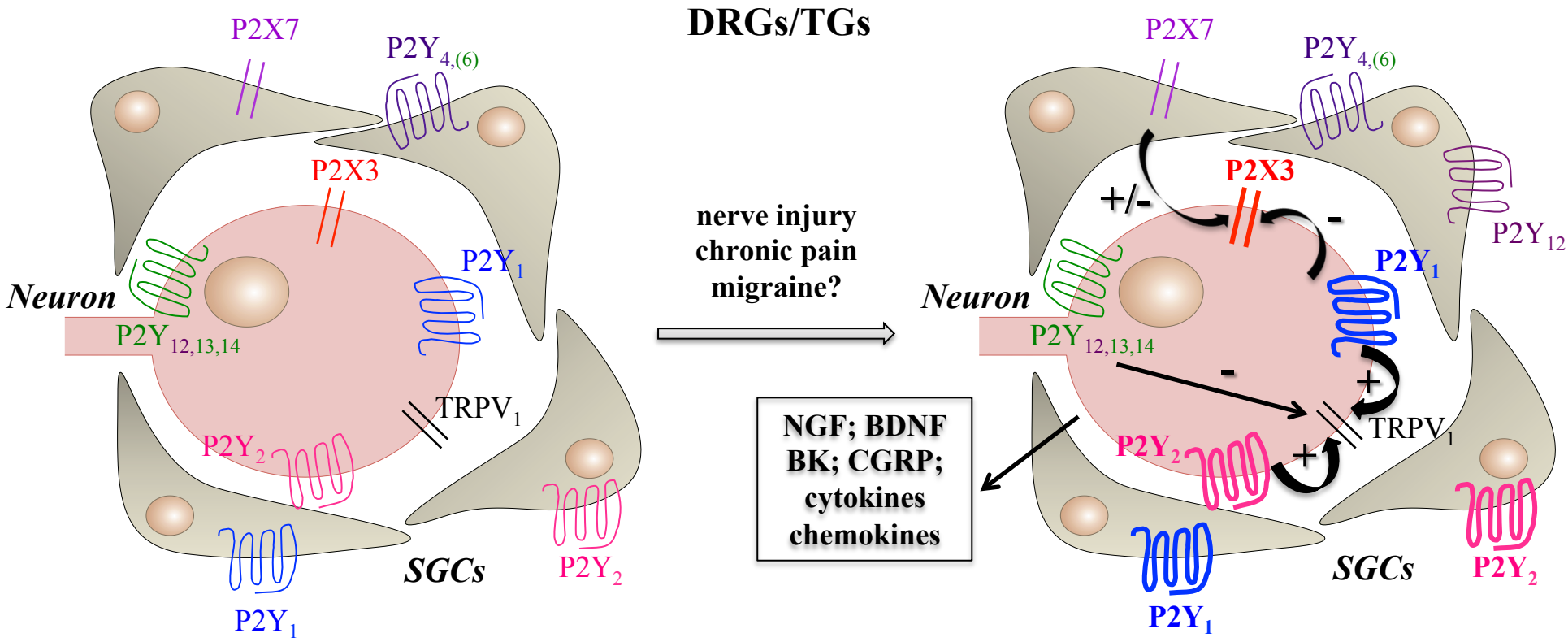
DRGs/TGs
P2X7
P2Y4,(6)
P2X3
P2Y1
Neuron
P2Y12,13,14
TRPV1
P2Y2
SGCs
P2Y1
P2Y2
nerve injury
chronic pain
migraine?
NGF; BDNF
BK; CGRP;
cytokines
chemokines
P2X7
P2Y4,(6)
P2X3
+/-
-
P2Y12
P2Y1
Neuron
P2Y12,13,14
-
+
TRPV1
+
P2Y2
SGCs
P2Y2
P2Y1

SPINAL CORD DORSAL HORN