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Update of P2X receptor properties and their pharmacology: IUPHAR Review 30

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Abbreviations: AD, Alzheimer's disease; BBB, blood-brain-barrier; Bz-ATP, dibenzoyl-ATP; DRG, dorsal root ganglion; h, human; KO, knockout; m, mouse; NANC, non-adrenergic, non-cholinergic; PAM, positive allosteric modulator; r, rat; R,

receptor; SE, status epilepticus; SNP, single nucleotide polymorphism; TM, transmembrane; TNP-ATP, trinitrophenyl-ATP; α,β-meATP, α,β-methylene ATP

Abstract

The known seven mammalian receptor (R) subunits (P2X1-7) form cationic channels gated by ATP. Three subunits compose a receptor-channel. Each subunit is a polypeptide consisting of two transmembrane regions (TM1, TM2), intracellular N- and C-termini, and a bulky extracellular loop. Crystallization allowed the identification of the 3D-structure and gating cycle of P2XRs. The agonist binding pocket is located at the intersection of two neighboring subunits. In addition to the mammalian P2XRs their primitive ligand-gated counterparts with little structural similarity have also been cloned. Selective agonists for P2XR subtypes are not available, but medicinal chemistry supplied a range of subtype selective antagonists, as well as positive and negative allosteric modulators. Knockout mice and selective antagonists helped to identify pathological functions due to defective P2XRs, such as male infertility (P2X1), hearing loss (P2X2), pain/cough (P2X3), neuropathic pain (P2X4), inflammatory bone loss (P2X5), and faulty immune reactions (P2X7).

1. INTRODUCTION

ATP, originally assumed to be exclusively the universal energy currency of cells, was proposed by Geoffrey Burnstock (1972) in his classic review to be an extracellular, non-adrenergic, non-cholinergic (NANC) neurotransmitter in smooth muscle organs of the gastrointestinal tract (see Verkhratsky et al., 2020 for a fuller description of the evidence). After the acceptance of "NANC neurotransmission", and the broadening of this hypothesis to the "co-transmission" idea (co-storage and co-release of ATP with acetylcholine or noradrenaline), purine and pyrimidine nucleotides were then recognized to be extracellular signalling molecules that co-ordinate the function of almost every cell in the animal/human organism (Burnstock & Knight, 2004). Receptors that are stimulated by these nucleotides have been classified into two types (Burnstock & Kennedy, 1985), the ligand-gated cationic channels termed P2X receptors (P2XRs) (seven mammalian subtypes: P2X1-7; Khakh et al., 2001), and the G protein-coupled P2Y receptors (P2YRs) (eight mammalian subtypes: $P2Y_{1,2,4,6,11\text{-}14}$; Abbracchio et al., 2006).

For P2YRs an excellent new IUPHAR review is available, describing in detail the pharmacological data available on this class of receptors (Jacobson et al., 2019). However, our knowledge on P2XR nomenclature and subunit properties was last summarized in an official IUPHAR review in 2001 (Khakh et al., 2001), with an interim, and non-official up-dating in 2009 (Jarvis & Khakh, 2009). Hence, the aim of this review is to present a critical update of the pharmacological properties as well as physiological and pathophysiological functions of P2XRs.

2. MEDICINAL CHEMISTRY OF P2X RECEPTOR LIGANDS

The mammalian P2XRs form homo- or heterotrimeric channels, each of which harbors three ATP binding sites. Table 1 summarizes the molecular and general pharmacological properties (agonist and antagonist potencies) of the seven P2XR subtypes. For this purpose, we have updated a most helpful Table from a review by Jarvis & Khakh (2009) by including more recently discovered receptor ligands, and introducing some recent developments (i.e. generation of knockout animal models).

P2XR agonists. Despite high conservation of the ATP binding site in each P2XR subtype, there are marked differences in the potency of ATP (**1**) (Table 1). It is active in the low micromolar to submicromolar range at all subtypes, except for the P2X7R, which requires ATP concentrations in the hundreds of micromolar range for activation. P2XR agonists structurally derived from ATP have been described (see Figure 1), but agonists with high selectivity for a single subtype are presently not available (Lambertucci et al., 2015; Jacobson & Müller, 2016). 2-Methylthio-ATP (**2**) and ATPγS (**3**) display a similar profile as ATP, but both compounds are metabolically more stable. α,β-Methylene-ATP (**4**) shows a preference for P2X1Rs and P2X3Rs with somewhat lower P2X4R potency and much lower P2X7R potency, while β,γ-methylene-ATP (**5**) is most potent at P2X1Rs and has only negligible potency at the other subtypes. 2'(3')-*O*-(4-benzoylbenzoyl)ATP (BzATP, **6**) is sometimes described as a selective agonist for P2X7Rs, but in fact it has the highest potency at P2X1Rs followed by P2X3Rs (for data see Table 1). Nonetheless, because it is approximately ten times more potent than ATP, it is frequently used for activation of P2X7Rs to avoid high, cytotoxic concentrations of ATP. 2',3'-Substitution with bulky residues, as in TNP-ATP (**7**) and the P2X3R antagonists **8** and **9** (DT-0111), or dinucleotide formation, as in diinosine pentaphosphate (Ip_5I, **10**), can abolish agonistic activity leading to P2XR antagonists.

Allosteric modulation of P2XRs by physiological ions, lipids, steroids and ethanol. P2XR function can be allosterically modulated by ions (e.g., Mg^{2+}, Ca^{2+}, Zn^{2+}; Table 1), steroids, bile acids and lipids, e.g., phosphatidylinositol polyphosphates such as $PI(4,5)P_2$ (Bernier et al., 2008). The PIPs bind to positively charged amino acids in the cytosolic C-terminal domain and inhibit P2X4R-mediated currents. The P2X4R, and to some extent the P2X2R, are inhibited by high ethanol concentrations (~100 mM) (Müller, 2015, Sivcev et al., 2019 Ilyaskin et al., 2019).

Positive allosteric modulators of P2XRs. Ivermectin (**11**, Figure 1), a CNS penetrant macrocyclic lactone used in veterinary medicine as an antiparasitic agent, interacts with various ion channels (Zemkova et al., 2014, Müller, 2015) and acts as a positive allosteric modulator (PAM) at P2X4Rs, facilitating the opening and retarding closing of the channel in the 100 nM - 3 µM range (Khakh et al., 1999; Priel and Silberberg, 2004). At a similar concentration (3 µM), ivermectin is also active at the human (h),

but not rat (r) and mouse (m) P2X7Rs (Nörenberg et al., 2012). Structural modification of several antagonists has produced derivatives with positive modulatory activity. MRS2219 (**12**) selectively potentiates ATP-induced responses at recombinant rP2X1Rs expressed in *Xenopus laevis* oocytes, with an EC_{50} of 5.9 µM (Jacobson et al., 1998). Among a series of P2X2R antagonists with an anthraquinone core structure, several derivatives showed positive allosteric modulation. PSB-10129 (**13**) was one of the most potent P2X2R-PAMs (EC_{50} = 489 nM), causing a 3-fold increase in the maximal ATP-elicited current (Baqi et al., 2011). The anthraquinone derivative Cibacron Blue (**14a**), which is one of the isomers present in Reactive Blue 2 (**14b**), is a PAM of hP2X3Rs and rP2X4Rs (Baqi et al., 2011). It is non-selective and may also interact with other P2XR subtypes. Recently, ginsenosides, e.g. **15**, structurally related to steroids and representing the main constituents of the medicinal plant, ginseng (*Panax ginseng*), were found to act as P2X4R PAMs (Dhuna et al., 2018). The development of PAMs may be a promising approach for drug development, since selectivity for orthosteric agonists will be difficult to achieve.

Non-selective P2X receptor antagonists. Moderately potent, non-selective P2XR antagonists include suramin, Reactive Blue 2 (**14b**, Figure 2), PPADS, and iso-PPADS. They are of limited use and should be replaced by more potent and selective antagonists that are now available. The ATP derivative trinitrophenyl (TNP)-ATP, (**7**) is very potent at P2X1Rs and P2X3Rs (low nanomolar IC_{50} values), much less potent at P2X2Rs and P2X4Rs, and virtually inactive at P2X7Rs (Dal Ben et al., 2018). Ip_5I (**10**) is most potent as a P2X1R antagonist and also blocks the P2X3R, but not the heteromeric P2X2/3R, while it potentiates the P2X4R (Lambertucci et al., 2015). Recently, aurintricarboxylic acid, a potent inhibitor of nucleases, was reported to strongly block P2X1Rs and P2X3Rs in a non-competitive manner (Obrecht et al. 2019).

P2X1 receptor antagonists. Only a few P2X1R-selective antagonists have been developed (Figure 2). Salicylamide derivatives with high potency and selectivity were recently described (Tian et al., 2020), representing small, uncharged molecules, which act as negative allosteric modulators (NAMs). Some of them, e.g. PSB-2014 (**16b**), only partially inhibited the human P2X1R. Suramin derivatives, e.g. NF279

(**17**) and NF023 (**18**), appear to be competitive antagonists. MRS2159 (**19**), derived from PPADS, binds covalently to the orthosteric binding site of the receptor (Erhard et al., 2003), but may also block P2X7Rs (Donnelly-Roberts et al., 2009).

P2X2 receptor antagonists. Only a few selective P2X2R antagonists have been developed (Figure 2). The anionic standard P2R antagonists PPADS, Reactive Blue 2 (**14b**), TNP-ATP (**7**), and suramin are moderately potent, non-selective P2X2R antagonists. The suramin derivative, NF770 (**20**), is more potent and selective for P2X2Rs than to the other P2XRs, and evidence was presented for a competitive mechanism of action (Wolf et al., 2011). Potent and selective P2X2R antagonists related to Reactive Blue 2 have been developed, such as PSB-10211 (**21**) and PSB-1011 (**22**) (Baqi et al., 2011).

P2X3 receptor antagonists. Many potent, selective P2X3R antagonists have been developed (Marucci et al., 2019; Ford, 2012; Müller, 2010) (Figure 2). One of the first was A-317491 (**23**), a tricarboxylate, which binds to the ATP binding site, acting as a competitive antagonist (Jarvis et al., 2002). It displays low peroral and CNS bioavailability and high plasma-protein binding. 2',3'-Benzylidene-ATP (**8**) and related ATP derivatives have submicromolar potency and some P2X3R selectivity (Dal Ben et al., 2019). The 3'-benzamido-ATP derivative, DT-0111 (**9**), was developed as a water-soluble P2X2/3R antagonist suitable for administration by inhalation (Pelleg et al., 2019). It has an IC_{50} value of 0.3 μM, as well as high selectivity, and represents a drug candidate for chronic obstructive pulmonary disease, chronic cough and overactive urinary bladder (Pelleg et al., 2019).

A series of allosteric antagonists were discovered in a high-throughput screening campaign centered on the drug trimethoprim, (for reviews see Müller, 2010; Müller, 2015). Gefapixant (**24**), named after Geoffrey Burnstock ("Gef" sounds the same as "Geoff"), has been advanced to clinical trials for chronic cough (Morice et al., 2019). Further potent and selective derivatives of this series include AF-353 (**25**) and AF-906 (**26**). AF-353 displays IC_{50} values of 6 nM (hP2X3R), 13 nM (rP2X3R) and 25 nM (hP2X2/3R), and is selective versus P2X1, P2X2, P2X5, and P2X7Rs (IC_{50} > 10 μM) as well as a range of other targets. It shows peroral bioavailability and is brain penetrant. AF-906 displayed superior pharmacokinetic properties and IC_{50} values of 2 nM (hP2X3R) and 5 nM (hP2X2/3R). The imidazopyridine derivative BLU-5937

(**27**) was reported to be selective for the homomeric P2X3R versus the heteromeric P2X2/3R and therefore, in contrast to Gefapixant, is expected not to affect taste sensing. The compound is being evaluated clinically for chronic cough and pruritus (Garceau & Chauret, 2019).

P2X4 receptor antagonists. The benzodiazepine derivative 5-BDBD (**28**, Figure 2) is a moderately potent (IC_{50} = 0.5 µM), selective allosteric P2X4R antagonist (Abdelrahman et al., 2016). It displays low water-solubility and is therefore not easily handled. NP-1815-PX (**29**) is a related compound with increased water-solubility (Matsumara et al., 2016). A possibly related compound, NC-2600 (structure not disclosed), entered clinical trials for neuropathic pain, but after a phase I study, no further development has been reported. The allosteric P2X4R antagonist, *N*-(benzyloxycarbonyl)phenoxazine (PSB-12054, **30**), exhibited an IC_{50} of 0.2 µM at the hP2X4R, but was less potent at rP2X4Rs (2.1 µM) and mP2X4Rs (1.8 µM) (Hernandez-Olmos et al., 2012). A drawback is its high lipophilicity and moderate water-solubility. A more water-soluble analogue is PSB-12062 (**31**), the *N*-(*p*-methylphenylsulfonyl)-substituted phenoxazine. It was similarly potent at hP2X4Rs (IC_{50} = 1.4 µM), rP2X4Rs (0.9 µM) and mP2X4Rs (1.8 µM) and showed selectivity versus P2X1Rs, P2X3Rs and P2X7Rs (Hernandez-Olmos et al., 2012). The urea derivative BX430 (**32**) was identified by compound library screening as an allosteric antagonist with an IC_{50} value of 0.5 µM at the hP2X4R. It showed selectivity versus other P2XR subtypes, but had no effect on mP2X4Rs and rP2X4Rs (Ase et al., 2015). The sulfonamide BAY-1797 (**33**) was recently presented as a compound with an IC_{50} of 210 nM at the hP2X4R and high selectivity (Werner et al., 2020). This antagonist shows high water-solubility and no brain penetration. The potent and selective P2X4R antagonist PSB-15417 (structure undisclosed), a brain-penetrant compound, showed high activity in animal models of neuropathic pain (Teixeira et al., 2018).

P2X7 receptor antagonists The P2X7R has been the most extensively investigated subtype for drug development, and numerous potent and selective, mainly allosteric, antagonists have been reported (Gelin et al., 2020). The sulfonate dye Brilliant Blue G blocks P2X7Rs and has been used in a number of studies due to its low cost. However, improved P2X7R antagonists with suitable pharmacokinetic properties are

available (Rech et al., 2016, Gelin et al., 2020) and should be used instead. As for many allosteric modulators, species differences have been observed for some P2X7R antagonists. The very first clinical studies were performed with allosteric P2X7R antagonists (e.g. AZD9056, **40**) for rheumatoid arthritis treatment, but their effectiveness for this indication was not convincing. Useful P2X7R antagonists (**34**-**45**, Figure 2) belong to a range of chemical compound classes. Some, e.g. **43**, show high brain-permeability. JNJ-54175446 (**43**) has been clinically evaluated for the treatment of major depression and bipolar disorders. ^{11}C and ^{18}F tracers (**44**, **45**) for positron emission tomography (PET) have been developed.

3. GENERAL STRUCTURE OF P2X RECEPTORS

To date, 27 high-resolution P2XR structures have been solved, all within the last decade and all obtained from truncated subunits, except for rP2X7, for which the full-length structure has been solved. Supported by structural data obtained from vertebrate and invertebrate receptors, there is now strong evidence that P2XRs share similar tertiary and quaternary architecture, further confirming the hypothesis that all P2XRs belong to the same structural and evolutionary group (Figure 3a-d).

Structures revealed a chalice-like, trimeric assembly of three subunit monomers, with the extracellular domain protruding 70 Å above the plasma membrane plane, and the transmembrane (TM) domain, which is comprised of six TM α-helices (two from each subunit) forming the ionic pore, extending approximately 28 Å into the membrane. The intracellular domain protrudes less than the extracellular domain, and contains the “cytoplasmic cap”, a highly intertwined region, rich in β-sheets, which is formed by domain-swapping of the N- and C-termini of all three subunits. The intracellular region of the rP2X7R contains two additional domains: the cysteine-rich C-cys anchor and the cytoplasmic ballast, both located at the C-terminal end (McCarthy et al., 2019) (Figure 3c).

Agonist sites. Three ATP-binding sites are found in the extracellular domain within large, interfacial pockets located ~40 Å from the TM domain. ATP-bound structures reveal that the phosphates of ATP bind to several highly conserved, positively charged (e.g. Lys70, Lys72, Arg298 and Lys316 in zfP2X4R) and polar (Asn296) residues (Figure 3e-g). Structures further showed that ATP unexpectedly adopts an

unusual U-shaped conformation, allowing the adenine base of ATP to be deeply buried in the binding pocket and recognized by polar (Lys70 and Thr189) and hydrophobic interactions (Leu191 and Ile232). The ribose ring of ATP is recognized by non-polar residues (Leu217), while the O1 and O2 atoms of the ring are rather solvent-accessible. Structures of other bound agonists, such as 2-methylthio-ATP (Mansoor et al., 2016), and CTP (Kasuya et al., 2017a), reveal similar binding modes and orientations, although subtle differences exist, in particular in the base recognition. Interestingly, the entrance to the binding pocket of the P2X7R (<11Å orifice) (McCarthy et al., 2019) is much narrower than that of other P2XRs, for example the P2X3R (17 Å orifice) (Mansoor et al., 2016). This may reduce drug accessibility to the binding pocket and so contribute to the three orders of magnitude lower potency of ATP at the P2X7R compared to other P2X subtypes. Nonetheless, these structural data strongly suggest that the molecular rules governing agonist recognition are highly conserved across P2XRs and species.

Antagonist sites. Molecular rules governing competitive antagonist binding seem less stringent than those of agonists. Structures of bound competitive antagonists, such as TNP-ATP or A-317491, reveal significant differences in both binding modes and orientations, when compared to agonist-bound structures. Although TNP-ATP and A-317491 occupy the orthosteric site, they bind more deeply in the binding cleft than ATP, adopting either a Y-shape (Mansoor et al., 2016) or an extended conformation (Kasuya et al., 2017b). Compared to the high structural constrains imposed by agonist binding, conformational flexibility of bound competitive antagonists may explain why they do not produce channel opening.

Allosteric sites. Structures with several bound non-competitive inhibitors also reveal molecular details of allosteric antagonism in P2XRs (Coddou et al., 2011; Habermacher et al., 2016), and at least two have been resolved at the level of atomic detail: One is located near the apex of the panda P2X7R (Karasawa and Kawate, 2016) and the other one just beneath the orthosteric binding site in the hP2X3R (Wang et al., 2018). Occupancy of these allosteric sites is thought to prevent mechanical motions involved in channel gating. Other allosteric sites are also present in P2XRs, including sites in the TM that are regulated by phospholipids

(Karasawa et al., 2017), but their definitive locations have not yet been confirmed by high-resolution structural studies.

Gating cycle. Of the 27 high-resolution structures, six have been solved in an apparent open channel conformation, bound to ATP, and three in a desensitized, closed channel state, bound to either ATP or 2-methylthio-ATP. Based on these structures, gating cycle models have been proposed for desensitizing (Mansoor et al., 2016) and non-desensitizing channels (McCarthy et al., 2019). Although these structures were all solved in detergents (i.e. in non-native phospholipid bilayer), they provide important clues on channel gating and desensitization mechanisms. Supported by experimental data (see references cited in (Habermacher et al., 2016)), it is thought that ATP binding induces a series of structural changes, from tightening of the binding pocket to the outward expansion of the six TM α-helices, leading to channel opening.

Interestingly, the helical pitch of the three innermost TM2 helices changes from an α- to a 3_{10}-helix during channel opening. Owing to the presence of the cytoplasmic cap, this helical stretching is energetically compensated (Mansoor et al., 2016), which results in the stabilization of the open channel state. However, it appears that during desensitization, TM2 helices recoil as the cytoplasmic cap dissembles. Therefore, the structural stability of the cytoplasmic cap appears to tune the rate and extent of desensitization, whereby fast-desensitizing P2X receptors have a less stable cap, compared to slowly or non-desensitizing receptors, such as P2X7Rs, which possess a more stable cap (McCarthy et al., 2019).

4. P2X RECEPTOR MOUSE MODELS

The major phenotypes of P2XR knockout mice are summarized below. For detailed information and references see Figure 4, Kaczmarek-Hajek et al. (2012), Nicke et al. (2019), http://www.informatics.jax.org/, and http://www.findmice.org/. Changes caused by the deletion of individual P2XRs will be discussed in detail in the specific P2XR sections.

Consistent with P2X1R expression, *P2rx1*^{tm1Chn} mice show a smooth muscle (90% reduced male fertility, slight hypertension) and a prothrombotic phenotype. Despite abundant P2X2R expression in neuronal and non-neuronal tissues, *P2rx2*^{tm1Ckn} mice

present a mild phenotype, with impaired neurotransmission, e.g. in pelvic afferent nerves, carotid sinus nerve and sensory ganglia. A role for cochlear P2X2R in noise adaption and hearing loss was found in *P2rx2*^{tm1Ckn} mice and in humans carrying a P2X2R mutation. *P2rx3*^{tm1Ckn} and *P2rx3*^{tm1Jwo} are less sensitive to inflammatory pain, but not acute noxious thermal (hot plate) and mechanical stimuli. However, using different assays, thermal hyperresponsiveness was observed and compensatory effects were suggested. Like *P2rx2*^{tm1Ckn} mice, *P2rx3*$^{-/-}$ mice showed bladder hyporeflexia and impaired peristalsis and additionally altered hippocampal synaptic plasticity. *P2rx2*/*P2rx3*$^{Dbl-/-}$ mice have gustatory deficits and show developmental abnormalities and high lethality due to pneumonia, probably resulting from reduced ventilatory responses to hypoxia. Surviving mice appear normal. *P2rx4*^{tm1Rass} and *P2rx4*^{tm1Ando} mice confirmed the involvement of spinal microglial P2X4Rs in chronic inflammatory and neuropathic pain. Investigation of *P2rx4*^{tm1Ando} mice also revealed a role of endothelial P2X4Rs in vascular functions, resulting in high blood pressure. In *P2rx4*^{tm1Rass} mice altered hippocampal synaptic plasticity and perceptual and socio-communicative deficits were described. A mouse model in which P2X4R-mCherry expression in the plasma membrane can be conditionally increased (*P2rx4*mCherryIN) revealed anxiolytic effects and learning deficits. Transgenic hP2X4R-overexpressing mice exhibit increased myocyte contractility, while cardiac-specific P2X4R knockout mice (*P2rx4*$^{tm1.1Ngc}$) show more severe heart failure. *P2rx4*^{tm1Dgen} showed abnormal macrophage function. P2X5Rs are widely distributed in murine tissues; osteoclasts from *P2rx5*$^{-/-}$ mice were shown to have deficits in inflammasome activation and osteoclast maturation under inflammatory conditions (Kim et al., 2017). *P2rx5*^{tm1Lex} mice showed altered immune cell numbers and learning/exploratory behaviour. *P2rx6*^{tm1Dgen} mice showed a pain phenotype.

At least seven *P2rx7*$^{-/-}$ mouse lines have been generated and show deficits in immune function, cytokine release and reduced inflammatory and neuropathic pain. *P2rx7* deletion is beneficial in numerous pathophysiological and inflammatory conditions. Furthermore, alterations in bone formation (*P2rx7*^{tm1Gab}) and an antidepressant-like profile (*P2rx7*^{tm1Lex}) were described. A floxed humanized P2X7R knock-in mouse (*P2rx7*$^{tm1.1(P2RX7)Jde}$) showed an abnormal sleep pattern upon hP2X7R deletion.

Despite invaluable information derived from the early knockout models, some caveats need to be considered: *P2rx7*^{tm1Ipch} and *P2rx7*^{tm1Gab} mice express alternative

splice variants and incomplete receptor transcripts, respectively. The P2X7k variant expressed in the *P2rx7*^{tm1Ipch} mouse is functional and highly expressed in T cells (Kaczmarek-Hajek et al., 2012). Also, most published knockout mice were generated using ES-cells derived from 129 mouse strains and the existence and possible functional effects of 129-derived passenger genes or mutations that remain upon back-crossing into other strains need to be considered, as shown for *P2rx4*^{tm1Rass} mice (Er-Lukowiak et al., 2020), which contain the "gain-of-function" P2X7L451P variant. Finally, compensatory effects cannot always be excluded in non-conditional knockout mice.

In 2007, the International knockout mouse consortium (IKMC) formed with the aim to knockout each of the >20.000 protein-coding mouse genes in C57BL/6N-derived ES cells. To this end, the KOMP, EUCOMM, NorCOM, and TIGM programs coordinated their strategies to acquire, generate, archive and distribute knockout strains and disseminate the respective data. Within KOMP, two high-throughput gene targeting pipelines were established: 1) a BAC-derived targeting vector-based approach (Valenzuela et al., 2003) to create null mutant alleles by preferentially deleting all sequences from the start ATG to the stop codon (used by the VelociGene group) and 2) a gene trapping approach (Skarnes et al., 2011) to create "ko-first/conditional-ready" alleles (Figure 4B) for tissue- or time specific gene deletion (used by Children's Hospital Oakland Research Institute, Wellcome Trust Sanger Institute, University of California at Davis (KOMP-CSD)). The latter approach is also used in the EU-funded EUCOMM program. In the succeeding EUCOMM Tools program, inducible forms of Cre recombinase (CreERT2) together with an EGFP reporter are now knocked-in into genes with useful expression patterns such as *P2rx7*$^{tm1(EGFP\_CreERT2)Wtsi}$.

Furthermore, standardized phenotyping projects were launched and so far, data for P2X2R (non-significant), P2X4R (clavicle morphology, heart weight), P2X5R (gait, preweaning lethality), P2X6R (cholesterol level, body fat), and P2X7R (cataract, eye morphology) are available at the International Mouse Phenotyping Consortium (IMPC) web site (www.mousephenotype.org).

Additionally, P2X reporter mice that express soluble fluorescent reporter proteins or fluorescent protein-tagged receptors are becoming available (Figure 4A). While both the BAC transgenic Tg(*P2rx4*-tdTomato)1Khakh (expressing soluble tdTomato) and the *P2rx4*mCherryIN knock-in mouse (conditionally expressing mCherry-tagged

P2X4) reliably reported P2X4R expression (Xu et al., 2016; Bertin et al., 2020), two BAC transgenic P2X7R reporter mice (Tg(P2rx7-EGFP)FY174Gsat and Tg(RP24-114E20-P2X7-His-StrepEGFP)Ani (expressing soluble and P2X7-fused EGFP, respectively), show highly divergent expression patterns (Kaczmarek-Hajek et al., 2018), further demonstrating the need for careful characterization of mouse models, in particular those generated in high-throughput approaches. Most recently, a knockin $P2X2^{Cre}$ mouse was described, which upon crossing with Cre-sensitive reporter mice reveals P2X2 expression (Kim et al, 2020).

5. CLONED PRIMITIVE P2X RECEPTORS

The cloning of P2XRs from primitive organisms including amoeba and algae, demonstrate the early utilization of ATP as a fast transmitter molecule (Fountain & Burnstock, 2009). Those cloned to date have low protein sequence homology with mammalian P2XRs, typically 20-40%, and as such, a different nomenclature has been devised from that of mammalian P2XRs. This system includes a prefix of an italicized short form of the species the receptor was cloned from, and in cases where multiple subtypes are identified, a letter is denoted in subscript (e.g. *Dd*$P2X_A$, *Dictyostelium discoideum* P2XR subtype A). The National Centre for Biotechnology (NCBI) accession number is given for each cloned receptor in parenthesis as they are introduced in the following sections. Bioinformatic analysis predicts further primitive P2XRs (Fountain & Burnstock, 2009), but this section describes those that have been cloned and are reported to form functional P2XRs.

Dictyostelium discoideum is a soil-living amoeba that transitions to a multicellular developmental lifecycle. Five P2XRs have been cloned, *Dd*$P2X_A$ (XM_640286), *Dd*$P2X_B$ (XM_638738), *Dd*$P2X_C$ (XM_638739), *Dd*$P2X_D$ (XM_631676) and *Dd*$P2X_E$ (XM_631865). *Dd*$P2X_C$ does not form functional channels following heterologous expression in HEK293 cells or *Xenopus laevis* oocytes (Fountain et al., 2007). *Dd*$P2X_D$ forms functional channels under low external Na^+ conditions (Baines et al., 2013). The functional receptors display a rank order in sensitivity to ATP of *Dd*$P2X_A$ (EC_{50} 97 µM) > *Dd*$P2X_B$ (EC_{50} 266 µM) > *Dd*$P2X_E$ (EC_{50} 511 µM). For *Dd*$P2X_A$, β,γ-imido-ATP (EC_{50} 15 µM) and α,β-methylene-ATP (EC_{50} 95 µM) are full agonists and

BzATP is a weak partial agonist (25% activation at 3 mM). β,γ-Imido-ATP is a full agonist at *Dd*P2X$_B$ receptors (EC_{50} 85 μM) and weak partial agonist at *Dd*P2X$_E$ receptors (22% activation at 3 mM). *Dd*P2X$_A$, *Dd*P2X$_B$ and *Dd*P2X$_E$ receptors are insensitive to suramin, PPADS and TNP-ATP, but inhibited by Cu^{2+} (*Dd*P2X$_A$, IC_{50} 40 nM; *Dd*P2X$_B$, 85% inhibition at 100 nM; *Dd*P2X$_D$, 30% inhibition at 100 nM; *Dd*P2X$_E$, 70% inhibition at 100 nM).

Further P2XRs with variable sensitivities to P2XR ligands have been described in *Schistosoma mansoni, Ostreococcus tauri, Monosiga brevicollis, Hypsibius dujardini, Boophilus microplus,* and *Lymnaea stagnalis* (Fountain, 2013).

6. P2X1 RECEPTOR

P2XR1 gene and structure. The *P2RX1* gene (ENSG00000108405) is on human chromosome 17p13.3, is 2,662 base pairs long, has 12 exons and encodes a protein of 399 amino acids. The *Ensembl Gene* database lists 4 splice variants and 262 species orthologues. In common with all other P2XR subtypes, 102 species orthologues are present in placental mammals and they are also present in birds, reptiles and fish, but not *Caenorhabditis elegans*, *Drosophila melanogaster* and *Saccharomyces cerevisiae*.

The homomeric P2X1R is a rapidly-desensitizing, non-selective cationic channel, with a relatively high permeability to Ca^{2+} (Egan & Khakh, 2004). Recombinant P2X1 subunits also form functional heteromultimers with the P2X2, P2X4 and P2X5 subunits, but this has not as yet been reported *in vivo,* with the exception of P2X1/5 (see Kennedy, 2015).

Expression. P2RX1 mRNA and P2X1R protein are widely expressed throughout the body (Burnstock & Knight, 2004; Kennedy, 2015). P2X1 is the predominant P2X subunit present in most smooth muscle tissues, including vas deferens, arteries and urinary bladder. Recently, Mahaut-Smith et al. (2019) created a P2X1R-eYFP knock-in mouse, enabling receptor expression to be viewed in live cells. Confirming earlier reports, P2X1R-eYFP fluorescence was seen in urinary bladder and arterial smooth muscle cells, platelets and megakaryocytes, but was absent from the CNS. Furthermore, the receptor is highly mobile within the plasma membrane and likely

present in lipid rafts, cholesterol-rich microdomains that are involved in receptor signalling and trafficking.

Neurotransmission. P2X1Rs mediate the actions of ATP when it is released as an excitatory cotransmitter with noradrenaline from sympathetic and with acetylcholine from parasympathetic nerves. Initial evidence depended upon P2X1R desensitization by α,β-meATP or inhibition by antagonists, but the clearest evidence is provided by gene knockout (see Kennedy, 2015 and references therein). This greatly decreased the amplitude of sympathetic contractions of mouse vas deferens and was associated with a 90% fall in fertility. Simultaneous knockout of α_{1A}-adrenoceptors caused total infertility. Thus P2X1Rs clearly play a crucial role in male reproductive function. Sympathetic, purinergic cotransmission in arteries mediates vasoconstriction, but its contribution to mean arterial blood pressure is unclear.

Parasympathetic nerves mediate contraction of urinary bladder detrusor smooth muscle, causing voiding of urine. In most species, atropine only partially inhibits these contractions and P2X1R knockout abolished the remaining response. Interestingly, P2X1R antagonists failed to mimic the effect of P2X1R knockout and it was suggested that ATP may act at both the homomeric P2X1R and at the P2X1/4R heteromer to elicit urinary bladder contraction (Kennedy et al., 2007).

Thrombosis. P2X1Rs mediate Ca^{2+} influx in platelets, but P2X1R knockout mice do not show spontaneous bleeding or increased bleeding time (Hechler et al., 2003). Fewer knockout mice died, however, in an *in vivo* model of acute obstruction of the lung microcirculation. Furthermore, thrombi formed by localized damage to arterioles were smaller and easily dispersed. Thus platelet P2X1Rs may contribute to thrombi formation, particularly in arterioles, which are narrow and associated with a high shear stress.

Dysfunctional urinary bladder. Atropine abolishes neurogenic contractions of the healthy urinary bladder in humans, but atropine-resistant contractions appear with increasing age and in chronic disorders, such as interstitial cystitis, idiopathic detrusor instability and overactive bladder syndrome. They are abolished *in vitro* by prolonged exposure to α,β-meATP, indicating mediation by P2X1Rs, though this has yet to be been confirmed using P2X1R antagonists (see Kennedy, 2015).

Inflammation. ATP released by stressed or damaged cells, or in response to inflammatory stimuli, acts via P2X4Rs, P2X7Rs, P2Y1Rs, P2Y2Rs, P2Y6Rs as a Damage-Associated Molecular Pattern (DAMP) signalling molecule to elicit proinflammatory responses in macrophages and neutrophils (Di Virgilio et al., 2020). Lecut et al. (2012) reported that P2X1R knockout increased mortality due to lipopolysaccharide (LPS)-induced endotoxemia, but Maître et al., (2015) saw a decrease, whilst there was no difference in the mortality rate from septic shock induced by uropathogenic *Escherichia coli* (Greve et al., 2017). The reasons for this variability are unclear, but may reflect differences in the serotype of the pathogenic stimulus used.

Platelets also contribute to inflammation by facilitating immune cell recruitment and activation. In a mouse model of colitis, platelet depletion or P2X1R knockout caused intestinal bleeding, leading to macrocytic regenerative anaemia, whereas neutrophil depletion reduced blood loss (Wéra et al., 2020). Thus platelet and neutrophil P2X1Rs may have protective roles in the inflamed intestine.

P2RX1 single nucleotide polymorphisms (SNPs) in cancer. The *HIVE Lab* database lists 86 unique *P2RX1* SNPs in cells from multiple types of cancer that change the P2X1R protein sequence. Whether these contribute to the development and/or maintenance of cancer is unknown, but many are predicted to be “probably damaging”. A significant reduction in *P2RX1* mRNA expression was identified in several cases, but it is unclear if this is a cause or effect of the cancer.

7. P2X2 RECEPTOR

The P2RX2 gene. The first *PRX2* cDNA encoding the rP2X2 subunit protein (UniProt ID P49653.1) was expression-cloned from pheochromocytoma cells using *Xenopus laevis* oocytes (North, 2002). The Ensembl database locates the *hP2RX2* gene (ENSG00000187848) on chromosome 12 (between 132,618776 and 132,622,388 bp) and predicts eight splice variants. The *P2RX2* genomic structure consists of 11 exons and ten introns and is highly conserved among human and rat.

Homotrimeric and heterotrimeric P2X2R proteins. P2X2 subunits assemble during their endoplasmic reticulum-bound synthesis into homotrimers (Nicke et al., 1998,

Aschrafi et al., 2004). The contacts between the subunits relevant for trimerization are located in the ectodomain, while the TMs support assembly by restricting the folding space (Duckwitz et al., 2006). P2X2 subunits are co-expressed with other P2X subtypes in many cell types; in recombinant systems they can co-assemble to functional and stable heterotrimers, such as P2X2/1R, P2X2/3R, P2X2/5R and P2X2/6R (Hausmann et al., 2015).

There is no direct X-ray or cryo-EM structure of a PX2R available, but X-ray templates from the zfP2X4R (see Section 3.) enabled building P2X2R homology models, which proved to be reliable. Achievements of these models include: (1) understanding P2X2R channel gating; (2) localization of potency-determining residues of the P2X2R antagonist NF770 (a suramin derivative) by homology docking; (3) disclosure of lateral fenestrations as ion access pathways to the channel pore; (4) identification of ionic coordination of ATP^{4-} into its binding pocket as an opening mechanism to break a salt bridge that stabilizes the closed state (Hausmann et al., 2015). The subtype-specific signatures of the homotrimeric P2X2R channel are (1) fast activation by external ATP, (2) virtually no activation by up to 300 µM external α,β-meATP, and (3) among all P2XR subtypes, the most stable steady-state current during prolonged ATP exposure, with slow or no desensitization (North, 2002). The main active form of ATP at the P2X2R is free ionic ATP (ATP^{4-}; EC_{50} 2.0 ± 0.7 µM in divalent-free solution); the Mg^{2+}-complexed form $MgATP^{2-}$ binds with much lower affinity and is thus largely ineffective in opening the P2X2R (Li et al., 2013).

P2X2R expression and physiological functions. *P2RX2* mRNA and P2X2R protein are expressed abundantly throughout the body on neurons and non-neuronal cells (Cockayne et al., 2005). The Human Brain Atlas (http://proteinatlas.org) points to a particularly high *P2RX2*/P2X2R expression in the hippocampal formation. Compared to the widespread expression in the nervous system, the behavioral phenotype in P2X2R knockout mice is remarkably inconspicuous in terms of general excitability of the CNS and sensory and motor function (Cockayne et al. 2005). Essential physiological P2X2R functions are their contribution to the sensitivity of the carotid body to hypoxia by stimulating afferent fibers of the sinus nerve (Rong et al. 2003) and their involvement in taste perception in an epithelial-to-neuronal mode of

signaling. Exposure of oral taste receptor cells to taste stimuli releases ATP that activates P2X2R and P2X3R, which are co-expressed in the taste buds that innervate the tongue (Cockayne et al. 2005). P2X2R/P2X3R double knockout mice are taste-blind to all taste stimuli, while responses to touch, temperature and menthol remain unaffected. However, single knockout of either P2X2Rs or P2X3Rs only slightly reduces taste responses (Finger et al. 2005). This implies that in addition to the heterotrimeric P2X2/3R, the homotrimeric P2X2R and/or P2X3R must also be involved in taste perception. A plausible explanation is that presynaptic homotrimeric P2X2Rs are needed to stimulate ATP secretion via an autocrine positive feedback (Huang et al. 2011).

P2X2R and auditory system. The P2X2R is abundantly expressed in the cochlea, the sensory hair cells of the organ of Corti, the tectorial membrane, the Reissner's membrane and spiral ganglion neurons. Sustained elevated noise levels release ATP into the cochlear endolymph via connexin hemichannels. This ATP activates P2X2Rs on epithelial cells lining the endolymphic compartment. The induced inward current reduces the endocochlear potential, and, consequentially, hearing sensitivity. In short, ATP is an auditory neurotransmitter that regulates hearing sensitivity via the P2X2R (for references see (Mittal et al., 2016)).

The Orphanet database for rare diseases and orphan drugs assigns *P2RX2* to a single disease-causing germline mutation, autosomal dominant deafness DFNA41. A genomic analysis of a DFNA41 family in China revealed a $V^{60}L$ mutation in the *hP2RX2* gene (Yan et al., 2013). Heterozygous family members experienced accelerated noise-induced hearing loss to high frequencies in adolescence. $V^{60}L$-hP2X2 subunits assemble as a constitutively-active, ATP-insensitive channel (George et al., 2019). A second mutation most likely causing autosomal dominant deafness, $G^{353}R$-hP2X2R, was detected in an Italian family. $G^{353}R$-hP2X2R exhibits alterations in sensitivity to ATP, inward rectification, and ion selectivity (George et al., 2019). Altogether, these results establish an essential role of the P2X2R for the preservation of hearing.

8. P2X3 AND P2X2/3 RECEPTORS

P2RX3 and P2RX3R genes and the respective homomeric and heteromeric proteins. The ensemble database reports *mP2rx3* (ENSMUSG00000027071), *rP2rx3* (ENSRNOG00000008552), and *hP2RX3* (ENST00000604659) cDNAs encoding full receptor proteins. The *mP2rx3* has two and the *hP2RX3* only one protein coding splice variant. The rP2X3R protein was cloned from dorsal root ganglia (DRGs), which when expressed in *Xenopus laevis* oocytes, yielded a channel responding to ATP with a rapidly desensitizing current (Chen et al., 1995). Soon afterwards it was found that the co-expression of rP2X3R with rP2X2R yielded ATP-activated currents that slowly desensitized and resembled those recorded in rat nodose ganglia (Lewis et al., 1995). Apparently, sensory neurons of the DRG possess a mixture of P2X3R and P2X2/3R channels, while nodose ganglia possess only P2X2/3Rs. Both P2X3Rs and P2X2/3Rs respond to the agonist α,β-meATP with the typical inward currents, although P2X2Rs are insensitive to α,β-meATP. Originally it was assumed that P2X2/3Rs consist of an obligatory combination of two P2X3 and one P2X2 subunit (Jiang et al., 2003), but later an inverse combination of the two types of subunits [$(P2X2)_2$/$(P2X3)_1$] was shown to be also functional in expression systems for recombinant receptors (Kowalski et al., 2015).

P2X3R and P2X2/3R distribution and function. In addition to postsynaptic P2X3Rs and P2X2/3Rs located at the cell bodies of sensory neurons, presynaptic P2X3Rs and P2X2/3Rs have a facilitatory role to enhance glutamatergic neurotransmission from the central terminals of sensory neurons onto the cell bodies of spinal cord afferent neurons (Khakh & North, 2012). The regulation of P2X3R and P2X2/3R expression in pathophysiology is complex. Both up and down regulation of individual receptor subunits has been documented in various experimental models (North & Jarvis, 2013; Bernier et al., 2018). This variability is likely dependent on specific contextual influences, including neuroanatomical structure and biochemistry. The ability of ATP to evoke ectopic neuronal hypersensitivity may not be solely dependent on intrinsic P2X3R or P2X2/3R activation, since functional interactions with other P2Rs, ligand-gated ion channels, and multiple signaling pathways have been described (Bernier et al., 2018).

Important insights regarding the physiological roles of P2X2Rs, P2X3Rs and P2X2/3Rs were gained through the functional analysis of transgenic and transient gene disrupted (i.e. P2X3 antisense) mice (North and Jarvis, 2013). Genetic disruption of each of these individual P2XRs resulting in a complete loss of receptor expression has been shown to reduce sensory nerve function and a concomitant diminution of nociceptive and hyperactive bladder responses (North & Jarvis, 2013; Ford & Udem, 2013). Transgenic mice lacking both P2X2Rs and P2X3Rs also show alterations in taste sensitivity to bitter and sweet substances (Finger et al., 2005).

P2X3R antagonists in clinical concept trials. Studies using nonselective antagonists or agonist-induced down-regulation of P2X3Rs provided preliminary evidence for the roles of these receptors in sensory systems (Jarvis & Khakh, 2009). Subsequently, *in vivo* studies using P2X3R and P2X2/3R selective antagonists generated evidence that blocking these receptors leads to diminished nociceptive sensitivity in a variety of experimental pain models, reduced bladder reflexes and elevated bladder volume thresholds and reduced airway sensitivity in preclinical cough models (Ford & Udem, 2013; North & Jarvis, 2013).

Gefapixant (AF-219, MK-7264), the potent and reversible noncompetitive antagonist of P2X3Rs, is approximately 3-fold less potent at P2X2/3Rs (Richards et al. 2019). Gefapixant and closely related diaminopyrimidine class structural analogues inhibit P2X3R-dependent action potentials in afferent neurons innervating peripheral tissues in a variety of nociceptive, urological, and respiratory models (Richards et al., 2019; Ford & Udem, 2013).

Gefapixant is orally bioavailable, peripherally restricted and has suitable drug-like properties enabling exploration of its therapeutic potential in humans (Ford & Udem 2013). A consistent tolerability finding for gefapixant-treated patients is a high prevalence of altered taste sensitivity (dysgeusia; Smith et al., 2020). This appears to be dose-dependent, indicating a potential for optimization of a dose to maintain antitussive efficacy, while minimizing dysgeusia (Smith et al., 2020).

Based on the known physiological roles of P2X3Rs and P2X2/3Rs, their expression on taste buds (Finger et al., 2005) and the phenotype of double knockout mice lacking both P2X2Rs and P2X3Rs, gefapixant-mediated dysgeusia is likely mediated by block of the heteromeric P2X2/3Rs (Garceau & Chauret, 2019). First generation non-nucleoside P2X3R antagonists show little selectivity in blocking

homomeric P2X3Rs and heteromeric P2X2/3Rs (North & Jarvis, 2013). However, more recently discovered P2X3R antagonists, including the imidazo-pyridine, BLU-5937, have stereoselective preferential affinity at P2X3Rs, with several orders of magnitude lower affinity at P2X2/3Rs (Garceau & Chauret, 2019). BlU5937 is currently in a proof of concept trial for chronic cough.

9. P2X4 RECEPTOR

The P2RX4 gene, splice variants and SNPs. The P2X4R was identified as a distinct member of the P2X family of receptors in 1995-1996, when it was cloned from rat whole brain, hippocampus, and superior cervical ganglia cDNA libraries and characterized by heterologous expression. During 1997-2001, human (*hP2RX4,* ENSG00000135124), mouse, chick, and *Xenopus laevis* receptor cDNAs were also cloned and characterized. Subsequently, rabbit, dog, frog, and zebrafish P2X4Rs were identified (Kaczmarek-Hajek *et al.*, 2012). The *hP2RX4* gene is located at 12q24.32, close to the *P2RX7* gene. The crystal structure of P2X4R was resolved for the zebrafish receptor (Hattori & Gouaux, 2012; Kawate *et al.*, 2009; see Section 3.). P2X4 subunits form functional homotrimers and heterotrimers with P2X1 and P2X6 subunits in expression systems for recombinant receptors. *hP2RX4* and *mP2rx4* are alternatively spliced, but the shorter forms do not form functional channels (Kaczmarek-Hajek *et al.*, 2012). In addition, there are four non-synonymous coding SNPs in the *hP2RX4* gene, but only Tyr315Cys affects the receptor function (Stokes *et al.*, 2011).

Homomeric and heteromeric P2X4R proteins. Native and recombinant homomeric P2X4Rs activate rapidly and desensitize incompletely with moderate rate, both in an ATP concentration-dependent manner, and deactivate rapidly and independently of ATP concentration. Thus, the receptor functions as a non-selective cationic channel, and its permeability for Ca^{2+} is the highest among the family (Egan & Khakh, 2004). The P2X4R is one of the most sensitive receptors to ATP, whereas the 315Cys-P2X4 mutant is less sensitive to ATP (Stokes *et al.*, 2011). The homotrimeric and heterotrimeric P2X4Rs, but not most other P2XRs, are sensitive to ivermectin, which acts as a PAM of these channels (Zemkova *et al.*, 2015).

Homotrimeric and heterotrimeric P2X4Rs also undergo rapid constitutive and agonist-induced internalization into early endosomes and lysosomes and subsequent reinsertion into the plasma membrane (Bobanovic *et al.*, 2002). Internalization of P2X4Rs is clathrin- and dynamin-dependent and determined by the C-terminal interacting with adapter protein 2 (AP2); mutation of either the endocytic motif or the Tyr binding pocket of AP2 leads to accumulation of functional receptors in the plasma membrane. Native P2X4Rs in cultured rat microglia, macrophages, and vascular endothelial cells are localized predominantly in lysosomes, where they retain their functionality and subsequently move out to the plasma membrane. This finding led to speculation about their intracellular functions (Kaczmarek-Hajek *et al.*, 2012).

Distribution and function. P2X4Rs are abundantly expressed in neurons and glial cells of several brain regions, including olfactory bulb, cerebral cortex, subcortical telencephalon, cerebellum, hypothalamus, thalamus, midbrain, hindbrain, and ventricular structures. P2X4Rs are also present in spinal cord microglia, peripheral neurons, including somatosensory cortical, nodose ganglion, trigeminal, vestibular ganglion, and spinal cord neurons, in addition to Schwann cells. In the mammalian retina, the receptor was identified in both neurons and glia (Montilla et al., 2020). The neuroendocrine cells of the hypothalamus and pituitary gland and the endocrine cells of the thyroid and adrenal glands also express P2X4R (Bjelobaba *et al.*, 2015). In the cardiovascular system they are located in cardiac and vascular smooth muscle cells and endothelial cells (Ralevic, 2015).

Numerous studies have shown a role for P2X4Rs in allodynia associated with chronic neuropathic pain, a persistent pain arising from changes in spinal cord processing pathways (Inoue, 2019). After nerve injury, overexpression of P2X4Rs in Schwann cells was reported to promote motor and sensory functional recovery and remyelination via brain-derived neurotrophic factor (BDNF) secretion. Furthermore, the P2X4R has a role in neuroinflammation, the complex biochemical and cellular response occurring during infections of the brain and the spinal cord, with the participation of microglia, astrocytes, and endothelial cells. Spinal cord injury, brain ischemia, and trauma increase P2X4R expression in microglial cells, which could be involved in inflamed lesions in the brain that persist for days/weeks after an ischemic stroke. The P2X4R may also play a role in neurodegenerative diseases, such as

Parkinson's disease, Alzheimer's disease (AD) and multiple sclerosis; they are associated with neuroinflammation that is accompanied by P2X4R-dependent microglia activation. Expression of P2X4Rs is upregulated in activated microglia from rats with experimental autoimmune encephalomyelitis (a model of amyotrophic lateral sclerosis) and in human multiple sclerosis optic nerve samples and they appear to facilitate repair response after demyelination (Montilla et al., 2020). Finally, activated P2X4Rs stimulate electrical activity, Ca^{2+} signaling, and neuro-hormone secretion in neuroendocrine cells (Bjelobaba *et al.*, 2015).

Transgenic and knockout models (see also Section 4.). A transgenic mouse model overexpressing the human P2X4 subunit exhibited increased contractility of cardiomyocytes and greater global contraction performance in intact heart compared to WT animals (Hu et al., 2001). Overexpression of human P2X4R in a calsequestrin transgenic mouse model of cardiomyopathy significantly delayed heart failure progression and increased life expectancy by more than two-fold (Yang et al., 2004). The P2X4R also contributes to the control of large vessel tone through endothelial-dependent NO release and arterial smooth muscle relaxation. Consistently, P2X4R knockout mice and human carriers of low functional 315Cys-P2X4R have raised blood pressure (Braganca & Correia-de-Sa, 2020).

10. P2X5 RECEPTOR

P2rx5 receptor gene. The human *P2rx5* gene (ENSG00000083454), located on 17p13.2, occurs as two alleles: T allele and G allele. The T allele leads to mature transcription of a full-length P2X5 subunit of 444 amino acids, and assembly of functional P2X5R ion channels (Kotnis et al., 2010). The G-allele appears in samples of human genomic DNA, but not in other mammals (Kotnis et al. 2010). The 3'-splice site (GGTCGT***gg***gat) of exon 10 contains ***gg*** on the intronic side rather than ***gt***, at which RNA splicing occurs (Bo et al., 2003). This SNP results in a shorter subunit, which lacks the 22 amino acids encoded by exon 10 (Lê *et al*. 1997; Duckwitz et al. 2006). Assemblies of the exon 10-deleted hP2X5R are retained in the cytosol by the endoplasmic reticulum (Duckwitz et al. 2006).

P2X5R protein. rP2XR5 and mP2X5 subunits share 95% homology over their 455 amino acid length, and 62% homology with the full length hP2X5 subunit. rP2X5R and mP2X5R channels are remarkable for producing very small currents to supramaximal ATP concentrations, around 5% of the amplitude of inwards currents generated under equivalent conditions by other P2XRs expressed in cell lines or *Xenopus laevis* oocytes (Collo et al., 1996). Light was shed on the cause of the small ATP responses, using a series of chimeric P2X5Rs bearing rat peptide sequences replaced with human equivalents (Sun et al., 2019). Two rP2X5R chimeras yielded considerably larger ATP responses: rP2X5R-Chimera 3, Ile^{50}-Arg^{114} (72 pA/pF); rP2X5R-Chimera 5, Leu^{171}- Lys^{205} (162 pA/pF); *cf*. rP2X5R-WT (2 pA/pF). Single substitution experiments subsequently revealed that any one of three mutations (Ser191Phe, Phe195His and Val67Iso) significantly improved functionality of rP2X5Rs by improving ATP binding at its docking site (Sun et al., 2019).

When stimulated by ATP, P2X5R functions as a slowly-desensitizing, non-selective cationic channel (P_{Ca}/P_{Na} = 1.5). Unlike other P2XRs, the P2X5R is also permeable to chloride ions (P_{Cl}/P_{Na} = 0.5) (Bo et al., 2003).

P*2X5R function.* Functional P2X5Rs may play a supporting role in the inflammatory response. Gene deletion of P2X5R (*mP2rx5*$^{-/-}$) decreased inflammatory bone loss in the parietal calvarium (skull), *in vivo*, without affecting normal bone development and homeostasis (Kim *et al.* 2017). Additionally, expression levels of pro-inflammatory IL1β, IL6, IL17a and TNF-sf11 were significantly lower in *P2rx5*$^{-/-}$ mice compared to WT mice (Kim et al., 2018).

11. P2X6 RECEPTOR

P2rx6 gene and P2X6R protein. The encoding DNA for the protein subunit has been identified in the human genome (ENSG00000099957) at 22q11.21. P2X6R is an ATP-gated ion channel when fully glycosylated. In 25 years, only two groups have succeeded in characterizing functional rP2X6Rs (Collo et al., 1996; Jones et al., 2004). In both studies, around 5% of transfected HEK293 cells yielded agonist responses to ATP and other nucleotides and P2X6Rs functioned as slowly-desensitizing, non-selective cationic channels.

N-linked glycosylation of ion channels can affect subunit folding, oligomeric assembly, trafficking to the membrane, agonist binding and channel opening. The extracellular domain of P2X6R contains the N*X*S/T glycosylation motif at three sites, with asparagine (N) residues at positions 157, 187, and 202 of the rat isoform (Newbolt et al. 1998; Rettinger et al. 2000; Jones et al. 2004). Functional and non-functional rP2X6R proteins extracted from HEK293 cells were discriminated by the molecular mass of epitope-tagged P2X6 subunits of 70 kDa and 60 kDa MW, respectively (Jones et al. 2004). Treatment with *N*-glycosidase-F reduced the molecular masses to 50 kDa, which is the expected size of the non-glycosylated P2X6 protein subunits. Thus, the efficiency of subunit glycosylation may hold the key to whether or not P2X6R express functionally.

P2X6R distribution and function. There has been renewed interest in P2X6R expression, based on two observations. Firstly, glycosylation of P2X6 subunits may be more efficient in native cell types compared to expression systems, producing the 70 kDa P2X6 subunits identified in adult midbrain, atrium, kidney, thymocytes and urinary bladder (Jones et al. 2004). Secondly, non-glycosylated P2X6R was shown to be translocated through the nuclear pore complex to the nucleus of mouse hippocampus neurons, where it interacted with the splicing factor (SF3A1), to reduce the incidence of mRNA splicing (Diaz-Hernández et al. 2015). A recent clinical study has implicated P2X6R overexpression in the progression and poor prognosis of renal cell cancer in human patients (Gong et al. 2019).

12. P2X7 RECEPTOR

The P2RX7 gene, splice variants and SNPs. hP2X7R is encoded by the *hP2RX7* gene (ENSG00000089041) on the long arm of chromosome 12, at 12q24.31 (Bartlett et al., 2014), close to the *hP2RX4* gene (12.q24.32), while *mP2rx7* is located on chromosome 5. Several nonsynonymous, intronic, or missense SNPs have been reported in the *hP2RX7* gene. A number of P2X7R isoforms derived from alternative splicing were identified both in humans and in rodents (Bartlett et al., 2014). Some variants are expressed and functional, e.g. human P2X7BR, and mouse and rat P2X7R variant “k”.

Some SNPs occurring in the coding region cause gain or loss of receptor function, and are variably associated to different disease conditions. Linkage studies suggested that the SNP *rs2230912* coding for Gln460Arg-P2X7R is connected with major depression and bipolar disorder, although this has been questioned by others (Illes et al. 2019c).

The P2X7R protein. The P2X7R has the lowest affinity for ATP among all P2Rs, a feature that has often raised doubts on its pathophysiological role. However, it is now clear that at sites of inflammation or in cancer, the local extracellular ATP concentration can rise to levels close to those needed to stimulate the P2X7R (Di Virgilio et al, 2018a). In addition, some inflammatory factors may act as positive allosteric modulators, thus lowering the ATP threshold for P2X7R activation (Di Virgilio et al., 2018b).

It was suggested about twenty years ago that some P2XR channels (P2X2, P2X4, P2X7) exhibit progressive dilation during long lasting stimulation by ATP and that the generated pore is permeable to high molecular weight cationic dyes, such as NMDG, Yo-Pro, ethidium or propidium iodide (Di Virgilio et al. 2018b). However, later it was shown that this interpretation of the experimental data obtained by reversal potential measurements is probably misleading. Participation of associated channel-forming proteins has been implicated (e.g. pannexin-1 or connexin-43), but convincing evidence now supports the view that the P2X7R itself has the ability to form a large-conductance pore in the absence of any significant dilatation; simply the P2X7R-channel allows the passage of large cationic molecules immediately from its initial activation, but at a much slower pace than that of the small cations Na^+, K^+, and Ca^{2+} (Di Virgilio et al., 2018b).

An early argument brought up to support the assumed dilation of P2X7Rs was the facilitation of P2X7R currents during long-lasting or repetitive application of ATP or BzATP (Surprenant et al., 1996). However, this was later shown to be independent of the entry of cationic molecules via the receptor-channel and rather caused by a Ca^{2+}/calmodulin-dependent current facilitation through some P2X7R orthologs (rat, but not human) (Roger et al., 2010) or the secondary activation of a chloride current e.g. in macrophages (Janks et al., 2019).

The P2X7R in the immune system. The P2X7R is widely expressed by myeloid and lymphoid immune cells, as well as by mast cells (Di Virgilio et al., 2017). Platelets also express the P2X7R, albeit at low level. The best characterized immune response associated with P2X7R stimulation is leucine-rich repeat, pyrin domain containing 3 (NLRP3) inflammasome activation and IL-1β secretion, but several other key immune responses (e.g. release of additional pro- or anti-inflammatory cytokines or chemokines, generation of reactive oxygen species, promotion of chemotaxis, stimulation/inhibition of phagocytosis, destruction of intracellular pathogens, formation of multinucleated giant cell at inflammatory granulomas) are promoted by P2X7R stimulation. Due to the relevance of the P2X7R in macrophage responses, the association of major infectious diseases with *P2RX7* polymorphisms has been widely investigated, but with inconsistent results.

The P2X7R has a special place in the overall mechanism of IL-1β secretion since its gating by extracellular ATP allows the efflux of large amounts of cytosolic K^+ that in turn drives NLRP3 assembly and caspase-1 activation. In fact, the P2X7R is the most potent plasma membrane receptor triggering pro-IL-1β processing and release (Di Virgilio, 2017), and thus is a crucial initiator of inflammation. P2X7R ko mice are less prone to initiate inflammation in response to a variety of stimuli. Secretion of mature IL-1β is severely reduced, and as a consequence, initiation of the cascade of inflammatory cytokines is also impaired (Solle et al., 2001).

Human neutrophils express functional P2X7Rs coupled to NLRP3 activation (Karmakar et al., 2016). In these cells, P2X7R activity is required for efficient clearance of *Streptococcus pneumoniae*-sustained bacterial infection (Karmakar et al, 2016). Dendritic cells (DCs) are the immune cell types that express the highest level of P2X7R. Different responses are dependent on P2X7R function in DCs, most notably antigen presentation (Mutini et al, 1999). It is highly likely that the P2X7R is a key component of the DAMP-sustained stimulatory circuit whereby adjuvants potentiate antigen presentation (Di Virgilio, 2017).

Very recently, the P2X7R was shown to be necessary to establish long-lived memory $CD8^+$ cells, and thus play a major role in immunological memory (Borges da Silva et al., 2018). Certain Natural Killer (NK) cell subtypes also express high P2X7R levels that, as for $CD8^+$ T lymphocytes, have a major role in supporting energy metabolism and maintaining these lymphoid cells fit. In the gut, the P2X7R is expressed by T follicular helper cells (Tfh) where it participates in Tfh-B cell

communication (Perruzza, 2017). P2X7R activity is understood to be necessary for the differentiation of IL-17-producing T lymphocytes in models of experimental arthritis (Fan et al., 2016) and for the induction of IL-23-dependent psoriatic dermatitis (Diaz-Perez et al., 2018).

The P2X7R is an important link between inflammation and coagulation since stimulation of macrophage and DC P2X7Rs drive a large microvesicle-mediated release of tissue factor (TF), the initiating agent of the extrinsic coagulation pathway (Baroni, 2007). The P2X7R is one of the most potent triggers for the release of exosomes and plasma membrane-derived microvesicles containing a vast array of intracellular components and exposing a variety of surface markers (Sluyter, 2017). Furthermore, P2X7R stimulation promotes vascular endothelial growth factor (VEGF) release and supports angiogenic activity in vivo (Adinolfi, 2012). Participation of the P2X7R in cancer growth and metastatization is increasingly recognized (Di Virgilio, 2018a).

The P2X7R in peripheral organs. Outside the immune system, the P2X7R is expressed by many different cell types, such as keratinocytes, corneal cells, hepatocytes, intestinal epithelial cells, vascular endothelial cells, retinal ganglion cells, fibroblasts, osteoclasts, osteoblasts, vascular smooth muscle, and skeletal muscle (Bartlett, 2014; Sluyter, 2017). It can be safely concluded that the P2X7R is ubiquitously expressed throughout the body, albeit to different levels. While the role of the P2X7R in the immune system is well established, in other tissues it is more elusive.

P2X7Rs in the CNS. The mammalian CNS consists of neuronal and non-neuronal cells. The latter comprise mainly of glia (astrocyte-like cells, oligodendrocytes, microglia) and ependymal cells. Microglia are resident macrophages of the CNS, similar in function to blood-born peripheral immunocytes (monocytes/macrophages, lymphocytes), which cross the blood-brain barrier (BBB) only in case of massive infections or BBB damage of diverse origin. Microglia possess the highest density of P2X7Rs, surmounting that present in astrocytes/oligodendrocytes, whereas neurons appear to be devoid of this receptor (Illes et al. 2017). Effects attributed previously to the activation of neuronal P2X7Rs are now thought to be indirect, mediated by the

release/outflow of gliotransmitters, or other types of glial signalling molecules (Illes et al. 2019a).

Involvement of P2X7Rs in neurological and psychiatric illnesses. Although neurodegenerative illnesses have specific and distinct causative factors, they generate also an additional neuroinflammatory component that aggravates the primary condition. P2X7Rs of glial cells are intimately involved in neuroinflammation and, therefore, P2X7R antagonists have a favorable symptomatic impact in case of these illnesses. Glial (especially microglial) P2X7Rs are stimulated by large concentrations of ATP released from CNS cells under noxious conditions, both during acute injury (trauma, hypoxia/ischemia, epilepsy-induced seizures) and chronic neurodegenerative conditions (AD, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis) (Burnstock et al. 2011).

Epilepsy is typically thought to be the result of an enduring imbalance between excitation and inhibition in the brain (Engel et al. 2016). A major cause of long-lasting epileptic seizures termed "status epilepticus" (SE) is caused by the increased release of ATP. Kainic acid injection into the nucleus amygdala of mice induced seizure-like EEG activity; P2X7R blockers reduced electrographic seizures and cortical cell death. Conversely, when SE was induced by the systemic injection of pilocarpine, immediate seizure activity was increased, recurrent seizures following a one-time pilocarpine injection were facilitated, and this was thought to be due to the enhanced survival of hippocampal neural progenitor cells migrating into ectopic locations and generating a pathologic pace-maker (Rozmer et al. 2017).

The production of the neurotoxic molecules, β-amyloid (Aβ) and hyperphosphorylated tau have been assumed to cause AD and the cardinal clinical symptom, cognitive deterioration (Illes et al. 2019b). A diversity of investigations with primary microglial cultures and *in vivo* AD animal models suggested that Aβ induces an increase in $[Ca^{2+}]_i$ in microglia via P2X7R stimulation and the subsequent massive release of ATP (Sanz et al. 2009). A possible chain of events is that ATP released from damaged CNS cell types stimulates microglial P2X7Rs and releases IL-1β, as well as other cell products. The beneficial effects of BBB-permeable P2X7R antagonists in AD animal models also support the notion of pathological microglial activation in AD (Csolle et al. 2013).

As already mentioned, the SNP Gln460Arg-P2X7R is thought to be a predisposing factor for the affective diseases major depression and bipolar disease. In addition, various models of inescapable rodent stress lead to the generation of a behavioral reaction termed “learned helplessness” (Illes et al. 2019c). This is believed to be due to the increased secretion of adrenocorticotropic hormone (ACTH) and subsequently glucocorticoids thought to cause depressive-like behavior. Co-stimulation of microglial Toll-like receptors (TLRs) and P2X7Rs by DAMPs (ATP itself is the most ubiquitous DAMP) triggers NLRP3 activation and the associated IL-1β release, which is probably a major stimulus for the corticotropin-releasing hormone (CRH)-induced activation of the hypothalamic-pituitary-adrenal axis. In fact, P2X7R ko mice by themselves and WT mice after the application of P2X7R antagonists, exhibited an antidepressant-like profile in animal models of major depression and bipolar disorder (Basso et al. 2009).

13. CONCLUSIONS

P2XRs appear very early in phylogeny (algae, amoeba and basal fungi) and it is a fascinating property of these primitive receptors that, in contrast to their mammalian counterparts, they are expressed predominantly or even exclusively intracellularly. The mammalian P2X1-7Rs reside mainly in the plasma membrane and their ATP-induced opening and the subsequent cationic fluxes can regulate many essential cellular functions. Our knowledge of P2XR structure/function has greatly increased in the four decades elapsing since their discovery. Crystallization of the truncated zfP2X4R disclosed the structure of a chalice-shaped trimeric receptor that allows cations to flow through fenestrations to the vestibules near the ion channel, resulting in transmembrane ion fluxes. The zfP2X4R served as a pattern for homology modeling of mammalian P2XR-subtypes before they were crystallized and their 3D structure resolved.

Medicinal chemistry has made major contributions to the field by synthesizing subtype-selective antagonists and, with some temporal delay, positive and negative allosteric modulators. This is a pre-requisite of any conclusive experimental work and was therefore essential for moving the field ahead. Some of these antagonists were already prospective drugs in that they had favorable bioavailability after oral application and in case of an intended CNS activity, avidly crossed the BBB.

Indispensable for research activities was the generation of a battery of transgenic animals. Reporter mice that express soluble fluorescent reporter proteins or fluorescent protein-tagged P2XRs allowed targeted investigations of receptor-containing cells or their subcellular compartments.

Especially important was the assignment of deficiencies in individual P2XR-subtypes to certain diseases with a genetic background, and more recently population genetic studies aimed at the identification of loss-of-function SNPs pathogenically involved e.g. in affective diseases. In conclusion, structural deficiencies of P2XRs may underlay certain illnesses, and on the contrary, selective antagonists or negative allosteric modulators may correct the deleterious consequences of a pathological overstimulation by ATP (e.g. neuropathic/inflammatory pain or neurodegenerative illnesses). Although a P2XR-based widely used drug is still missing in our therapeutic repertoire, the pharmaceutical industry is working intensively in this field and there is strong hope of achieving a major breakthrough in the near future (Cully, 2020, Krajewski, 2020).

14. NOMENCLATURE OF TARGETS AND LIGANDS

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

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CONFLICT OF INTEREST

FDV is a member of the Scientific Advisory Board of Biosceptre Ltd, a UK-based Biotech involved in the development of P2X7-targeted therapeutic antibodies. Other Authors declare no competing interest.

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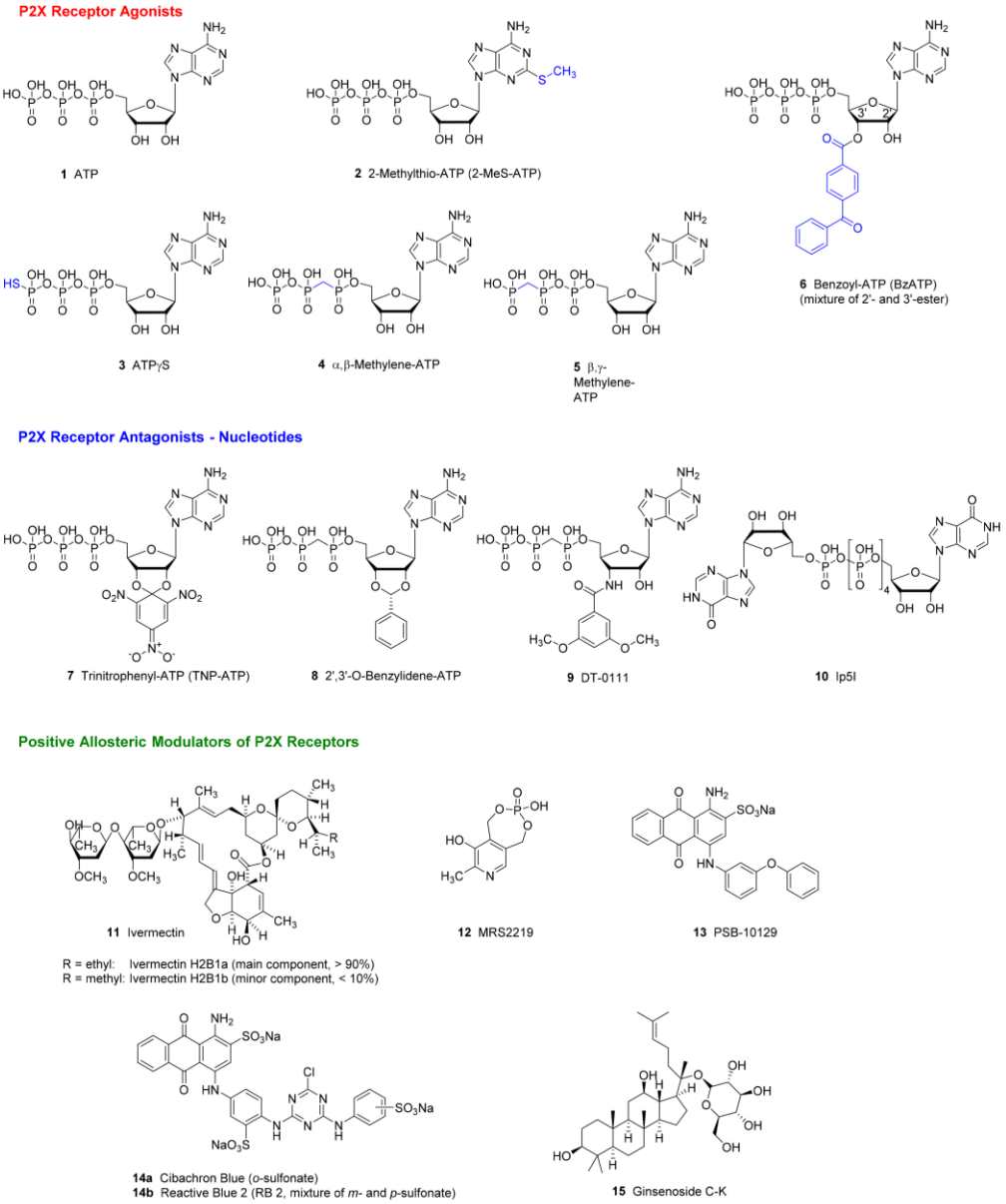

Figure 1. Selected P2XR agonists, nucleotide-derived antagonists, and positive allosteric modulators.

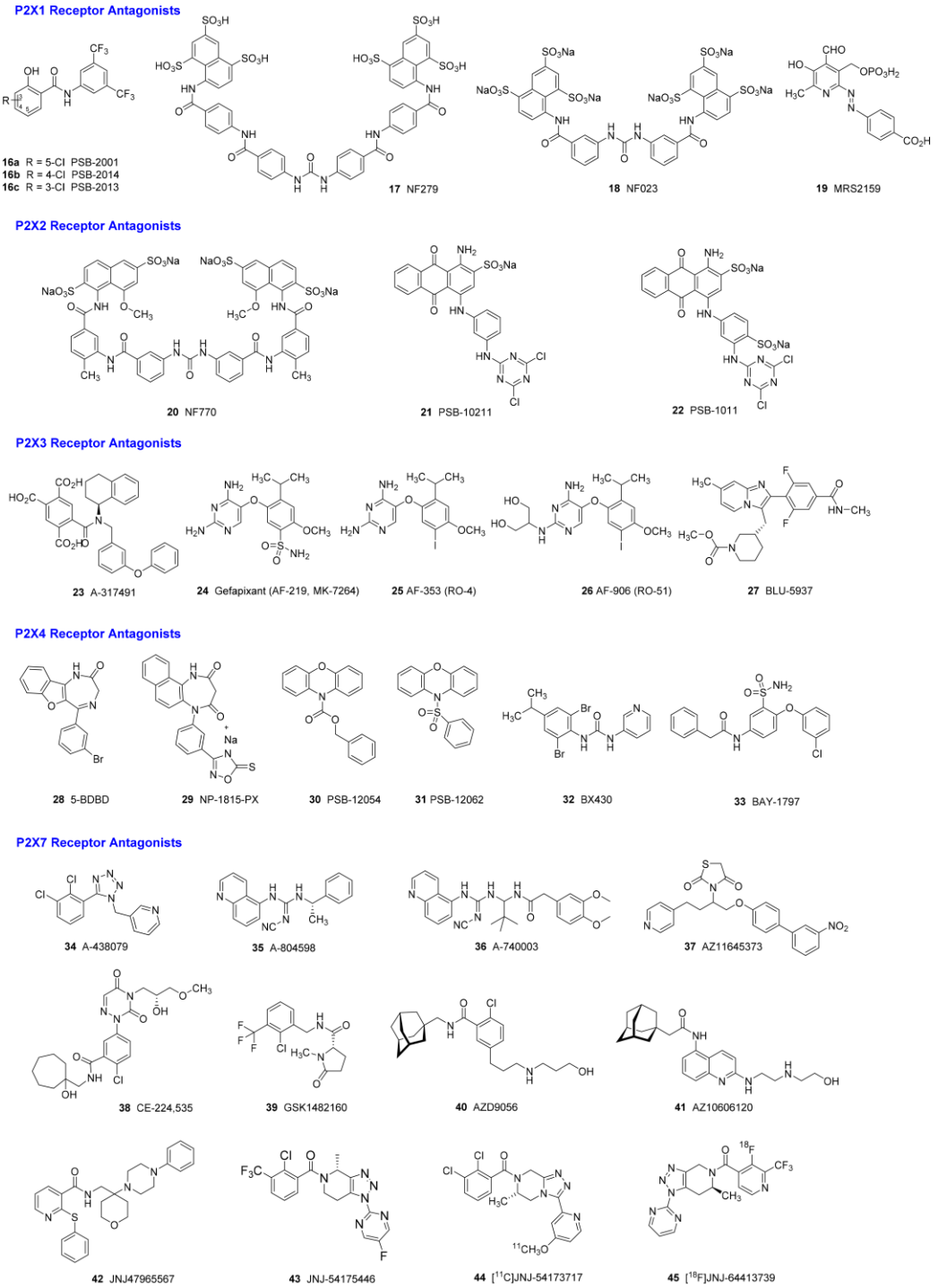

Figure 2. P2X receptor subtype-selective antagonists.

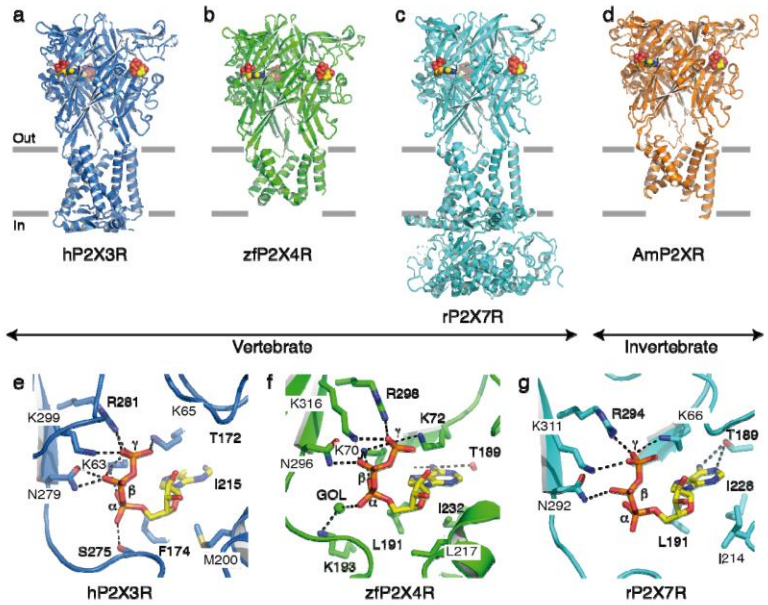

Figure 3. Structures of selected P2XRs. (a) Structure of hP2X3R bound to ATP (PBD ID: 5SVK) (Mansoor et al., 2016). The hP2X3R is shown in blue cartoon representation, and ATP is shown as spheres (carbon is yellow, oxygen red, nitrogen blue and phosphorus orange). Horizontal grey bars indicate the approximate location of the membrane bilayer defining the extracellular (out) and intracellular (in) milieu. (b) Structure of zfP2X4R bound to ATP (4DW1) (Hattori & Gouaux, 2012). The zfP2X4R is shown in green cartoon representation, and ATP is shown as spheres. (c) Structure of rP2X7R bound to ATP (6U9W) (McCarthy et al., 2019). The rP2X7R is shown in cyan cartoon representation, and ATP is shown as spheres. (d) Structure of the invertebrate AmP2XR bound to ATP (5F1C) (Kasuya et al., 2016). The AmP2XR is shown in orange cartoon representation, and ATP is shown as spheres. Note the structural similarity between vertebrate and invertebrate P2XRs. For structures having undergone heavy truncations, membrane spanning helices are lacking in their intracellular sides. (e-g) Close-up views of ATP-binding sites from hP2X3R (e), zfP2X4R (f) and rP2X7R (g). For comparison, views are taken from similar angles, and displayed residues are equivalent across P2XRs, except for S275 and K193. For those not directly contributing to ATP binding (distance > 3.5 Å), equivalent residues are not displayed (e.g. K64 in rP2X7R). ATP is shown in stick representation (carbon is yellow, oxygen red, nitrogen blue and phosphorus orange) with positions of α-, β- and γ-phosphate. The oxygen atom from a glycerol molecule (GOL) is shown in sphere representation. Black dashed lines indicate hydrogen bonding (< 3.5 Å). hP2X3: human P2X3R; zfP2X4: zebrafish P2X4R; rP2X7: rat P2X7R; AmP2X: Gulf Coast tick *Amblyomma maculatum* P2XR.

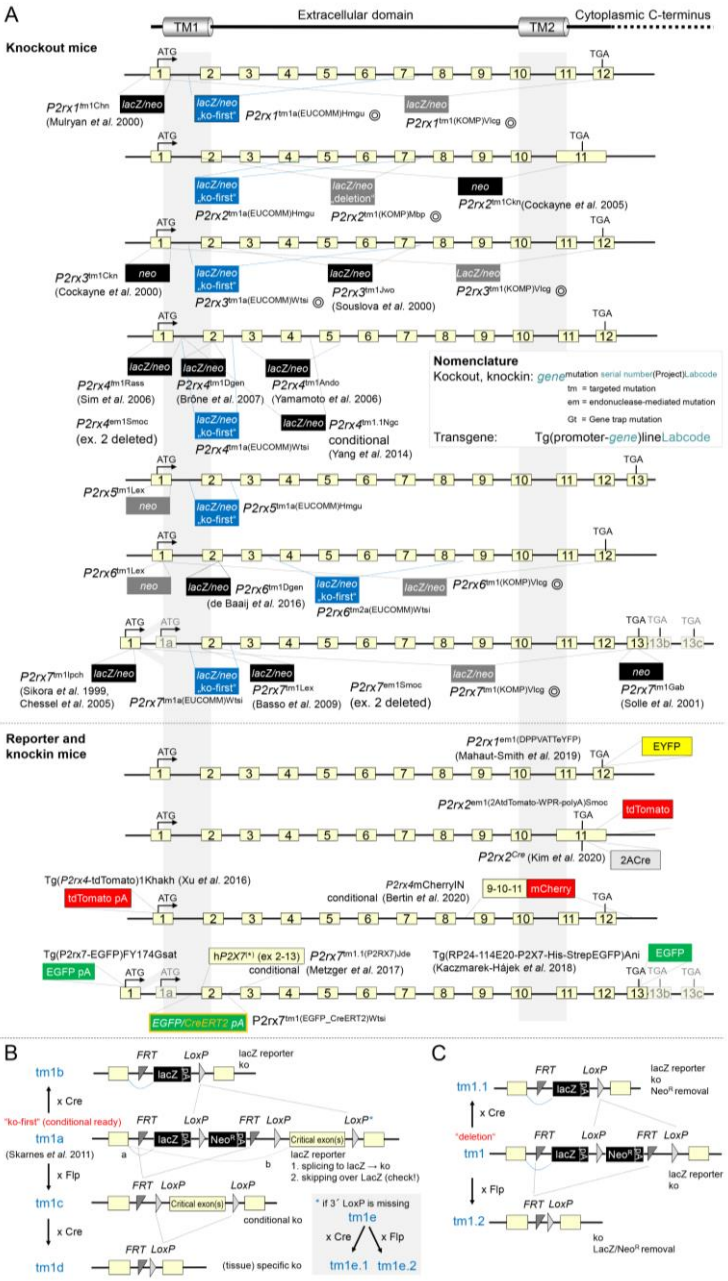

Figure 4. Currently available P2XR mouse models according to the literature and Mouse Genome Informatics (MGI)/International Mouse Strain Resource (IMSR). (A) Strategies to target *P2rx1-P2rx7* genes for the generation of knockout, knock-in, and transgenic mouse models. The nomenclature according to the current guidelines of the International Committee on Standardized Genetic Nomenclature for Mice is summarized in the inset and found on the mouse nomenclature home page (http://www.informatics.jax.org/mgihome/nomen/index.shtml). P2rx4mCherryIN is not named accordingly, yet. Light yellow boxes represent exons, black and coloured boxes represent introduced reporter/selection cassettes and/or cDNA. Circles behind the names indicate alleles that are only available in ES cells. In case of conditional strategies, only tm1a alleles („ko-first“) are shown. These can be further modified as described in (B). Further knockout strains are available from Taconic (deleted exons in brackets) for *P2rx1* (2-7), *P2rx4* (2-4), *P2rx5* (1), *P2rx6* (1-2), *P2rx7* (2-3) and from

TIGM (gene trap vector insertion in brackets) for *P2rx1* (IST14381H9), (IST12457B12) and *P2rx3* (IST10786C2). In addition, *P2rx2*$^{em1(IMPC)H}$, *P2rx4*$^{Gt(OST340739)Lex}$, and Gt(ROSA)26Sor$^{tm10(RNAi:P2rx7)Rkuhn}$ are available. Targeted reporter-tagged insertion with conditional (B) potential (ko-first, conditional ready) and reporter-tagged deletion alleles (C) and the respective nomenclature. Derivative alleles can be obtained through recombinase (Flp or Cre, as indicated) mediated changes (https://mpi2.github.io/IKMC-knowledgebase/2010/08/24/what-are-the-allele-types.html). The lacZ reporter is supposed to be spliced to the upstream exon 1 (A). However, skipping of lacZ (B) and splicing to the downstream (critical) exon (resulting in functional wt or hypomorph) cannot be excluded and needs to be experimentally determined. The critical exon is supposed to produce a frameshift mutation upon deletion. Note that tm1e represents an unplanned by-product of the original targeting strategy in which the 3´loxP site was lost during recombination but which might still be useful. For detailed information and references see Kaczmarek-Hajek et al., 2012; Nicke et al., 2018); http://www.informatics.jax.org/, and http://www.findmice.org/.).

Table 1.
Properties of recombinant homomeric P2X receptors and their pharmacology

	NC-IUPHAR subunit nomenclature						
	P2X1	**P2X2**	**P2X3**	**P2X4**	**P2X5**	**P2X6**	**P2X7**
Molecular properties[a]							
Gene name	*P2RX1*	*P2RX2*	*P2RX3*	*P2RX4*	*P2RX5*	*P2RX6*	*P2RX7*
Human chromosome location	17p13.3	12q24.33	11q12.1	12q24.32	17p13.3	22q11.21	12q24.31
Protein length (amino acids)	399	471	397	388	444	441	595
C tail length (amino acids)	41	113	56	29	82	87	240
Membrane expression	Good	Good	Good	Good	Poor	Usually no expression	Good
Desensitization (complete in)	Fast (<1 s)	Slow (>20 s)	Fast (<1 s)	Slow (>20 s)	Slow (>20 s)	–	Slow (>20s)
Activation-dependent endocytosis	Yes	No	–	Yes	–	–	–
Pharmacology[a]							
Agonists (EC$_{50}$ values (μM))[Ref. 1,Ref. 7]							
ATP **1**	0.56 - 0.70	2 - 8	0.5 - 1	1 - 10	0.44 - 10	12	100 (r)
2-MeSATP **2**	0.07 - 1	1	0.35	0.29; 4.5 (r); 1.35 (m)	0.5 - 10	9	178; 2000-4000 (m)
ATPγS **3**	2.3	1.5 (r)	0.7	2.3 (r)	0.5; 0.6 (r)	1.3 (r)	138
α,β-MeATP **4**	0.1 - 1	>300	0.74; 1 - 2 (r)	0.81; >100 (r,m)	160 - >300; 1.1 (r)	>100	>300
β,γ-MeATP **5**	2	>300	>300 (r)	>300	11.8 (r)	–	–
BzATP **6**	0.002	0.75	0.08	0.5; >100 (r); 2.9 (m)	6 - 40; >500; (partial, r)	25	5; 10 - 52 (r)
Antagonists (IC$_{50}$ values (μM))							
Non-selective antagonists							
Suramin[Ref. 1]	1 - 2	10	3	>300	2 - 3	–	>300
PPADS	1	1	1	>500	3	>100	10 - 50
RB-2 **14b**[Ref. 1]	30 (r)	0.4 - 0.5	–		18.3 (r)	–	–
Aurintricarboxylic acid	0.0086 (r)	21.7 (r)	0.0729 (r)	763 (r)	–	–	118 (r)
Nucleotide-derived antagonists							
TNP-ATP **7**[Ref. 1,Ref. 5]	0.006	1 - 2	0,001	1.5 - 15	0.45 (r)	–	>30
2',3'-O-Benzylidene-ATP **8**	0,002	5,5	0,08	0,49	40	25	52
DT-0111 **9**	–	–	0.3 (P2X2/3)	–	–	–	–
IP_5I **10**[Ref. 1]	0.003	>300	2.8	Potentiation	>30 (r)	–	
Non-nucleotide-derived antagonists							
P2X1 receptor antagonists							
PSB-2001 **16a**	0.019	>10	>10	0.156	–	–	0.175
PSB-2014 **16b**	0.0231	>10	>10	0.209	–	–	0.196
PSB-2013 **16c**	0.058	>10	>10	0.049	–	–	0.177
NF279 **17**[Ref. 1]	0.02 - 12	1	2	>300	–	–	3 - 20
NF023 **18**[Ref. 1]	0.2	>50	28.5; 8.5 (r)	>100	–	–	–
MRS2159 **19**	80	>100	>100	–	–	–	>100
P2X2 receptor antagonists							
NF770 **20**	1	0.019	0.08	>100	–	–	>100
PSB-10211 **21**[Ref. 2]	–	0.09 (r)	–	–	–	–	–
PSB-1011 **22**[Ref. 2]	0.42 (r)	0.08 (r)	0.49 (r)	>10 (r)	–	–	>10 (r)
P2X3 receptor antagonists							
A-317491 **23**	>10	>100	0,1	>100	–	>100	>100
Gefapixant (AF-219, MK-7264) **24**[Ref.	>10	0.100 - 0.250 (P2X2/3)	0.03	>10	>10 (r)	–	>10
AF-353 (RO-4) **25**	>10	>10	0.006	-	>10	–	>10
AF-906 (RO-51) **26**	>10	>10	0.002	>10	>10		>10
BLU-5937 **27**[Ref. 3, Ref. 4]	>20	>24 (P2X2/3)	0.025; 0.092 (r)	>20	–	–	>20
P2X4 receptor antagonists							
5-BDBD **28**[Ref. 7]	>10 (r)	>10 (r)	>10 (r)	0.35 - 0.5; 3.5 (r); 2.5 (m)	–	–	>10 (r)
NP-1815-PX **29**	>30	7.3	>30 (r)	0.26	–	–	>30
PSB-12054 **30**[Ref. 5]	6.5	>10	>10	0.19	–	–	>10
PSB-12062 **31**[Ref. 5]	>10	>10	>10	1.4	–	–	>10
BX430 **32**	>10	>10	>10	0,78	>10	–	>50
BAY-1797 **33**[Ref. 6]	>50	>30 /P2X2/3)	8.3	0.11 - 0.23 (h,m,r)	–	–	10.6
PSB-15417	10.3	>10	4.14	0.022; 0.037 (r); 0.087 (m)	–	–	2.13
P2X7 receptor antagonists							
A-438079 **34**[Ref. 1]	>100	>100	>100	>100	–	>100	0.06 - 0.5
A-804598 **35**	>100	>100	>100	>100	–	>100	0.010 (h,r,m)
A-740003 **36**[Ref. 1]	>100	>100	>100	>100	–	>100	0.04 - 0.05; 0.02 (r)
AZ11645373 **37**	>10	>10	>10	>10	>10	–	0.1
CE-224,535 **38**	>10	–	–	–	–	–	0.002 - 0.013; inactive (rodent)
GSK1482160 **39**	–	–	-	-	–	–	0.003
AZ9056 **40**	–	–	-	-	–	–	0.012
AZ10606120 **41**	–	–	-	-	–	–	0.0014
JNJ47965567 **42**	–	–	-	-	–	–	0.005
JNJ54175446 **43**	–	–	-	-	–	–	0.003
[^{11}C]JNJ-54173717 **44**	–	–	-	-	–	–	0.0016
[^{18}F]JNJ-64413739 **45**	–	–	-	-	–	–	0.015
Positive allosteric modulators (EC$_{50}$ values (μM))							
Ivermectin **11**	–	>30	>30	0,25	–	–	>30 (r, m), h↑
MRS2219 **12**	5.9 (r)	>100 (r)	>100 (r)	>100 (r)	–	–	–
PSB-10129 **13**	–	0.489	-	-	–	–	-
Cibacron Blue **14a**	IC$_{50}$ = 0.7 μM	–	Potentiation	Potentiation and block	–	–	–
Ginsenoside C-K **15**	–	–	-	8,5	–	–	1,1
Modulatory cations							
Zn^{2+}	–	Increase EC$_{50}$ = 7 μM	–	Increase 2 μM	–	–	–
H^+	Decrease pKa 6.3	Increase pKa 7.3	Decrease pKa 6.0	Decrease pKa 6.8	–	–	Decrease pKa 6.1
Ca^{2+}	No effect >100 mM	Block IC$_{50}$ = 87 mM	Block IC$_{50}$ = 15 mM	–	Decrease 7 mM	–	Decrease 3 mM
Physiology and pathophysiology							
Major cellular expression	Smooth muscle	Neurons	Pain sensing neurons	Neurons, microglia	Skeletal muscle	Broad expression	Immune cells
Major role	Neuroeffector transmission	Taste, hearing	Pain, bladder reflexes, taste	Vascular remodeling, neuropathic pain	Inflammatory bone loss	–	Inflammation, neurodegenerative illnesses
Model native cell type	Vas deferens, myocytes	SCG and myenteric plexus neurons	Small DRG neurons	Macrophages	Skeletal myocytes	–	Monocytes, macrophages, microglia
KO available?	Yes	Yes	Yes	Yes	Yes	Yes	Yes

a. Human (h) P2X receptor, unless noted (r, rat; m, mouse). The pharmacology values apply to homotrimeric receptors, unless noted. Bold nubers refer to chemical structures shown in Tables 1 and 2. Modified from Table 1 of Jarvis and Khakh (2009) with permission. Additional references are marked with numbers as shown below.
Ref. 1, Lambertucci et al., 2015; Ref. 2, Baqi et al., 2011; Ref. 3, Garceau and Chauret, 2019; Ref. 4, Marucci et al., 2019; ; Ref. 5, Olmos et al., 2012; Ref. 6, Werner et al., 2019.
SCG, superior cervical ganglion; DRG, dorsal root ganglion.