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REVIEW

Molecular and functional properties of P2X receptors—recent progress and persisting challenges

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Abstract ATP-gated P2X receptors are trimeric ion channels that assemble as homo- or heteromers from seven cloned subunits. Transcripts and/or proteins of P2X subunits have been found in most, if not all, mammalian tissues and are being discovered in an increasing number of non-vertebrates. Both the first crystal structure of a P2X receptor and the generation of knockout (KO) mice for five of the seven cloned subtypes greatly advanced our understanding of their molecular and physiological function and their validation as drug targets. This review summarizes the current understanding of the structure and function of P2X receptors and gives an update on recent developments in the search for P2X subtype-selective ligands. It also provides an overview about the current knowledge of the regulation and modulation of P2X receptors on the cellular level and finally on their physiological roles as inferred from studies on KO mice.

Keywords Molecular · Functional properties · P2X receptors · Physiology · Pharmacology · Protein interactions · KO mice

Introduction

Adenosine 5'-triphosphate (ATP) is an essential macromolecule for all life forms and most likely evolved under the pre-biotic conditions prevalent on the primitive earth [1]. Research of the past 40 years has shown that ATP is not only a principal energy source and component of nucleic acids inside the cell but also plays a crucial role in intercellular communication [2]. This possibly oldest transmitter is involved in both fast and slow communication between cells by activating ionotropic P2X (ligand-gated ion channel receptors) or metabotropic P2Y (G-protein coupled receptors) receptor families [3–5]. P2X receptors are present in virtually all mammalian tissues and mediate a large variety of responses from fast transmission at central synapses, contraction of smooth muscle cells, platelet aggregation, and macrophage activation to proliferation and cell death, to only name a few [6]. After a short introduction of P2X receptor phylogeny, we will summarize the current information about their structure and function, synthesis and protein interactions, and focus on the recent developments in P2X receptor pharmacology. In addition, we will provide an overview of some physiological P2X receptor functions that are inferred from genetically modified mice and other in vivo models. For more detailed information on P2X receptor function in other systems, their distribution and signalling, a variety of excellent and comprehensive reviews are available [7–21].

P2X receptors in different species

Since 1994, seven mammalian P2X cDNAs (P2X1-P2X7) have been cloned [19, 22, 23]. Subsequently, P2X receptors were found to be also widely distributed among all vertebrate animals [24]. However, low sequence homology has made it difficult to determine potential homologues in invertebrate species. Since the identification of the first invertebrate P2X receptor in parasitic trematode *Schistostoma mansoni* [25], P2X receptor family members have also been discovered in

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more primitive life forms such as the unicellular amoeba *Dictyostelium discoideum* and the green algae *Ostreococcus tauri* [24, 26, 27], which is the smallest known free-living eukaryote [28, 29] (Fig. 1). Interestingly, *D. discoideum* P2X receptors are localized in the membrane of the intracellular contractile vacuole [27, 30]. These findings demonstrate that P2X receptors must not only be considered in the context of the plasma membrane but that at least phylogenetically older P2X receptors may have an intracellular ion channel function [27].

Evidence is accumulating that P2X receptors arose at the same time or even before the appearance of G-protein coupled P1 (adenosine) or P2Y receptors [31]. However, despite extensive bioinformatics efforts, no prokaryotic P2X receptor has been identified so far [24, 32], suggesting that structurally different ATP receptors evolved in bacteria and that the P2X receptors were not derived from a prokaryotic ancestor [31]. Considering the presence of the P2X channels in the photosynthetic *O. tauri* and the significance of ATP-mediated signalling in plant physiology [33, 34], it is also astonishing that there is no evidence for P2X counterparts in higher plants such as *Arabidopsis thaliana*. More sequenced genomes and experimental data are necessary to completely exclude the possibility of the existence of the P2X receptors homologues in prokaryotes and higher plants. Functional P2X receptors have been identified in unicellular choanoflagellates (*Monosiga brevicollis*), which are the closest known relatives of the animal kingdom [26, 35]. Despite this fact, P2X-like protein sequences appear to be absent in some commonly used model systems such as the yeast *Saccharomyces cerevisiae*, the nematode *Caenorhabditis*

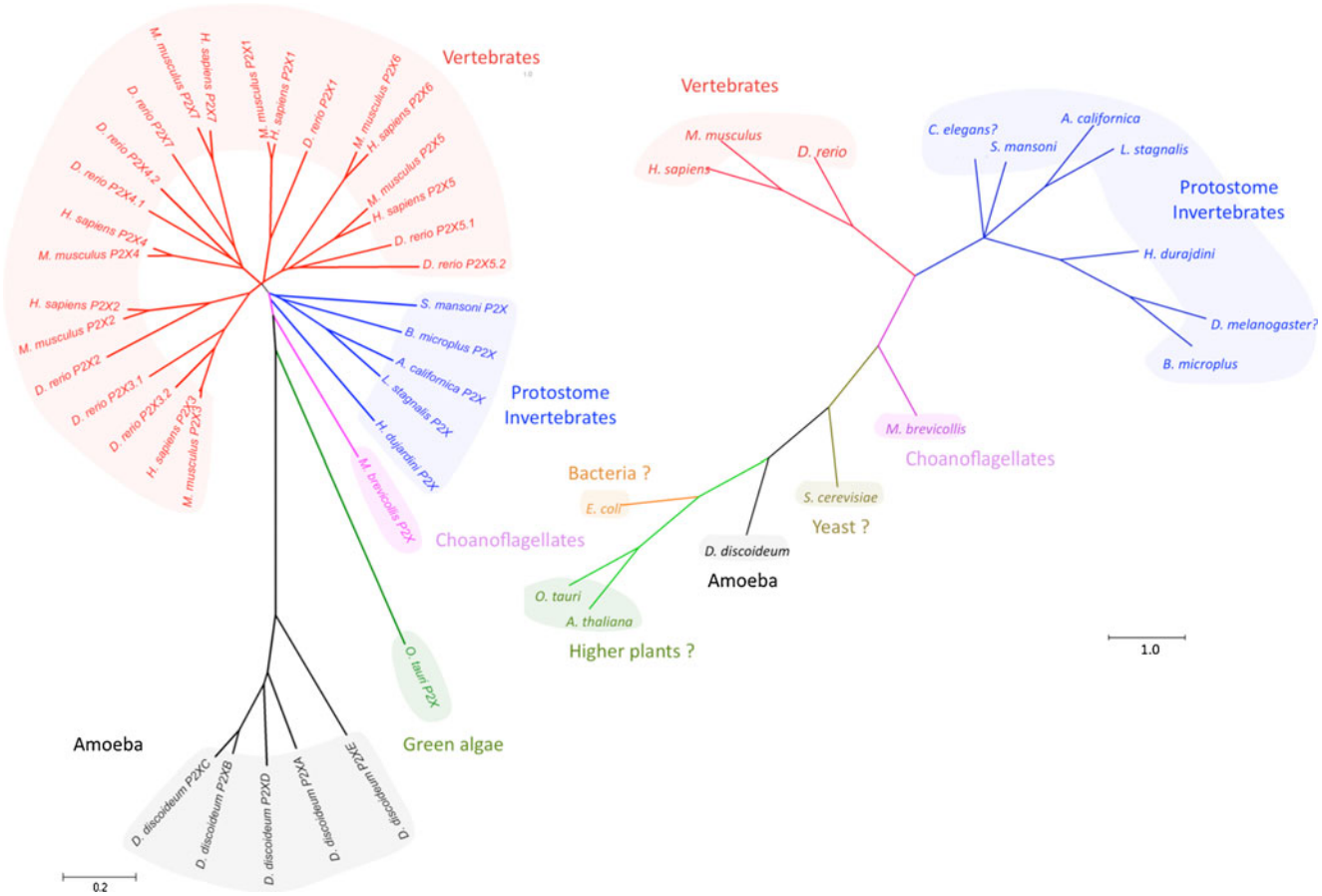

Fig. 1 Evolutionary relationship of P2X receptors and common model organisms. *Left panel*: Unrooted neighbor-joining phylogeny of identified P2X protein sequences. The tree was constructed using the MEGA program (http://www.megasoftware.net/). The scale *bar* indicates the genetic distance in percent sequence divergence. *Right panel*: A phylogenetic tree showing the relationship between organisms in which P2X receptors are present and common model organisms in which P2X receptors have not been identified (indicated by question mark). The tree was created by hand and edited using the program Dendroscope (http://ab.inf.uni-tuebingen.de/software/dendroscope/) based on the information given in King et al. [35]. The following P2X receptor sequences were used: *D. discoideum* (XP_645378.1, XP_643830.2, XP_643831.1, XP_636768.1, XP_636957.2), *M. brevicollis* (EDQ92249.1), *S. mansoni* (CAH04147.1), *H. dujardini* (ACL14328.1), *B. microplus* (ADO64254.1), *A. californica* (AAR28669.1), *D. rerio* (NP_945333.1, NP_945334.1, NP_571698.1, NP_945337.2, AAH42317.1, AAI62598.1, NP_945336.1, NP_945335.1), *M. musculus* (AAF68968.1, AAK95327.2, AAH23089.1, AAC95601.1, AAK49936.1, NP_035158.2, NP_035157.2, AAI62774.1), *H. sapiens* (NP_002549.1, NP_733782.1, NP_002550.2, NP_002551.2, NP_002552.2, NP_005437.2, NP_002553.3), *O. tauri* (CAL54489.1). We thank Steve Ennion for providing the sequence of *L. stagnalis* and Henrik Krehenwinkel for phylogenetic analysis

elegans, and the arthropods *Anopheles gambiae*, *Apis mellifera*, and *Drosophila melanogaster* [10, 24, 25, 36]. The absence of genes coding for P2X receptors in these animal groups is in contrast to the potent pharmacological actions of nucleotides in these species, suggesting that novel receptors are still to be discovered [31]. Indeed, a first arthropod P2X receptor (BmP2X from the cattle tick *Boophilus microplus*) has been described most recently [37]. The presence of P2X receptors in other members of this group could be anticipated, since functional P2X receptors have been identified in the tardigrade species *Hypsibius dujardini* [36] which, together with arthropods and nematodes, belongs to the common superphylum Ecdysozoa. These new findings support the postulate that the P2X genes have not been lost in an ancestor of the Ecdysozoa but rather disappeared independently in nematodes and maybe some arthropods before they diverged from Tardigrada [36].

Nevertheless, it is still not clear if other, not sequenced members of nematodes and arthropods are also void of P2X channels. Notably, P2X homologues have also been identified in the CNS of *Aplysia californica* and *Lymnaea stagnalis*, which are members of the superphylum Lophotrochozoa, a sister clade to the Ecdysozoa [38, 39]. According to the present state of knowledge, it seems that the development of the seven mammalian P2X genes was a relatively recent phenomenon and occurred after the branching between vertebrates and invertebrates (Fig. 1) [36]. Taken together, further identification of P2X receptors in various organisms, especially primitive ones, will be necessary to better understand the evolutionary gaps mentioned above and eventually trace the phylogenetic history of P2X receptors. Moreover, new sequences from different P2X family members provide useful information to decipher their structure–function relationships.

Molecular structure and function of P2X receptors

Primary structure and subunit topology

The seven cloned human and rat P2X subunits are between 379 (rat P2X6) and 595 (rat and human P2X7) amino acids long and share 35–54% sequence identity. All have a common topology with two transmembrane (TM) domains, a large extracellular ligand binding loop, and intracellular N and C termini. The extracellular domain connecting the two TMs constitutes the largest part of the polypeptide. An important feature is the presence of ten Cys residues conserved among all vertebrate receptors and bound in five disulfide bridges [40, 68]. In addition, all rat subunits contain three to six consensus sequences for N-linked glycosylation (see section "Synthesis and trafficking of P2X receptors"). The N termini are similar in length (20–30 amino acids) and contain a consensus site for protein kinase C (PKC) phosphorylation [41]. The C termini differ in length between 26 (P2X6) and 239 (P2X7) amino acids and exhibit only sequence relatedness for the first 25 amino acid residues, indicating that they might serve subunit specific properties [21]. They contain several motifs involved in trafficking and stabilization of the receptors in the plasma membrane and specific protein interactions (for further details, see sections "Synthesis and trafficking of P2X receptors"and "Regulation and protein interactions of P2X receptors"). The primary sequence of P2X receptors shares no significant homology with other ligand-gated ion channels, ATP-binding proteins, or other known proteins.

Quaternary structure and subunit assembly

Early electrophysiological measurements in dorsal root ganglion (DRG) neurons predicted that there are at least three ATP molecules needed to open a P2X channel [42]. Subsequent single-channel analysis of P2X2 receptors supported this idea [43]. The first biochemical evidence for a trimeric organization of P2X receptor channels came from cross-linking studies and blue-native PAGE analysis of P2X1 and P2X3 receptors heterologously expressed in oocytes of *Xenopus laevis* [44]. The trimeric architecture was confirmed by atomic force microscopy [45], electron microscopy and single particle analysis [46, 47], and finally, by crystallization of the first P2X receptor, the P2X4 subtype from zebrafish (zP2X4) [48]. Although P2X receptors share the TM topology and trimeric quaternary structure with the epithelial Na^+ channels (ENaC)/DEG (epithelial sodium channels/degenerin) superfamily of proteins they show no significant amino acid sequence relationships or similarities in the extracellular domain [48–50].

Heterologously expressed P2X receptors have been characterized extensively in terms of their biophysical and pharmacological properties (for a recent review, see [12]), and there is good evidence for homomeric P2X1, P2X2, P2X3, P2X4, and P2X7 receptors in native tissues [12]. However, P2X receptor properties also often do not match with those observed in native tissues, suggesting that P2X receptors occur naturally as both homo- and hetero-oligomers [51]. Indeed, only the P2X7 subunit appears unable to form heterotrimeric channels with other subunits [52, 53]. In contrast, the P2X6 receptor is the only subunit virtually unable to form homo-oligomers [45, 54]. In addition to heteromerization, splice variants and the presence of more than one functional P2X subtype in many cell types can contribute to the diversity of P2X receptor signalling. For P2X5 receptors, which occur in humans as a non-functional splice variant, species-specific differences in heterologous expression efficiency and functional properties such as ion permeability are observed [55–62]. Regarding heteromeric receptors, the best evidence and most comprehensive data

exist for P2X2/3 receptors. For these, a subunit stoichiometry of one P2X2 and two P2X3 subunits has been demonstrated [63, 64], and their presence and importance in native tissues has been shown in numerous studies, e. g., [8, 10, 65, 66] (see also section "P2X3"). Nevertheless, there is also good evidence that, in acutely isolated cortical astrocytes, heteromeric P2X1/5 receptors mediate the ATP-elicited currents, as these match the pharmacologic and kinetic properties of heterologously expressed P2X1/5 receptors [67]. Hardly any of the four other heteromeric subunit combinations characterized in heterologous expression systems (P2X1/2, P2X1/4, P2X2/6, P2X4/6) has been convincingly verified in native tissues. For details on their functional and pharmacological properties, see Coddou et al. and Nicke et al. [12, 51].

Crystal structure of the P2X receptor

A major breakthrough in P2X receptor research was the crystallization of the first P2X receptor by Kawate et al. [48], which provided a fundamentally new ion channel structure. Screening a variety of P2X receptor isoforms, a zP2X4.1 receptor containing three point mutations (C51F/N78C/N187R) and lacking the N and C termini (ΔP2X4-B, PDB entry 3H9V) was found optimal for crystallization and could be resolved at 3.1 Å. The homotrimeric receptor resembles a chalice, with the large extracellular domain protruding ~70 Å above the membrane plane and displays a right-handed twist if seen from the cytoplasmic side. The TM region has an hourglass shape formed by the six helices. Due to weak electron densities in the inward facing thirds of TM1 and TM2, these were less well resolved, resulting in unclear side chain orientations in these regions. The zP2X4 structure has been compared with the shape of a dolphin, in which the TM helices and the extracellular region form the flukes and the upper body, respectively (Fig. 2). Attached to the body domain, a head domain, a dorsal fin, and right and left flippers have been defined. The body domain is structurally rigid, characterized by a β-sandwich motif, with extensive contacts between the sandwich-forming β-sheets. The structure confirmed the existence of the five proposed disulfide bridges [40, 68], three of which are located in the head domain.

Ligand binding sites

ATP binding Unlike other ATP-binding proteins, P2X receptors lack consensus sequences for ATP coordination [20]. Before the P2X4 crystal structure became available, extensive studies on P2X1, P2X2, P2X3, and P2X4 receptors [15, 16, 69–74] employed mutagenesis-based approaches to localize the agonist binding site (for recent reviews, see Coddou et al. and Evans [12, 16]). These

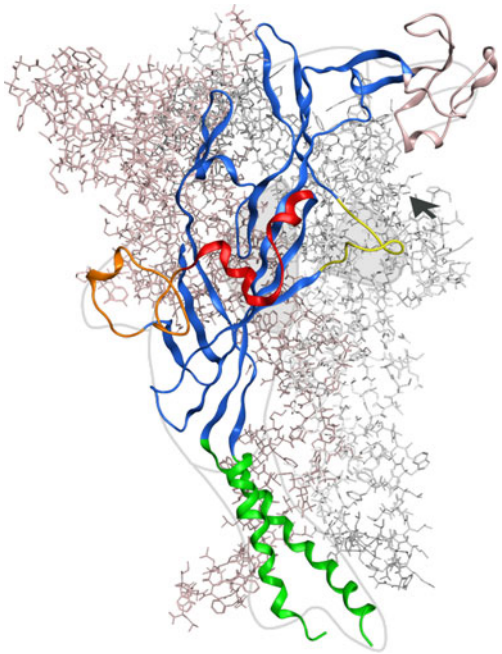

Fig. 2 Homology model of the homomeric P2X2 receptor. The homotrimeric rP2X2 receptor structure is shown from the side, i.e., parallel to the membrane plane. Two subunits are shown as *pink or gray* sticks; one subunit is *highlighted as ribbon representation* with depiction of α-helices, β-sheets, and coil regions. The *dolphin-like shape* of this single subunit [48] (body, *blue*; fluke, *green*; head, *pink*; dorsal fin, *orange*; right flipper, *red*; left flipper, *yellow*) is emphasized by an *overlay* of a *grey dolphin cartoon*. The *arrowhead* indicates one of the three possible ATP binding pockets. The rP2X2 receptor homology model based on the X-ray structure of the zP2X4.1 receptor was generated using the MOE2008.10 software. For further details, see [93]. The figure was generated and kindly provided by Achim Kless, Grünenthal GmbH, Global Drug Discovery

studies led to the conclusion that the ATP binding pocket is generally conserved within the P2X receptor family, and positively charged amino acid residues coordinate the negatively charged phosphate oxygens of ATP. In particular, Lys68, Lys70, Arg292, and Lys 309 (P2X1 numbering) were shown to be of importance for ATP potency [15, 71, 75]. In addition, conserved aromatic residues Phe185/Thr186 (P2X1) and Asn290/Phe 291 (P2X1) of a conserved NFR motif were shown to contribute to agonist action [76] and proposed to be involved in the coordination of the adenine ring [77, 78]. Studies using P2X2, P2X3, or P2X4 receptors revealed that conserved corresponding residues are responsible for ATP binding in these P2X receptors [69–74]. However, non-conserved amino acid residues contribute to the heterogeneity in pharmacological properties and play an equally important role to conserved residues in defining P2X receptor function [16, 78–82].

In a disulfide cross-linking study, it was shown that coexpressed P2X1 K68C and F291C mutants form an intersubunit cross-link in the absence but not in the presence of ATP, indicating that the ATP binding site is located at the

interface of two adjacent subunits [83]. This is in line with functional studies on binding site mutants in the P2X2/3 heteromer, which suggested that residues from different subunits interact in agonist binding [64]. All these findings are in good agreement with the position of the relevant amino acids in the crystal structure of the zP2X4 receptor. Based on this structure, it appears that the ATP binding site is formed by deep intersubunit grooves, which are 45 Å away from the TM domains and surrounded by the conserved residues implicated in ATP binding. These residues are provided by the "body domain" and the "left flipper" of one subunit and the "dorsal fin" of the neighboring subunit (Fig. 2). The Cys-rich "head" domain of the first subunit projects over this binding site [48]. For recent reviews, see Coddou et al., Evans, and Browne et al. [12, 16, 84].

However, the crystal was obtained in the absence of ATP, and therefore, its exact mode of binding is unknown. Likewise, the conformational changes governing channel opening and desensitization remain elusive. The proposed position of the ATP binding site was supported by cysteine scanning mutagenesis and homology modelling using the zP2X4 crystal structure as template for a P2X1 homology model [85]. Its localization was further corroborated by a functional study showing that the thiol-reactive ATP-analogue NCS-ATP can be covalently attached to introduced cysteine residues (N140C or L186C) located at two adjacent subunits within the proposed ATP binding cavity in the P2X2 receptor [86]. Interestingly, covalent attachment of NCS-ATP to these introduced cysteines resulted in agonist-bound states that differ in the ability to gate the channel, suggesting the existence of at least two binding modes of ATP and allowing speculations on the reaction scheme of P2X ligand binding and opening [86].

Antagonist binding Although selective competitive P2X receptor antagonists, like NF449 or A-317491 are known, studies dealing with the molecular action of these antagonists are infrequent. Instead, several studies of antagonist binding are performed with the non-selective negatively charged antagonists PPADS [16, 87–90] and suramin [75, 76, 90, 91]. As several reviews describing these data are available [12, 16, 84], we will focus here on the recent findings on the molecular action of the P2X1 receptor selective antagonist NF449 and the P2X2 receptor antagonist NF770 which were obtained by mutagenesis combined with docking studies on homology models based on the zP2X4 receptor structure.

A study on the antagonistic action of NF449 and suramin at the P2X1 receptor [92] identified a cluster of positively charged residues (^{136}KAKRK^{140}) at the base of the Cys-rich head domain that is responsible for the P2X1-selective antagonism of NF449 and absent in P2X2 receptors. However, these residues are not exclusive determinants of the selective antagonism by NF449, since reciprocal mutations in the P2X2 receptor only modestly increased the NF449 sensitivity, suggesting a more complex interaction with other non-conserved residues [92]. Nevertheless, this study highlights the importance of the Cys-rich region for normal channel function and ligand binding at human P2X1 receptors [92], as already proposed from studies on the *D. discoideum* P2X receptor that lacks this region [27, 30].

Structure–activity relationship analysis of suramin derivatives and in silico docking studies using a P2X2 receptor homology model revealed that residues important for potent antagonism such as Arg290 or Gly72 are also important in ATP action at P2X2 receptors [93] (Fig. 3). Furthermore, this study highlights the role of strong ionic interactions, for example, between the acidic groups of suramin derivatives and positively charged amino acid residues (Lys71, Lys246, Lys279, and Arg290) in the ATP-binding site, as suggested

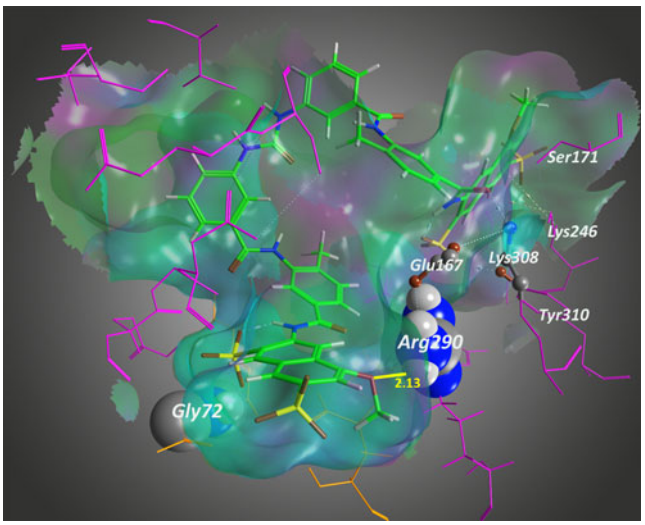

Fig. 3 Proposed binding of the antagonist NF770 to the P2X2 receptor. The suramin derivative NF770 (7,7-(carbonylbis(imino-3,1-phenylenecarbonylimino-3,1-(4-methylphenylene)carbonylimino))bis(1-methoxy-naphthalene-3,6-disulfonic acid) tetrasodium salt)) is shown within the rP2X2 receptor binding pocket. Selected residues of the rP2X2 receptor binding site are shown as pink sticks, side chains of Gly72, Arg290, Glu167 and Lys308 are shown as ball and stick or space filling. NF770 is directed by a Gly72-sulfonate group (yellow/brown sticks) interaction to orient spatially in a way that the methoxy group oxygen (brown stick) comes into close apposition to Arg290. This way, a hydrogen bond can form that is a key determinant of the interaction of NF770 with the rP2X2 receptor. The close distance of 2.13 Å between the methoxy group and Arg290 (yellow bar) appears to account for the strong binding. The rP2X2 receptor homology model based on the X-ray structure of zP2X4.1 was generated by MOE2008.10. The receptor model was kept rigid during the docking computation, whereas the NF770 was allowed to remain flexible. For further details, see Wolf et al. [93]. The figure was generated and kindly provided by Achim Kless, Grünenthal GmbH, Global Drug Discovery

for the interaction with the phosphate oxygens of ATP [16, 64, 72, 84].

Thus, several additional and subtype-specific amino acid residues have been identified that play an important role for ligand selectivity and contribute to a comprehensive mapping of the orthosteric ligand binding site. This knowledge certainly will facilitate future ligand optimization by means of homology-model-based docking computation.

P2X receptors are also modulated by a variety of compounds including divalent cations, protons, lipids, steroids, ethanol, and ivermectin. As these allosteric modulatory sites of P2X receptors have been excellently reviewed recently [11, 12], they are not further considered here.

Ion permeation pathway and channel opening

Several cysteine scanning mutagenesis studies suggested that TM2 lines the central ion-conducting pore and includes the channel gate at Thr336 (P2X2 numbering) and that TM1 is positioned peripheral to TM2 [94–98]. This spatial arrangement of the two TM domains was confirmed by the zP2X4 structure [48] and is quite similar to that found in the ASIC1 channel [49]. The TM helices within a subunit are oriented antiparallel to one another and are angled ~45° from the membrane plane with the inner TM2 helices defining most of the ion conducting pathway. They are surrounded by the peripheral TM1 helices, which make most of the contacts with the lipid bilayer [48, 49]. Along the threefold axis of symmetry of the P2X4 crystal structure, Kawate et al. identified four cavities, three in the ectodomain (upper-, central- and extracellular vestibules) and one located intracellularly (intracellular vestibule).

Based on the X-ray structure, the ion channel gate is presumably formed by residues Leu340 and Asn341 (corresponding to Ile332 and Asn333 in P2X2) on the extracellular side and by Leu346 and Ala347 (corresponding to Leu338 and Thr339 in P2X2) on the intracellular side of TM2. The closest association of the TM2 helices is Ala344, representing the center of the gate [48]. However, the weak side-chain density of the X-ray structure of the cytoplasmic terminus of TM2 complicates the side chain localization within the zP2X4 channel model. In subsequent studies, the pore and the gate of the P2X2 receptor were independently mapped, and potential opening movements were proposed [99–101]. Systematic mutagenesis of charged TM2 residues in combination with single channel analysis revealed that the side chains of Asn333, Thr336, and Ser340 (P2X2 numbering) are exposed to the permeation pathway within the open channel. It was proposed that the gate is formed by amino acid residues Asn333 to Thr339 and that the TM2 helices undergo a rotation and separation during channel opening [99]. Likewise, Keceli and Kubo found that the TM2 residue Thr339 orients towards the center of the permeation pathway. In addition, they provided evidence that residues Tyr43, Phe44, and Tyr47 in TM1 are oriented toward the pore-forming TM2 and interact with Ile328, thus stabilizing the closed state of the channel. This interaction is released in a voltage-dependent manner during gating of the channel [100]. By substituted cysteine accessibility analysis with the rapidly reversible Cd^{2+}, Kracun et al. identified residues Thr339, Val343, Asp349, and Leu353 of TM2 lining deeper parts of the pore in the open state [101]. The different position of the P2X2 channel gate to that proposed by Li et al. [102] may be due to the use of the Cd^{2+} as a thiol modifying agent which allows to probe the rates of modifications of introduced cysteines and which is also smaller than the previously used thiol reactive methanethiosulfonate (MTS) compounds.

The question of how ions access the TM region of the channel has also been addressed. The zP2X4 structure suggests two pathways by which extracellular ions could enter the extracellular vestibule that allows access to the TM ion channel region [48]. First, three lateral fenestrations above the TM domains might allow cations to access this region. Second, ions might pass along the threefold axis of symmetry through the conspicuous upper and central vestibule to enter the extracellular vestibule and the TM channel region [48]. By using homology models of the human P2X1, rat P2X2, or human P2X4 receptor, residues that line the central and the lateral pathway were substituted by cysteine residues, and their reactivity to thiol-reactive MTS reagents during current recordings was investigated [85, 103, 104]. These studies concluded that ions enter the channels via the lateral pathway. Chambers along the central pathway were proposed to have a regulatory function [103], and the equivalent spacing of the three lateral portals was suggested to split the ion flow and thus minimize ion diffusion [104].

As the zP2X4 structure was obtained in the absence of ATP, it most likely represents the closed resting state of the channel [48]. Without a structure of the open state, the conformational changes involved in channel opening are difficult to predict. The mutagenesis and modelling studies suggest a dilation of the gate by a rotation [99] of the TM2 helices or by intrahelical movements resulting in less bending and a steeper position within the lipid bilayer [101]. Isoform specific amino acid differences in the region of the gate may account for variations in the occlusion point [101]. The proposed rotation and sliding of the TM2 helices against each other that leads to their separation is consistent with the functional finding that P2X2 receptor channel opening is prevented when Ile 328 (at the outer end of TM2) is tethered by an engineered disulfide to Val48 in TM1 [105].

A more recent cysteine scanning mutagenesis study using Ag^{+}, Cd^{2+}, and MTS reagents of different size suggests that the internal region of TM2 must move a large distance towards the central axis of the pore during opening, indicating that the pore-forming TM2 helices straighten from the steeply angled orientation toward the normal of the bilayer to open the channel [106]. According to the current view, ligand binding may be transduced into channel opening (resulting from TM movements) through the two structurally rigid β-sheets of the body domain ('connecting rods') of each subunit rising from the TM1 and TM2 [84]. The polar residues Glu63 and Arg274 localized within β-sheets β1 and β12, respectively, were identified to form an intersubunit salt-bridge that is likely to stabilize the closed state of the P2X2 channel [107]. Disulfide-bridge formation between the substituted cysteines E63C and R274C was reduced in the presence of ATP, suggesting that ATP binding might trigger relative movements of adjacent subunits at the level of Glu63 and Arg274, allowing the TM helices to open the channel [107]. Since these residues are not conserved in other subunits, subtype-specific mechanisms might govern channel opening.

Channel desensitization

Based on whole cell recordings of heterologously expressed proteins, P2X receptors can be divided in rapidly desensitizing (P2X1 and P2X3) and slowly desensitizing (P2X2, P2X4, P2X5, and P2X7) receptors [10, 19]. The extent of desensitization of specific P2X receptors is of great physiological relevance as it determines the time course of P2X receptor signal transduction and regulates the responsiveness in the sustained presence of ATP. Desensitization represents the transition into a, most likely, agonist-bound closed form. It is followed by the recovery process that requires agonist unbinding and a conformational change from the agonist-free desensitized to the resting state [20]. The fact that the presence of high-affinity binding sites for ATP, α,β-meATP, and TNP-ATP appears to be associated with fast desensitization, and slow recovery from desensitization is in agreement with studies showing that the P2X1 receptor needs to open before it can go into the desensitized state, and that unbinding of the agonist from the ligand-bound desensitized state is the rate-limiting step for recovery from desensitization [20, 108, 109]. Consistent with these data, unbinding of [^{32}P]-ATP from P2X3 receptors mirrored the rate of recovery from desensitization [20, 110].

The molecular mechanisms controlling desensitization are not yet understood and apparently involve multiple receptor segments (intracellular, TM, and extracellular domains) and possibly also interactions with other proteins or intracellular messengers [20, 37, 111]. Chimeras composed of parts of desensitizing and non-desensitizing P2X receptors indicate the involvement of the N-terminal- and TM1 domains [109, 112, 113] and a short N-terminal part of the ectodomain [114]. A recent study showed that substitution of solely intracellular N- or C-terminal parts of desensitizing P2X receptors by corresponding parts of non-desensitizing receptors is sufficient to obtain at least partial desensitizing or non-desensitizing receptors [37]. As shown by analysis of P2X2 splice variants and subsequent mutagenesis data, intracellular C-terminal parts next to TM2 contribute to the rate of desensitization [115, 116]. In the hP2X4 receptor, two residues in the proximal end of the C terminus, Lys373 and Tyr374, were found to accelerate desensitization [117]. Furthermore, it was shown that the positive charges of Lys365 and Lys369 within the C-terminal domain of P2X2 receptors are responsible for the interaction with membrane phosphoinositides and regulate desensitization of P2X2 receptors [118]. The extent of C-terminal controlled desensitization was suggested to influence the efficacy of the agonists [119]. Interestingly, the study of Bavan et al. showed that the penultimate C-terminal charge of the arginine residue of the BmP2X receptor is responsible for the slow desensitization kinetics but not the current run-down during repetitive ATP applications, indicating that run-down and desensitization are governed by distinct mechanisms [37].

In addition to the above described contribution of the intracellular C-terminal part, disruption of the putative conserved PKC phosphorylation site (see also section on "Phosphorylation of P2X receptors") in the N terminus (^{18}TXK^{20}) by T18A or K20T mutations led to fast desensitization of P2X2 receptors [41, 121]. In contrast, the K20C mutation did not affect the P2X2 desensitization kinetics, indicating that this is a structural rather than charge effect [105]. The corresponding mutations in the P2X1 or P2X3 receptors result in rudimentary functional or non-functional receptors, respectively [41, 121, 122]. In support of the involvement of cytosolic components, inactivation properties of P2X2 receptors have been shown to differ greatly between measurements in excised patches and in whole cell mode [123].

In conclusion, these data suggest that desensitization is determined mostly, but not exclusively by the N- and C-terminal P2X receptor segments. Since the crystal structure of the zP2X4 is lacking these intracellular termini, we cannot infer their possible structural involvement in these processes.

Pharmacological characteristics of P2X receptors

Agonists The primary agonist of all homomeric and heteromeric P2X receptors is ATP. Regardless of the species-

dependent differences, the amount of ATP necessary to elicit the half maximal response (EC_{50}) varies between submicromolar concentrations for P2X1, P2X3, and P2X5, and low micromolar concentrations for P2X2, P2X4, and P2X6 receptors [7, 124]. The P2X7 receptor requires exceptionally high agonist concentrations with an EC_{50} value for ATP higher than 100 μM [79, 125]. Remarkably, at this receptor, ATP is only a partial agonist. Extracellular Mg^{2+} ions diminish the agonist response at P2X7 receptors, an observation that has been interpreted as ATP^{4-} being the active agonist. Further experiments are needed to define whether this applies to other members of the family and to clarify to which extent divalent cations act directly at the receptor as negative modulators. In the absence of extracellular Mg^{2+} and other divalent cations, hP2X7 receptors were shown to have high and low affinity sites for free ATP^{4-} with apparent dissociation constants of 4 and 220 μM, respectively [126]. In addition to ATP, most P2X receptors are activated by diadenosine polyphosphates or related dinucleotides and some nucleoside triphosphates such as CTP and GTP [7, 12]. By contrast, the breakdown products of ATP, ADP, AMP, adenosine, or UTP and UDP activate P2X receptors either weakly or not, further corroborating the importance of the interaction with the three phosphate groups [12].

Early pharmacological studies have used the non-hydrolyzable ATP analogue α,β-meATP to differentiate between fast and slowly or non-desensitizing P2X receptors in smooth muscle and sensory neurons [4]. After cloning and heterologous expression of the seven subtypes, the P2X1 and P2X3 receptors were found to be sensitive to α,β-meATP ($EC_{50} \leq 1$ μM), [17, 18, 66]. Heteromeric assemblies, which contain P2X1 or P2X3 subunits and heteromeric P2X4/6 receptors also show α,β-meATP-sensitivity ($EC_{50} \leq 10$ μM) [17, 66]. At P2X7 receptors, 2'-3'-*O*-(4-benzoylbenzoyl)-adenosine 5'-triphosphate (BzATP) is a more potent agonist than ATP (EC_{50} ~10 μM) [79, 125]. In addition, BzATP activates particularly P2X1, P2X2, and P2X3 receptors with high potency [17, 127]. Furthermore commonly used ATP derivatives are ATPγS, which activates all P2X receptors with exception of the P2X7 receptor, and 2-MeS-ATP, which activates most P2 receptors but not adenosine (P1) receptors [7, 12].

A further peculiarity exists for the P2X7 receptor: For the mouse P2X7 receptor, it was shown that it can be activated by low concentrations of extracellular NAD. This process involves ADP-ribosylation of the P2X7 Arg125 by ecto-ADP-ribosyltransferase and results in constitutive channel activation [128, 129].

Antagonists Research in the P2X field has for a long time been hampered by the unavailability of truly selective antagonists [66]. P2X receptors have attracted widespread interest as therapeutic targets, e.g., for chronic inflammatory diseases and pain. In particular, P2X3 and P2X7 receptor antagonists have been developed and demonstrated antinociceptive or antiinflammatory effects in animal models of these diseases [17, 130]. In basic research, a variety of more or less selective compounds and their derivatives have been used, including dyes (e.g., phenol red, reactive red, reactive blue II, trypan blue, Evans blue, and brilliant blue), the antitrypanocidal drug suramin, the photoreactive agent ANAPP3, the cross-linking reagent DIDS, and the pyridoxal-5-phosphate analogue PPADS [7, 12, 17, 66, 127, 131]. In addition, trinitrophenyl-substituted nucleotides, especially TNP-ATP, are potent antagonists at P2X1, P2X3, and heteromeric P2X2/3 receptors [132]. A considerable additional problem are species-dependent differences in the action of both agonists and antagonists at P2X receptors (reviewed in Gever et al. and Donnelly-Roberts et al. [66, 79]). For example, the isoquinoline compounds KN-62 and KN-04 antagonize mouse [133] and human [134] P2X7 receptors but are inactive at the rat isoform [135].

Since the available P2X receptor antagonists have been extensively reviewed [7, 12, 17, 66, 127], we will focus here on more recently described and novel compounds that give new insights in ligand binding of P2X receptors and/or have proven to be useful tools in animal models of diseases or even progressed into clinical studies in man.

The potential of suramin as a lead structure for development of selective and/or potent P2X receptor antagonists has been shown in several studies [93, 136–138]. Its derivative NF449 is currently the most potent and highly selective P2X1 receptor antagonist ($EC_{50} < 1$ nM) [137, 139]. Together with suramin related compounds, such as NF770, which is a potent but less selective P2X2 receptor antagonist, it helped to understand competitive action of suramin and the basis of their subtype specificity [92, 93] (see section "Ligand binding sites"). Evaluation and optimization of anthraquinone derivatives related to reactive blue 2 yielded the first potent and selective P2X2 receptor antagonist PSB-1011 [140].

The first selective and highly potent dual inhibitor of P2X3 and P2X2/3 receptors, A-317491, showed strong antinociceptive effects in vivo in rodent models of chronic inflammatory and neuropathic pain [141], thus providing an important proof of concept. It was not pursued as a drug candidate due to its poor distribution into the central nervous system [66, 142]. Selective dual inhibition of P2X3 and P2X2/3 receptors has also been achieved by the nanomolar potent and orally bioavailable diaminopyrimidine derivatives RO-3, RO-4 (recently redesignated AF-353), and RO-51 developed at Roche [17, 142–144]. In particular, AF-353 (previously known as RO-4) was shown to bear a favorable pharmacokinetic profile and excellent antagonist

potency and selectivity for P2X3 and P2X2/3 receptors [142]. Furthermore, it was able to attenuate bone cancer pain behavior in rats [145]. RO-85, an orally bioavailable drug-like P2X3 receptor antagonist, is selective for the P2X3 receptor over the P2X2/3 and other P2X receptor subtypes [146]. Three additional P2X3 and P2X2/3 selective diaminopyrimidine derivatives (compounds A, B, and C) with nanomolar potency were recently published by GlaxoSmithKline [147]. Compound B was shown to exhibit significant effects in the CFA model of chronic inflammatory pain [147]. AF-219 is another P2X3 receptor antagonist and the lead compound of Afferent Pharmaceuticals. It has successfully completed two phase-I clinical studies and progressed into phase II clinical testing (personal communication and see release at www.afferentpharma.com, accessed Aug. 22nd, 2011).

5-BDBD (5-(3-Bromophenyl)-1,3-dihydro-2 H-benzofuro-[3,2-e]-1,4-diazepin-2-one) was developed by Bayer Healthcare as a P2X4 receptor antagonist for treatment of arteriosclerosis and restenosis [148]. However, its impact is still unclear.

The strong evidence for P2X7 receptor involvement in pain and inflammation boosted interest in the pharmacology of this receptor, and selective P2X7 receptor antagonists have been frequently discovered in the past few years [17, 149]. Selectivity and in vitro activity at heterologously expressed human (and partly rat) P2X7 receptors has been shown for A-804598 and further compounds from Abbott [150–152], AZ11645373 [153]; several compounds from GlaxoSmithKline [154–156]; and compounds from Pfizer [157]. The newly developed nanomolar potent P2X7 receptor-selective antagonists GSK314181A (and further GSK compounds), A-740003, A-438079, and A-839977 have in addition been shown to have in vivo analgesic effects in rodent models of inflammatory pain [158–164]. The AstraZeneca compound AZD9056 was the first P2X7 receptor antagonist that entered clinical trials and was well tolerated in phase I studies [165]. Unexpectedly, it failed to show significant efficacy in the treatment of rheumatoid arthritis in a phase IIb clinical study, suggesting that the P2X7 receptor is not a therapeutically useful target in rheumatoid arthritis [166]. It is currently in clinical testing for the treatment of osteoarthritis, chronic obstructive pulmonary disease, and inflammatory bowel disease [165]. Likewise, CE-224535, a P2X7 receptor antagonist from Pfizer was reported to have no effect in phase II studies for the treatment of rheumatoid arthritis and is now studied for treatment of other conditions, such as pain and Alzheimer´s disease [167]. Furthermore, the P2X7 receptor antagonist GSK1482160 from GlaxoSmithKline has recently finished phase I clinical testing (ClinicalTrials.gov identifier: NCT00849134). The P2X7 receptor antagonist EVT-401 from Evotec is under development in the companion animal market for the treatment of inflammatory conditions (http://www.evotec.com, accessed 22 Aug 2011).

Also, currently approved drugs have been identified that act on P2X receptors. For instance, aminoglycoside antibiotics have been shown to block P2X2 receptor channels [168]. Several antidepressants, in particular, paroxetine, were shown to inhibit ATP-evoked rat and human P2X4 receptor-mediated responses [169]. Furthermore, intrathecally injected paroxetine produced significant antiallodynic effects in a rat model of neuropathic pain. In contrast, the tricyclic antidepressant amitriptyline exhibited only weak or no P2X4 inhibitory activity [169, 170]. However, a recent study indicates that the antidepressants act indirectly by interfering with P2X4 receptor trafficking [171] rather than directly blocking the receptors. Lately, the approved H_1 antihistaminic clemastine was shown to act as a positive modulator of the P2X7 receptor [172].

Synthesis and trafficking of P2X receptors

Cellular activity depends on the interaction between membrane receptors and intracellular signalling pathways and is critically regulated by the spatial and temporal distribution of the membrane receptors. For example, the control of receptor function by protein biogenesis, trafficking, and redistribution represents a central process in synaptic plasticity.

P2X receptor synthesis The appearance of functional ion channels in the plasma membrane follows a complex series of events, including specific oligomerization of protein subunits as well as post-translational folding and modification. Like other membrane proteins, P2X receptors are synthesized and core glycosylated in the rough ER and, upon complex glycosylation in the Golgi apparatus, are trafficked to the plasma membrane in a constitutive or regulated pathway of vesicle exocytosis [173]. In both cases, specific vesicle docking to target membranes is achieved by distinct members of the SNARE (soluble *N*-ethylmaleimide-sensitive factor (NSF) attachment protein receptors) protein family. This process is counterbalanced by a clathrin-mediated endocytosis of receptors to the endosome where they are further sorted into vesicles, depending on their final destination (degradation or recycling). P2X polypeptides assemble quickly into trimers since monomeric subunits or intermediate dimeric assembly states were never observed with metabolically labelled P2X1 protein expressed in oocytes [44]. Besides formation of disulfide bonds [40, 68], post-translational modification by *N*-linked glycosylation is important for delivery of functional channels to the plasma membrane. The seven rat P2X subunits contain three to six consensus sequences for *N*-linked glycosylation in

their extracellular domain. Systematic mutagenesis studies at the P2X1 [174], P2X2 [175], P2X3 [176], and P2X7 receptors [177] suggest that a minimum number of two *N*-glycans is essential for efficient plasma membrane targeting. The glycosylation site equivalent to Asn 170 in P2X3 is the best conserved among the P2X subtypes and appears to play also a critical role for receptor function [71, 77, 176, 177], which might be explained by its close location to the proposed ATP binding site.

P2X receptor trafficking A YXXXK motif in the C terminus is common to all P2X subunits. It is located downstream of the second TM domain, except for the P2X7 subunit where a cysteine-rich domain of 18 amino acids lies between the second TM domain and this motif [178]. The YXXXK motif regulates the surface expression of P2X receptors, and accordingly, its mutation significantly limits the trafficking of homomeric P2X receptors. Trafficking of mutant subunits is rescued by heteromerization with wild-type subunits. The YXXXK motif is proposed to stabilize membrane inserted P2X receptors, rather than affect ER exit [178]. Unstable mutants are rapidly internalized and directed to the lysosomal pathway for destruction. The expression and plasma membrane transport of P2X receptors is highly regulated by cell activity and maturation. However, the trafficking mechanisms for individual P2X receptors are poorly understood, and hardly any interacting proteins controlling these processes have been identified so far.

P2X1 receptors P2X1 receptors show fast desensitization and long recovery periods until full reactivation is possible. The desensitization and recovery appears to be determined by two mechanisms: first, intrinsic receptor properties leading to fast conformational changes upon ATP binding and slow unbinding of ATP allowing a delayed return to the resting state. In addition, agonist-induced internalization and redistribution of receptors between plasma membrane and intracellular compartments has been described for heterologously expressed GFP-tagged P2X1 receptors [179, 180] and native P2X1 receptors in smooth muscle [181]. A recent fluorescence recovery after photo-bleaching (FRAP) study suggests that both a constitutive brefeldin A-sensitive and an agonist-induced dynasore-sensitive trafficking pathway contribute to the recycling of P2X1 receptors [182].

P2X2 receptors Agonist-induced receptor clustering associated with increased current responses and dendritic morphology changes, but no net internalization or externalization has been demonstrated for GFP-tagged P2X2 receptor expressed in embryonic hippocampal neurons [183]. Interestingly, this effect was not seen if the PKC consensus site of P2X2 was disrupted by a T18A mutation. Supporting evidence for agonist-induced clustering of P2X2 receptors is provided in a recent study on spinal cord neurons [184]. Here, a proportion of P2X2 receptors appear to directly interact with and stabilize $GABA_A$ receptors, which in turn help their trafficking to extrasynaptic localizations in the plasma membrane.

P2X3 receptors Expression of P2X2 and the fast desensitizing P2X3 receptors is upregulated in DRG neurons from rats with peripheral inflammation [185], resulting in increased ATP-responses and sensitization of the neurons to ATP. An increase in plasma membrane trafficking of P2X3 receptors was found to be responsible for this effect [186]. Upon electrical stimulation to mimic the injurious state, CaMKII is likewise upregulated and has been shown to promote trafficking of P2X3 receptor in the plasma membrane [187]. A recent study on transfected HEK cells and primary cultures of DRG neurons found that the P2X3 receptor undergoes rapid constitutive endocytosis and is predominantly localized in intracellular compartments labelled by the late endosome/lysosome marker lamp1. Upon agonist application, the level of functional receptors in the plasma membrane is rapidly upregulated [188]. In trigeminal neurons, the trafficking to the plasma membrane and activity of P2X3 receptors was shown to be regulated by calcitonin-gene-related peptide and nerve growth factor via PKA and PKC, respectively [189].

P2X4 receptors Trafficking processes have been best characterized for the P2X4 receptor. Upregulation of P2X4 receptors in spinal microglia, as a result of peripheral nerve injury, has been shown to be an important determinant of neuropathic pain [190, 191]. If heterologously expressed in neurons, this receptor undergoes rapid constitutive- and agonist-induced internalization into early endosomes and lysosomes from where they are subsequently reinserted into the plasma membrane [192]. Internalization of the P2X4 receptor is clathrin- and dynamin-dependent and determined by a non-canonical endocytic motif (YXXGL) downstream of the conserved YXXXK motif and a canonical YXXV motif. This YXXGL motif was shown to interact with adapter protein 2 (AP2), and mutation of this endocytic motif or the Tyr binding pocket in the μ2 subunit of the AP2 clathrin adaptor protein complex resulted in accumulation of functional P2X4 receptors in the membrane [193]. An intact endocytosis motif also appears to be required for the enhancement of P2X4 receptor currents by protein kinase A, suggesting that the endocytotic pathway is regulated by phosphorylation [194]. Similarly, impairment of P2X4-endocytosis by the positive modulator ivermectin has been suggested as one mechanism underlying enhancement of P2X4 receptor responses [195]. However, this mechanism is controversially discussed [196].

In addition to the non-canonical endocytic motif, a N-terminal dileucine-type motif appears to contribute to

lysosomal targeting of P2X4 receptors [197]. Native P2X4 receptors in microglia, macrophages, and endothelial cells are localized primarily in lysosomes where their *N*-glycans protect them from degradation. Stimulation of lysosome exocytosis by ionomycin-induced rise in intracellular Ca^{2+} concentration or methylamine-induced rise in lysosomal pH enhanced P2X4 expression and responses at the plasma membrane. This suggests that the lysosomal pool of P2X4 receptors can be mobilized to upregulate P2X4 responsiveness of these cells [197]. Likewise, lysosome-localized P2X4 receptors were inserted into the plasma membrane in lipopolysaccaride (LPS)-activated C8-B4 microglia, a cell line of cerebellar origin. Interestingly, this lysosomal secretion was reduced by clinically relevant concentrations of antidepressants, providing a possible explanation for their effectiveness in neuropathic pain models [171]. In contrast, activation of human alveolar or rodent macrophages by IFN-γ plus LPS or TNF-α resulted in decreased P2X4 responses while induction of lysosomal secretion (pH increase of intracellular vesicles by chloroquin) or phagocytosis (ingestion of zymosan particles) increased the appearance of functional P2X4 receptors in the plasma membrane [198]. Also in bone-marrow-derived macrophages, large amounts of P2X4 receptors were predominantly localized intracellularly, and treatment with the endocytosis inhibitor dynasore did not enhance surface expression, suggesting a much less dynamic trafficking than in microglia [52].

The predominant intracellular localization of some P2X receptors, in particular, the P2X4 subtype, makes it tempting to speculate about intracellular functions of P2X receptors in certain cell types. In simple eukaryotes, for example, P2X receptors localized in intracellular membranes were shown to be involved in osmoregulation [27] even though playing only a minor role [30]. It remains to be determined whether mammalian P2X receptors can also fulfil specific intracellular roles [52, 199].

P2X5 and P2X6 receptors Recombinant rodent and zebrafish P2X5 receptors show low current responses despite good expression on the protein level. In contrast, chick and bullfrog P2X5 receptors show good functional expression [57, 200–202]. The reason for this is not clear. The human P2X5 receptor is non-functional due to the deletion of exon 10 but gives good responses if the missing sequence is included, as in some individuals that carry a polymorphism in which the critical intronic splice site is preserved [55].

Heterologous expression of functional rat P2X6 receptors has been reported in only a low percentage of HEK cells [200, 203] and not at all or only at the detection limit in *Xenopus* oocytes [204, 205]. The mouse and human isoforms of P2X6 also do not express well [206]. Biochemical analysis revealed that the majority of heterologously expressed P2X6 subunits failed to form trimers and were retained in the ER [45, 53, 54]. Homotrimeric assembly and trafficking to the plasma membrane could be enhanced by mutation or deletion of an uncharged region in the P2X6 N terminus [207]. Differential glycosylation of P2X6 subunits in HEK cells has also been proposed to account for inconsistencies in the functional expression of this subtype [203].

P2X7 receptors Together with P2X4 receptors, P2X7 receptors are predominantly expressed in endothelial and epithelial cells, and cells of the immune system where the level of functional P2X7 receptors in the plasma membrane is also tightly regulated. In monocytes and lymphocytes, for example, they are localized intracellularly and appear to be recruited to the plasma membrane during differentiation of monocytes into macrophages [208–210]. In macrophages and microglia, they appear predominantly at the cell surface [52]. Two basic amino acid residues (Arg578, Lys579 [211]) within an LPS-binding motif (residues 573–590 [212]) have been shown to be critical for efficient surface localization, presumably by stabilizing the receptor in the plasma membrane. Likewise, truncations or mutations (residues C572G, R574G, F581G) in an overlapping region between residues 551 and 581 [213] in the P2X7 C terminus abolished surface expression, and it was suggested that they contain an ER retention/retrieval motif. In agreement with the importance of this region, the I568N polymorphism in this domain [214] causes deficits in surface expression. In rat submandibular gland, a fraction of P2X7 receptors was found in lipid rafts [215]. Also in alveolar epithelial cells, P2X7 receptors were found to co-localize with caveolin-1, and deletion or suppression of this protein resulted in a strong reduction of P2X7 immunoreactivity [216]. A study on transfected HEK cells and macrophages [217] found that palmitoylation is involved in the correct targeting of P2X7 receptors into lipid rafts and correlates with its plasma membrane expression. While more distal groups of cysteine residues (Cys477, Cys479, Cys482/Cys498, Cys499, Cys506/Cys572, Cys573) are essential, juxtamembrane cysteine residues (Cys371, Cys373, Cys374) also appear to be involved in palmitoylation. Interestingly, the essential residues include Cys572 and Cys573, which are located in the above-mentioned regions [211–213]. Palmitoylation-deficient mutants were retained in the ER, and it was concluded that palmitoylation is required for P2X7 receptor maturation. Agonist-induced down regulation of P2X7 receptors was shown in RAW macrophage-like cells [218].

Regulation and protein interactions of P2X receptors

Apart from yet unidentified subunit combinations or splice variants, transient or permanent physical interactions with

associated proteins can account for diversity in P2X receptor properties. Transiently associated proteins include proteins involved in protein synthesis and maturation, such as enzymes involved in glycosylation and chaperones, as well as proteins that participate in the trafficking and stabilization of the receptor at specific membranes, such as adaptor, anchoring, and scaffolding proteins (see section "Synthesis and trafficking of P2X receptors"). In addition, the functions of a mature receptor can be modified by intracellular signalling molecules, by enzymes, such as kinases, and by cross-talk with other receptors or membrane proteins.

Phosphorylation of P2X receptors

In addition to several *N*-linked glycosylation sites, P2X receptors contain a conserved putative PKC phosphorylation site (Thr-X-Arg/Lys). Disruption of this N-terminal PKC site in the P2X1 receptors alters the time course of desensitization, suggesting that desensitization is regulated by phosphorylation [122]. Indeed, basal P2X1 receptor phosphorylation was demonstrated by [^{32}P]orthophosphate labelling in HEK293 cells expressing this receptor. However, the effect on desensitization was found to be indirect and rather involves phosphorylation of an accessory protein [219]. Potentiation of P2X1 receptor-mediated responses by the PKC activator phorbol 12-myristate 13-acetate (PMA) or stimulation of coexpressed mGluR1α receptors was abolished after disruption of the N-terminal phosphorylation motif or by mutations within the C-terminal region between His355-Tyr370, indicating regulatory roles of both the N-terminal and C-terminal domains [220].

In *Xenopus* oocyte-expressed P2X2 receptors, the phosphorylation motif likewise controls the desensitization kinetics and phosphorylation of Thr18 was demonstrated with a phosphothreonine–proline-specific antibody [41]. In contrast, direct phosphorylation of P2X2 receptors expressed in *Xenopus* oocytes or HEK293 cells could neither be detected by immuno-blotting nor by in vitro and in vivo phosphorylation assays in another study [121]. Nevertheless, the role of the ^{18}ThrProLys^{20} motif for desensitization kinetics was confirmed. Three studies on P2X3 receptors have shown that the PKC activator PMA increases P2X3 receptor-mediated current amplitudes, but, in contrast to the findings with P2X1 receptors, no phosphorylation was detected [121, 221, 222]. Interestingly, a PKC consensus site in the P2X3 ectodomain was shown to be regulated by ecto-PKC, resulting in changes of the $\alpha\beta$-meATP-induced current responses [223, 224].

A cAMP-dependent protein kinase A-mediated regulation of the P2X4 receptor function via C-terminal motifs was also shown [194]. For P2X7 receptors, it was reported that receptor activation results in dephosphorylation of Tyr343 within the second TM domain, suggesting its basal phosphorylation [225].

Clustering of P2X receptors and interactions with ion channels

Several studies provide functional evidence for interactions between homotrimeric P2X2 receptors. Properties such as mean open times, open channel noise [226], potentiation by Zn^{2+}, and pH, as well as the EC_{50} value for ATP appear to depend on receptor density [227]. Also, the ability to form large pores and inward rectification properties were shown to depend on the P2X2 expression level [228] and to be influenced by mutation of amino acid residue Ile328 in the second TM domain. A physical interaction between P2X2 receptors can be inferred from biochemical experiments that show an increased tendency of this receptor to form higher-order complexes [54]. Functional and physical interactions between P2X7 and P2X4 [229–231] and recently also P2X2 and P2X4 receptors [232] have been observed, although heterotrimerization between these subunits was excluded [52, 232, 233]. Together, these data suggest that some P2X trimers can interact with each other either directly or via clustering molecules. Whether these interactions have physiological relevance or represent overexpression artifacts remains to be determined. Interestingly, P2X4 and P2X7 could be coprecipitated with the extracellular matrix component biglycan and soluble biglycan-induced clustering of P2X4 and P2X7 receptors with Toll-like receptor (TLR) 2/4 was found to underlie the activation of the inflammasome by this component [234].

A wealth of functional and biochemical evidence exists for interactions between P2X receptors and various members of the Cys-loop superfamily of ligand-gated ion channels. Functional interactions resulting in cross-inhibition have been described in native and/or recombinant systems between P2X receptors and γ-aminobutyric acid receptors [235–238], nicotinic acetylcholine receptors [239–244], and 5-hydroxytryptamine receptors [245, 246]. There is evidence that the P2X2 receptor, via its C terminus, physically interacts with $GABA_AR$s and $GABA_CR$s [235, 238] and that co-transfection of P2X2 subunits modulates their targeting in transfected hippocampal neurons and spinal cord neurons [184]. Similarly, two (Tyr374, Val375) and three (Gln386-Thr388) amino acid residues in the P2X3 and P2X4 C termini, respectively, enable an inhibitory cross-talk with $GABA_A$ receptors in DRG [247] and hypothalamic neurons and thus regulate synaptic transmission [248]. A detailed analysis of the interaction between P2X2 receptors and the $\alpha4\beta2$ nAChR by FRET combined with total internal reflection fluorescence microscopy indicates that both channels are closely associated (approximately 80 Å apart), suggesting that they form functional dimers of two receptor complexes [249].

Coexpression of P2X subunits with ENaC resulted in mutual regulation of channel trafficking in *Xenopus* oocytes [250]. More recently, a close interaction between P2X

receptors and another member of the amiloride-sensitive Na^+ channel family, the acid-sensing ion channel (ASIC), was reported in sensory neurons [251]. According to this study, the electrically quiet P2X5 receptor forms a molecular complex with ASIC3 and increases its pH sensitivity, thereby forming a coincidence detector for low pH and ATP in muscle ischemia. Finally, a functional Ca^{2+}-dependent interaction between P2X receptors and *N*-methyl-D-aspartate (NMDA) receptors has been described in hippocampal pyramidal neurons and appears to play a role in the modulation of synaptic plasticity [252, 253].

Much attention was raised by the finding that the P2X7 receptor and the hemichannel pannexin-1 could be co-purified from transfected HEK cells and that a functional interaction of both proteins was shown in different cell types. Based on these data, it was concluded that pannexin-1 constitutes the "P2X7 pore" and is required for processing of caspase-1 and subsequent release of mature IL-1β [254]. Other studies, however, could not confirm this finding [255–258], and the pore-forming mechanism remains to be elusive (see section "Non-specific pore formation").

Interactions of P2X receptors with other proteins

Due to their particular longer C termini that can be used as baits in "pull-down" assays, most interactions have been determined for P2X2 and P2X7 receptors. Using the C terminus of the P2X2 receptor as a bait in GST-pull-down or yeast two-hybrid assays, βIII tubulin, myelin basic protein [259], heat-shock protein 90 (HSP90), vacuolar-type H^+-adenosine triphosphatase, NSF, tubulin 1α, vesicle amin transport protein 1 (VAT1), glutamic acid decarboxylase synapsin IIb, glutamine synthetase, visinin-like protein 1 (VILIP1) [260], as well as Fe65 and Fe65-like adaptor proteins were identified as associated proteins [261]. βIII tubulin was found to bind to a prolin-rich segment (371–412) in the P2X2 C terminus.

Fe65 is a brain-enriched multidomain adaptor protein containing one WW protein interaction domain and two phosphotyrosine binding/interacting domains (PTB/PID). It has been shown to interact with amyloid precursor proteins and to be involved in brain development [262]. Interaction with the P2X2 receptor appears through the WW domain and the alternatively spliced P2X2b that lacks a C-terminal segment of 69 amino acid residues was not able to interact with Fe65. Co-localization of the P2X2 receptor with Fe65 at postsynaptic specializations of excitatory synapses in the hippocampus was shown by immunogold labeling, and both proteins could be co-precipitated from rat brain extracts. Functional analysis showed that pore dilation of the P2X2 receptor (see section "Non-specific pore formation") was inhibited upon co-expression of Fe65 [261].

VILIP-1 is a member of the neuronal EF-hand Ca^{2+}-sensor protein family. It has been shown to interact also with a nicotinic ion channel and plays a role in regulating cAMP levels, cell signalling, and membrane trafficking [263]. P2X2 receptors and VILIP1 were co-localized in deep cerebellar nuclei, and the dentate gyrus and both proteins could be co-immunoprecipitated from brain extracts. Co-expression of VILIP1 lowered the ATP sensitivity of P2X2 receptors and increased its membrane expression, peak responses, and diffusion in the plasma membrane. Further analysis indicated that a constitutive interaction via the P2X2 juxtamembrane region of the C terminus exists and is increased in an activation- and Ca^{2+}-dependent manner, which could constitute a molecular feedback mechanism [260].

The multiple P2X7 receptor functions appear to be particularly dependent on its C terminus and associated proteins but only comparatively limited information on its interaction with other proteins is available. In rat but not in human P2X7 receptors, a Ca^{2+}-dependent facilitation of P2X7 receptor responses was identified, and calmodulin could be co-immunoprecipitated with agonist-stimulated P2X7 receptors. By mutagenesis, a novel calmodulin binding motif was identified [264, 265].

Upon immunoprecipitation of P2X7 receptors overexpressed in HEK cells, 11 proteins were co-isolated including cytoskeletal proteins (supervillin, β-actin, and α-actinin), chaperones (HSP70, HSC71, and HSP90), the integrin β2 subunit, the extracellular matrix protein laminin α3, the scaffolding protein MAGuK, and the signalling molecules PI4K and receptor phospho-tyrosine phosphatase β (RPTPβ) [225]. The interaction with HSP 90 was further characterized [266]. Using a HEK cell library in a yeast two-hybrid assay, the epithelial membrane protein 2 and related proteins were found to directly interact with the P2X7 receptor C terminus [267].

In a more recent immunopurification study, two non-muscle myosins, NMMHC-IIA and myosin VA, were isolated from monocytic THP-1 cells and P2X7-transfected HEK cells, respectively. In line with the above study, an interaction with protein-tyrosine phosphatase, β-actin, and heat-shock proteins was also found, and in addition, the ubiquitin ligaseRo52, InsP6 and PP-IP5 kinase 1, myosin regulatory light chain, nucleoprotein TRP, tubulin, and nucleoside diphosphate kinase B were identified [268]. P2X7 receptors were shown to co-localize with NMMHC-IIA in HEK cells and functional characterizations by flow cytometry suggest that agonist-induced dissociation of the receptor is required for pore formation while the intact complex is required for phagocytosis in transfected HEK cells, human monocytes, and mice macrophages [269].

For P2X4 and P2X6 receptors, an interaction with the endothelial cell-specific adhesion molecule VE-cadherin was found in human endothelial cells [270].

In a recent co-purification study, a close interaction of the P2X1 receptor with the actin cytoskeleton was found and shown to occur via the P2X1-amino-terminus. The interaction was suggested to contribute to a localized signalling environment in lipid rafts [271].

In addition to the above-described protein interactions, for which in many cases evidence for a physical interaction was provided, P2X receptors have also been shown to functionally interact with a range of other proteins including G-protein-coupled receptors. For further details, please refer to Koeles et al. [272].

Investigation of the tissue-specific subunit composition of native P2X receptors and their protein–protein interactions has partly been hampered by the lack of P2X antibodies that are specific and suitable for immunoprecipitation. Such studies appear highly important in view of the entirely unknown composition of P2X receptor complexes in neuronal membranes where neither their subunit composition nor their enrichment at synaptic sites has been analyzed in detail.

P2X receptor signalling

Ion flux

P2X receptors are essentially non-selective cation channels permeable to small monovalent and divalent cations. P2X receptor activation generally leads to a change in membrane potential initiating subsequent cellular events. For instance, P2X receptor-mediated changes of the membrane potential in neurons presynaptically modulate neurotransmitter release [273–277] or postsynaptically result in fast excitatory signalling [18, 278]. The involvement of P2X receptor-mediated currents in signalling processes of virtually all cells, tissues, and organs is extensively reviewed elsewhere [3, 18, 21, 279]. Nevertheless, beside the direct change of the membrane potential, a major physiological mechanism by which activated P2X receptors control cellular functions is elevation in intracellular calcium concentration ($[Ca^{2+}]_i$) both directly by Ca^{2+} permeation and indirectly by facilitation of voltage-gated Ca^{2+} channels [111, 280, 281]. The fractional Ca^{2+} currents of recombinant P2X receptor subtypes were systematically analyzed by Egan and Khakh and vary between 2.7% and 12.4% (P2X1, 12.4%; P2X2, 5.7%; P2X3, 2.7%; P2X4, 11.0%; P2X5, 4.5%; P2X7, 4.6%; P2X2/3, 3.5%; P2X2/6, 7.7%; P2X4/6, 11.3%), which for some receptors is larger than the Ca^{2+} permeability of acetylcholine-, serotonin-, or glutamate-gated channels [282]. In contrast to the highly Ca^{2+}-permeable NMDA receptors, however, P2X receptors can mediate Ca^{2+} influx at resting or low membrane potentials when NMDA receptors are not active. The increase in $[Ca^{2+}]_i$ activates a broad range of second messenger systems and signalling cascades and can trigger manifold short- and long-term cellular events. For instance, P2X receptors participate in synaptic transmission in the hippocampus by providing a component of the excitatory input to CA1 pyramidal neurons, in which the activation of P2X receptors generates calcium influx that does not require cell depolarization [253]. Inhibition of P2X receptors on these CA1 pyramidal neurons facilitates the induction of long-term potentiation (LTP), indicating that P2X receptors act via calcium influx as a dynamic low-frequency filter within the hippocampus [252, 253].

Non-specific pore formation

It is generally assumed that ionic selectivity is an invariant property of specific ion channels. Several examples exist, however, of channels that have dynamic selectivity filters. These include proton-gated channels, cardiac sodium channels, and some Kv channels [283]. More recently, the TRPV1 receptor has been shown to dilate into larger pores that are permeable to the large fluorescent dye YO-PRO-1 [284]. The physiological significance of these permeability changes remains elusive.

For the slowly desensitizing P2X2, P2X2/3, P2X4, and P2X7 receptors, the development of an additional permeability state which allows the passage of the large cation *N*-methyl-D-glucamine (NMDG) and fluorescence dyes such as the cationic propidium dye YO-PRO-1, and ethidium has also been observed upon repeated applications or in the continuous presence (~30 s) of agonist [283, 285, 286]. This permeability change can also be monitored by a change in the reversal potential if experiments are performed in extracellular NMDG, an organic cation that generally does not efficiently permeate ion channels but does so during ATP-activated channel dilation [283, 285–287]. The two permeability states are referred to as I_1 (permeability to small cations) and I_2 (permeability to larger cations) [283, 287]. In the P2X4 receptor, they could be separated by exchange of the conserved residue Gly347 in TM2: Mutation into a tyrosine residue resulted in channels that lacked the large permeability I_2 state while mutation into a positively charged residue strongly reduced the I_1 current [283]. In the P2X2 receptor, mutation of residues Asn333, Thr336, Leu338, and Gly342 (analogous to Gly347 in P2X4) in TM2 into alanine residues appeared to favor opening of the I_2 permeability state [285]. In a systematic alanine scanning mutagenesis study, a total of ten residues in the two TM domains (Phe31, Arg34, Gln37, Lys53, Ile328, Ile332, Ser340, Gly342, Trp350, Leu352) were identified to perturb transition from the I_1 to the NMDG permeable I_2 state [288]. Of these, Ile328 had also been shown in a previous study to be critical for expression level-dependent changes of the P2X2 receptor permeation properties [228].

Taking the orientation of the previously defined selectivity filter (Thr336, Thr339, and Ser340) [282] and the constraint that Val48 and Ile328 are close to each other [63, 289] as a basis, the I_2 state-specific hits were mapped onto helical wheel representations. In agreement with an effect on protein–protein interactions in the TM region, the residues were found at the interface of neighboring TM1 and TM2 domains of adjacent subunits [288], and it was proposed that the permeation pathway could dilate by helix tilting, rotation, or bending as assumed for other channels [290].

An additional mutagenesis study showed that the pore dilation occurs only in rat but not mouse P2X2 receptors and is dependent on specific residues in the C-terminal domain, suggesting that changes in the permeation pathway during opening to the I_2 state require conformational changes in the C terminus [287]. Interestingly and in support of this finding, the I_2 state appears to be inhibited by interaction of the P2X2 receptor C terminus with the beta-amyloid precursor protein-binding protein Fe65 [261]. In addition, channel activity and pore dilation appear to be regulated by the interaction of membrane-bound phosphoinositides with the proximal region of the P2X2 receptor C-terminal domain [118]. Cytosolic gating motions in the N- and C-terminal domains were also shown and further analyzed by FRET studies with fluorescent proteins and FlAsh-labelled receptors. These studies also revealed that the pore dilation is not dependent on pannexin-1, which was proposed to be involved in pore dilation of P2X7 receptors [255, 291]. Together, all these data suggest that, at least in the P2X2 receptor, the pore dilation is an intrinsic property of the receptor. Most recently, it was shown that colchicine inhibits pore dilation but not ATP-gated currents of P2X2 and P2X7 receptors in oocytes and macrophages [292].

The P2X7 receptor shows a permeability increase with similar kinetics as the P2X2 and P2X4 receptors [285, 286]. In addition, the sustained agonist application leads to cell lysis and apoptosis [293, 294]. These features have not been observed with P2X2 and P2X4 subtypes and require the C terminus while YO-PRO-1 uptake in P2X7 receptors is strongly reduced but not abolished if the C terminus is removed [125, 213]. It remains to be answered whether the dilation of the P2X7 ion channel [285] reflects a property common to the P2X2 and P2X4 receptors. Based on patch-clamp measurements in the cell-attached configuration, it has been suggested that P2X7 receptor-associated pore formation might require ancillary proteins (e.g., either hemi-channels, or the maitotoxin-associated pore [295]) whose activation is dependent on the production of diffusible second messengers such as Ca^{2+} or MAP kinases [296, 297]. A study on P2X7-transfected HEK cells showed that NMDG permeability measured by reversal potential shifts and YO-PRO-1 uptake measured by fluorescence intensity could be differentiated: NMDG permeability but not YO-PRO-1 uptake was inhibited by both normal sodium concentration in the extracellular medium or deletion of a cys-rich 18-amino acid segment in the juxtamembrane C-terminal region of the receptor [298]. From these data, it was concluded that the NMDG permeability is an intrinsic channel property while YO-PRO-1 uptake requires a distinct permeation pathway. In addition, another study found that both a cationic and an anionic dye permeation pathway were opened by P2X7 receptor activation [299]. Since blockade of pannexin hemichannels inhibited P2X7 receptor-associated dye uptake in HEK cells and macrophages while its overexpression resulted in increased dye uptake and both proteins were co-purified from transfected HEK cells, pannexin was suggested to interact with the P2X7 receptor and to be the cause of pore formation [254]. However, more recent data do not confirm this hypothesis [255–258]. In support of an NMDG pore that is intrinsic to the P2X7 channel are two studies by Yan et al. who carefully investigated the complex biphasic current responses observed upon prolonged activation of P2X7 receptor which consist of a fast current increase in the millisecond range and a slowly increasing high-amplitude current that peaked after tens of seconds and had been associated with the presence of two ATP binding sites of different affinity [126]. Yan et al. showed that this slow current component temporally coincided with the shift in reversal potential in NMDG-containing extracellular solutions, and an immediate NMDG permeability was observed when residue Thr 15 in the P2X7 N terminus was mutated to residues with larger side chains (Glu, Lys, or Trp) [257]. Based on the activation and deactivation kinetics at different agonist concentrations and on the sensitization properties of the P2X7 receptor, a gating model was proposed in which occupancy of ATP binding sites controls channel conductance [258]. Involvement of the N-terminus in generation of an NMDG-permeable pore is further suggested by the properties of a P2X7 N-terminal splice variant, which also shows immediate NMDG permeability [300]. In conclusion, although the processes of pore dilation are still very poorly understood, the above data suggest that NMDG permeability increases in P2X2, P2X4, and P2X7 receptors are based on a common molecular mechanism intrinsic to the ion channels. Whether this dilated I_2 state is also responsible for dye uptake remains a matter of debate [301].

Inconsistent with an intrinsic channel property, however, is the fact that neither for P2X2 and P2X4 receptors nor for P2X7 receptors permeability states corresponding to the dilated channels have been observed in single channel recordings [172, 287, 302], and in P2X2 receptors, pore formation properties varied between experiments [287]. This could in part be due to the fact that optimal conditions for production of the I_2 state (very low extracellular Ca^{2+} and concentrations of ATP >10 μM) [283, 285] might not have been systematically explored.

Interestingly, a G496A polymorphism in the human P2X7 receptor [303] has been identified that produced loss of function in flow cytometry assays (ethidium uptake, apoptosis, Ba^{2+} influx) but revealed no differences in the electrophysiological properties of the heterologously expressed channel [304]. Likewise, conflicting data were found for the L451P polymorphism in the mouse P2X7 receptor which showed reduced pore formation and cell death if native thymocyte or T cell preparations were investigated by flow cytometry [305, 306], but no difference to wild-type (wt) when heterologously expressed variants were analyzed in fluorometry imaging assays [79] or in patch clamp experiments (own unpublished data). These data support the involvement of cell-specific and C terminus-dependent mechanisms in the formation of dye-permeable pores.

P2X7-specific signalling

A range of downstream cellular events have been identified upon P2X7 receptor activation. These include release of cytokines, cytoskeletal rearrangements, and plasma membrane protein shedding and cell death via necrosis and apoptosis as well as trophic effects, cell proliferation, and differentiation (e.g., [293, 307–309]). The cellular mechanisms underlying these various effects are dependent on the cell background and are very incompletely understood. Interactions with multiple intracellular signalling pathways have been shown. These include activation of phospholipases A2 and D and coupling to protein kinases PKC, Src, JNK (stress-activated protein kinase) and the ERK and p38MAP kinases, as well as the rho-associated protein kinase (ROCK). For a comprehensive picture of P2X7 signalling in microglia, refer to Kettenmann et al. [310]. For additional information, refer to Erb et al., Duan et al., and Lenertz et al. [311–313]. Only two of the above-mentioned effects are briefly mentioned in the following.

Secretion of IL-1β and other cytokines The cytokine IL-1β is released by macrophages and other immune cells and represents an important mediator of inflammation. It is transcribed as the inactive precursor pro-IL-1β in response to inflammatory stimuli (e.g., LPS that acts via TLR and NFkB activation). Processing of pro-IL-1β into IL-1β involves the protease caspase-1, which in turn is proteolytically generated from procaspase-1 in a process that involves different multimeric "inflammasome complexes" that oligomerize and activate caspase-1 in response to a second specific stimulus [314, 315]. These can be the toxins nigericin and maitotoxin or high ATP concentrations in case of the NALP3 inflammasome [316]. Early studies had already shown that ATP is an efficient stimulator of IL-1β maturation and release [317–320] and that K^+ efflux appears to be involved in this process [320]. By generation of the P2X7−/− mouse [321–323], it was confirmed that the IL-1β release (and release of other IL-1 family members) is a consequence of P2X7 receptor activation. For a detailed review, see Ferrari et al. [324].

However, the molecular mechanisms that lead from K^+ depletion to NALP3 inflammasome activation and IL-1β release and how pannexin is involved in this process remain unclear [301, 325]. P2X7-mediated microvesicle shedding [326, 327] has been suggested to play a role in interleukin release.

Effects on extracellular and intracellular membranes A variety of changes in the plasma membrane composition and morphology have been observed upon P2X7 receptor activation [327]. These include the redistribution of phosphatidylserine to the extracellular leaflet of the plasma membrane (PS-flip), plasma membrane protein shedding (CD26L, CD23, CD 27) by matrix metallo proteases, and plasma membrane blebbing and microvesicle release. The so-called PS-flip represents an indicator of apoptotic cell death but has also been involved in physiological processes such as maturation and differentiation. In addition, P2X7 receptors have effects on intracellular organelles and membranes. For example, regulation of phagosome fusion with lysosomes has been shown to be involved in bacterial killing (for details on P2X7 regulation of extracellular and intracellular membrane responses refer to Qu and Dubiak [327]).

Physiological functions of P2X receptors inferred from genetically modified animals and other in vivo models

P2X receptor isoforms have been found to be widely but specifically distributed among different tissues in the vertebrate body. Expression patterns of P2X receptors have initially been evaluated at the mRNA level using Northern blot, RT-PCR, and in situ hybridization analysis [23, 61, 205, 328–330]. Following the development of P2X receptor subtype-specific antibodies, many of these findings have been verified at the protein level by Western blotting and immunohistochemistry [331–337]. It has to be mentioned, however, that reliability of some of these antibodies have been questioned [338, 339]. In addition, the lack of subtype-selective and metabolically stable agonists as well as truly potent and specific antagonists for some P2X subtypes has made the molecular identification of individual P2X receptor subtypes in native tissue preparations and determination of their function a challenging task. Thus, the identities and in vivo roles of many P2X receptors are still not completely understood or speculative. Dramatic progress has been made

by the use of knockout and transgenic animals. The following part of this review will provide an overview of the P2X receptor distribution and their physiological roles determined using both genetically engineered animal models and transient knockdown approaches (Table 1).

P2X1

The P2X1 receptor sequence was originally cloned from a rat vas deferens cDNA library [23] and subsequently isolated from human urinary bladder and platelets as well as mouse urinary bladder and vas deferens [340–343]. This receptor is most highly expressed in smooth muscle cells of various organs, including urinary bladder, vas deferens, and arteries [331, 336, 344–346]. Significant P2X1 receptor levels are also found in megakaryocytes and blood platelets [342, 347, 348]. P2X1 mRNA has been detected in apoptotic thymocytes but, surprisingly, not in peripheral T cells [349]. The precise localization of P2X1 receptors in the CNS remains unsettled as the P2X1 receptor antibodies were shown to exhibit similar immunostaining patterns in the CNS of wt and P2X1 knockout mice [338]. However, contribution of P2X1 subunits to functional responses in cortical astrocytes was reported [67, 350], and P2X1 receptor expression, detected by immunostaining, was shown to be downregulated in the hippocampus by TNP-ATP treatment [351]. Peritoneal mouse macrophages were shown to express functional P2X1 receptors absent in P2X1−/− mice [352]. In addition, human lung mast cells were shown to express functional P2X1 receptors [330].

The P2X1 encoding gene consists of 12 exons and is located close to the P2X5 gene on chromosome 10 in the rat, chromosome 17 in the human, and chromosome 11 in the mouse genome [340, 341, 343]. In most cases, the distribution profiles described for P2X1 receptors correlate with phenotypic data from P2X1 loss- and gain-of-function mouse models. P2X1-deficient mice were generated through the targeted replacement of a fragment encoding a part of

Table 1 Major physiological functions of different P2X receptor subtypes determined using knockout (KO), P2X2/P2X3 double knockout (DKO), and transgenic mice (TG) mice

Subtype	Phenotype (mouse model)	Physiological function	Reference
P2X1	Male infertility (KO)	Smooth muscle contraction	[344]
	Impaired kidney function (KO)	Renal autoregulation	[355–357]
	Reduced arterial thrombosis (KO)/prothrombotic phenotype (TG)	Platelet activation	[361, 362]
P2X2	Impaired synaptic facilitation (KO)	Regulation of transmitter release in hippocampus	[275]
	Reduced inflammatory pain (KO, DKO)	Nociceptive signalling	[65]
	Impaired peristalsis in small intestine (KO)	Intestinal neurotransmission	[394]
	Attenuated ventilatory response to hypoxia (KO, DKO)	Carotid body function	[396]
	Urinary bladder hyporeflexia (KO, DKO)	Sensory neurotransmission	[65]
	Impaired taste sensing (DKO)	Gustatory signalling	[433–435]
	Abnormal skeletal neuromuscular junctions (KO)	Endplate formation	[399]
P2X3	Impaired hippocampal LTD (KO)	Regulation of synaptic plasticity	[426]
	Reduced pain responses (KO, DKO)	Nociceptive signalling	[65, 416, 417]
	Impaired peristalsis in small intestine (KO)	Intestinal neurotransmission	[423]
	Urinary bladder hyporeflexia (KO, DKO)	Sensory neurotransmission	[65, 416]
	Impaired temperature sensitivity (KO)	Thermal sensation	[417, 425]
	Impaired taste sensing (DKO)	Gustatory signalling	[433–435]
P2X4	Decreased hippocampal LTP (KO)	Regulation of synaptic plasticity	[455]
	Reduced inflammatory and neuropathic pain (KO)	Modulation of chronic pain (regulation of BDNF and PGE2 release from activated microglia/macrophages)	[191, 460, 461]
	Higher blood pressure, lack of vascular remodelling, decreased flow-induced release of NO (KO)	Regulation of vascular tone	[456]
	Improved heart function (TG)	Control of contractility of the cardiomyocytes	[468–472, 474]
P2X7	Abolished IL-1β release, diminished inflammatory responses (KO)	Pro-inflammatory cytokine release	[322, 323]
	Reduced inflammatory and neuropathic pain (KO)	Immune cells activation	[321]
	Skeletal abnormalities (KO)	Bone metabolism	[552, 553]
	Reduced fluid secretion in salivary gland and pancreas (KO)	Regulation of exocrine gland secretion	[556, 557]

exon 1 (including the initiation codon ATG) by a lacZ/Neo cassette [344] (Fig. 4). In line with an important function in the vas deferens, these knockout mice revealed a drastic (up to 90%) reduction in male fertility. This was associated with a low sperm count in the ejaculate caused by the lack of P2X1 receptor-mediated vas deferens contraction [344]. Consistent data have been reported in guinea pig, rat, and human by functional and pharmacological analysis of isolated vas deferens preparations [6, 353, 354].

Renal P2X1 receptor-mediated pressure-induced afferent arteriolar autoregulatory responses were studied in P2X1−/− mice using the in vitro blood perfused juxtamedullary nephron technique [355–357]. It was suggested that P2X1 receptors expressed on afferent arteriolar smooth muscle cells contribute to the modulation of the tubuloglomerular feedback mechanism (TGF). Accordingly, macula densa cells of the juxtaglomerular apparatus respond to the increase in luminal tubular fluid NaCl concentration through release

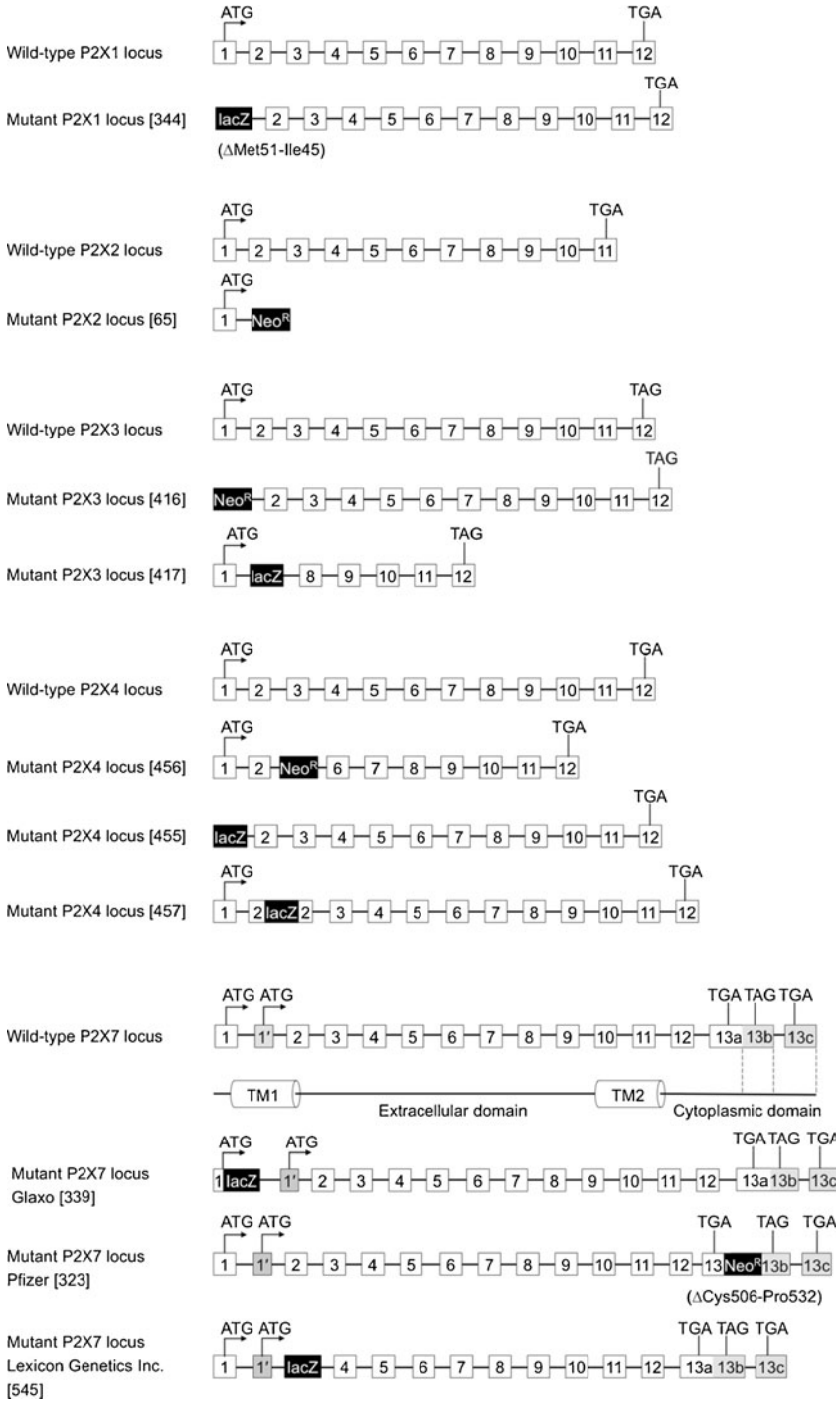

Fig. 4 Summary of published P2X receptor−/− mice and the targeting strategies used for their generation. Location of TM encoding exons are only shown for the P2X7 receptor, but, due to the strong conservation of exon-intron boundaries in P2X receptor encoding genes, can be transferred to the other subtypes. The figure also illustrates alternative exons (1′and 13b/c) identified in the rodent P2X7 gene [300, 525] and explains how the P2X7K splice variant derived from exon 1′ can escape the gene deletion strategy used in one of the available P2X7−/− mouse lines. Note that all cassettes indicated with lacZ represent actually lacZ-Neo^R cassettes

of ATP that, via the P2X1-mediated vasoconstriction of afferent arterioles, leads to changes in the glomerular filtration rate [331, 355–358]. These in vitro findings were confirmed in vivo by data from whole kidney blood flow experiments that show pharmacologically the importance of the P2X1 receptor activation for whole kidney autoregulation [359]. However, a recent study indicates that the direct activation of P2 purinergic receptors by ATP is not a major cause of TGF-induced vasoconstriction in vivo [360]. Therefore, additional studies are required to fully understand the involvement of P2X1 receptors in renal hemodynamics.

Analysis of the P2X1 knockout mice further indicated an involvement of P2X1 receptors in platelet function and thrombus formation [361]. Mice lacking the P2X1 receptor display reduced arterial thrombosis under conditions of high shear stress and exhibit reduced platelet aggregation in low collagen concentrations. About 20% of the analyzed P2X1−/− mice showed also markedly prolonged bleeding times [361]. Involvement of the P2X1 receptor in platelet function was also shown in a P2X1 transgenic mouse model in which human P2X1 cDNA was overexpressed under the control of the megakaryocyte-specific murine glycoprotein IIb (GPIIb) promoter [362]. This resulted in a mild prothrombotic phenotype. Accordingly, transgenic platelets displayed enhanced secretion and aggregation in vitro, in response to low doses of collagen, convulxin, and the thromboxane A2 mimetic U46619 or shear stress [362]. These observations are consistent with in vivo results obtained in a model of pulmonary thromboembolism, which demonstrated increased lethal thrombosis in hP2X1 transgenic mice compared with wt animals [362]. The involvement of P2X1 receptors in platelet activation during hemostasis or thrombosis make it an important therapeutic target. For more detailed information concerning P2X1 receptor function in platelet, see Hu et al. and Mahaut-Smith et al. [363, 364].

P2X2

The P2X2 cDNA was initially cloned from a rat pheochromocytoma PC12 cell line [22]. The P2X2 receptor is one of, if not the, most widely distributed subtype of the P2X receptor family. Its abundant expression has been found in both the central and the peripheral nervous systems [278, 365]. Particularly high expression levels were described in the olfactory bulb, cerebral cortex, basal ganglia, diencephalon, mesencephalon, cerebellum, medulla oblongata, and dorsal horn area of the spinal cord [200, 335, 337, 366–369]. Moreover, a significant P2X2 expression with importance for sensory neurotransmission has been described in both sensory and autonomic ganglion neurons of the peripheral nervous system [65, 200, 368, 370–378]. In addition, multiple non-neuronal tissues, such as adrenal medulla (chromaffin cells), urothelium, vasculature smooth muscle, skeletal muscle (during development and regeneration), cardiac muscle, and interstitial cells of the vas deferens, have been shown to express significant amounts of the P2X2 receptor subunit [22, 336, 337, 345, 379–385].

The P2X2 gene comprises 11 exons and lies together with the P2X4 and P2X7 genes on chromosome 12 in the rat and human and on chromosome 5 in the mouse genome. Multiple alternatively spliced transcripts of the P2X2 receptor have been detected [115, 368, 386–393]. The described variants arise from C-terminal alternative-splicing events. The functional, but fast desensitizing P2X2b (or P2X2-2) receptor isoform, in which exon 11 is partially deleted, appears to be conserved between human and rodents. Other identified splice variants include P2X2c, P2X2d, P2X2e, P2X2f, and P2X2g in rat [115, 368, 386, 387, 389], P2X2e in mouse [391, 392], P2X2-3 in guinea pig [388, 390], and P2X2c and P2X2d in human [393]. Some of these appear to be non-functional. It seems likely that various P2X2 receptor isoforms co-assemble with other P2X subunits to form heteromeric channels with modified properties [391].

Mice lacking the P2X2 subunit were generated by targeted deletion of a region spanning from exon 2 to exon 11 and its replacement with a floxed sequence encoding a neomycin resistance gene [65, 275] (Fig. 4). Despite the wide distribution of the P2X2 receptor, P2X2 knockout mice displayed only small differences in body weight compared with wt animals and were visibly and histopathologically normal for up to 1 year of age. Likewise, urinalysis, blood chemistries, and peripheral blood cell counts did not show significant differences between P2X2+/+ and P2X2−/− mice [65].

Analysis of P2X2 knockout mice revealed impaired peristalsis in the small intestine, most likely due to the absence of P2X2 receptor-mediated synaptic transmission in the myenteric plexus [394] but not in the mouse colon [395], indicating that the P2X2 subunit is not required for propulsive motility in the mouse colon. Also, an involvement in ATP-evoked synaptic facilitation of hippocampal interneurons [275] was reported, and a pivotal role of P2X2 subunit containing receptors in normal carotid body function and in ventilatory response to hypoxia was demonstrated in P2X2−/− mice. The mice showed reduced responses of the carotid sinus nerve to hypoxia and markedly attenuated ventilatory responses to hypoxia [396].

An involvement of P2X2-subunit-containing receptors in sensory transmission has early been proposed [397] and was confirmed in P2X2−/− mice. In agreement with the co-expression of P2X2 and P2X3 subunits on DRG neurons, functional analysis of wt and P2X2−/− mice revealed the contribution of P2X2 subunits to ATP-induced sustained but not transient (fast desensitizing) responses of DRG and

nodose ganglia neurons. Sympathetic neurons of the superior cervical ganglion from P2X2−/− mice exhibited no response to αβ-me-ATP, indicating an exclusive expression of P2X2 subunits in the form of homomeric P2X2 receptors [65], which is in good agreement with former pharmacological studies of cultured autonomic ganglia neurons [376, 398]. In the formalin-induced model of chemical nociception, P2X2 subunit deficiency leads to significant attenuation in the persistent but not acute phase of the formalin response [65]. Also, urinary bladder hyporeflexia and decreased activities of pelvic afferent nerves in response to bladder distension were observed in these mice [65].

Further experiments with P2X2 knockout mice have demonstrated a role for P2X2 receptor-dependent signalling in the development and maintenance of skeletal neuromuscular junctions [399]. The involvement of P2X2 receptor in the late stages of endplate formation is consistent with the expression pattern of the P2X2 subunit during skeletal muscle development and muscle fiber regeneration [382–384].

P2X3

The P2X3 sequence was originally cloned from a rat DRG cDNA library [400, 401] and subsequently also from a human heart cDNA library [88] and mouse genomic library [402]. In rodents, predominant and developmentally regulated P2X3 receptor expression has been demonstrated on small- to medium-diameter sensory neurons within DRG as well as nodose and trigeminal ganglia by Northern-blot analysis, in situ hybridization or immunohistochemistry [373, 374, 380, 400, 401, 403–408]. This very restricted pattern of P2X3 subunit distribution has been associated with P2X3-mediated nociceptive sensory nerve responses to ATP released from inflamed or damaged tissues [187, 333, 409]. The P2X3 subunit has also been detected in the spinal cord within the superficial laminae of the dorsal horn [373, 385, 404, 405]. In addition, P2X3 receptor expression has been found in both the urothelium and suburothelium of rat urinary bladder [410]. In humans, the P2X3 receptor has so far been reported in heart and spinal cord at the mRNA level and in dorsal root ganglia, intestine (myenteric plexus neurons), urinary bladder (urothelium and suburothelium), and dental pulp at the protein level [88, 410–415].

The gene encoding the P2X3 subunit contains 12 (human and mouse) to 13 (rat and zebrafish) exons and was mapped to chromosome 3 in rat, chromosome 2 in mouse, and chromosome 11 in human [88, 402].

An important role of the P2X3 channel in nociceptive signalling was confirmed using two independently engineered P2X3 knockout mouse lines. One line was generated by targeted replacement of the fragment, containing the ATG translational start site and exon 1, with a floxed neomycin-resistance gene [416]. In a second strain, a fragment ranging from exon 2 to exon 7 was replaced by an IRES-LacZ-MC1-Neo cassette [417] (Fig. 4). Both mice strains display significantly attenuated responses in the acute and persistent phases of the formalin-induced pain test [416, 417]. Likewise, pain caused by intraplantar injection of ATP is also greatly diminished in the P2X3−/− mice [416]. A significant role of P2X3 receptors in pain responses with reduction of agonist-induced mechanical hyperalgesia and tactile allodynia as well as reduced pain responses in neuropathic or inflammatory pain models is in agreement with studies using P2X3 siRNA [418, 419], antisense oligonucleotides [420], or pharmacological P2X3 receptor inhibition [141] in rats.

Deletion of the P2X3 gene in mice also had a significant effect on sensory function in the urinary bladder as evidenced by marked bladder hyporeflexia, resulting in greatly reduced voiding frequency and substantial increase in bladder capacity [65, 416]. Further studies demonstrated attenuated responses of pelvic afferents to bladder distension and intravesical injection of P2X agonists (ATP or α,β-meATP) in P2X3−/− mice [421]. It was concluded that bladder filling and subsequent distension induces release of ATP from the urothelium, which, via P2X3 receptors, triggers the mechanosensory signal transduction and excitation of afferent nerve fibers [421, 422]. Similar to the P2X2 null mutation, P2X3 deficiency also resulted in impaired peristalsis in the small intestine [423] but not in the mouse colon [395]. In addition, P2X3−/− mice exhibited a blunted response of gastric vagal afferents to fluid distension of oesophagus and stomach [424].

In line with an important function in sensory systems, both P2X3−/− models also showed an enhanced thermosensory phenotype [417, 425] and were unable to differentiate the intensity of non-noxious ‘warming’ stimuli [417]. However, it should be noted that thermal hyperresponsiveness observed in P2X3 null mice could not be reproduced by subcutaneous administration of A317491, a P2X3-selective antagonist [425]. Therefore, it has been suggested that long-term absence of P2X3 receptor is necessary to develop such a thermosensory phenotype or that compensatory changes contribute, at least in part, for the P2X3−/− phenotype [425]. Finally, analysis of synaptic plasticity in P2X3−/− mice has indicated that P2X3 receptor might be involved in the induction of long-term depression (LTD) at hippocampal synapses [426].

Besides the mouse models mentioned before, analyses of P2X3 receptor function has been performed in the zebrafish model system. Three groups reported the identification of orthologues of the mammalian P2X3 subunit in zebrafish [120, 427, 428]. The zebrafish orthologue, zP2rx3a (zP2X3, also termed zP2rx3.1), is located on chromosome 14, whereas zP2rx3b (or zP2rx3.2) is located on chromosome 1 [429]. Similarly to mammals, the expression of the zP2X3 receptor

has been mainly detected on sensory neurons. Very high levels were found in neurons of the trigeminal ganglia but also in the Rohon-Beard cells of the dorsal spinal cord [428, 429]. A non-neuronal expression of zP2X3 receptor has been found within lateral cranial ectodermal cells in zebrafish embryos [430, 431]. In order to characterize the function of zP2X3 receptor during development, morpholino oligonucleotide-mediated knockdown of this channel was performed [430]. These studies revealed that the zP2X3 subunit is required for normal craniofacial development and sensory neurogenesis because its loss in embryos led to craniofacial defects, such as malformation of pharyngeal skeleton and disrupted epibranchial ganglia formation. The specificity of this phenotype was confirmed by additional morpholino oligonucleotides, which target another sequence of the zP2RX3 gene and by partial rescue of the mutant phenotype by co-injection of rP2X3 RNA [430].

P2X2 and P2X3 subunits are co-localized in many neurons, particularly within dorsal root ganglia, nodose ganglia, nucleus tractus solitarius, and taste buds [373, 380]. Double knockout P2X2/P2X3$^{Dbl-/-}$ mice were generated by breeding the compound heterozygous offsprings obtained upon crossing of P2X3−/− (lacking the ATG translational start site and exon 1 [416]) with P2X2+/− mice [65]. Surprisingly, about 90% of these P2X2/P2X3$^{Dbl-/-}$ mice died in the early postnatal period with various abnormalities including: distended bladders, enlarged hearts, pronounced atrophy or hypocellularity of lymphohematopoietic organs, and lack of lymphoid follicles in the spleen and mesenteric lymph node [65]. Bacterial bronchial pneumonia was identified as the leading cause of mortality. Those P2X2/P2X3$^{Dbl-/-}$ mice that survived into adulthood (approximately 10%) were found to be normal in appearance and weight. In contrast to P2X2−/−, but similarly to P2X3−/− mice, the survived double mutant mice had reduced pain behavior in both phases of the formalin test [65] and provided evidence that the P2X2 and P2X3 subunits are the predominant P2X family members on mouse sensory neurons, since their deletion led to loss of virtually all ATP-activated currents in DRG and nodose ganglia.

Consistent with findings in P2X2 and P2X3 single knockout mice, P2X2/P2X3$^{Dbl-/-}$ mice developed urinary bladder hyporeflexia, decreased pelvic afferent fiber activity in response to bladder filling [65], and reduction in pelvic afferent response to colorectal distension [432]. Further studies highlighted a crucial role of the P2X2 subunit in ventilatory response to hypoxia, since this is significantly decreased in both P2X2−/− and P2X2/P2X3$^{Dbl-/-}$ mice but not in mice deficient in only the P2X3 receptor [396].

Finally, studies on P2X2/P2X3 $^{Dbl-/-}$ mice showed that ATP serves as a primary neurotransmitter in taste buds [433]. P2X2/P2X3$^{Dbl-/-}$ mice displayed a loss of peripheral gustatory nerve responses to salt, sweet, sour, bitter, and umami [433, 434]. Despite this profound taste deficit, avoidance of caffeine, and citric acid was comparable to that observed in wild-type controls, suggesting involvement of either non-gustatory or non-purinergic mechanisms for these taste stimuli [433, 435]. In contrast to double mutant mice, single P2X2−/− or P2X3−/− animals exhibited only moderate changes in taste-mediated behavior, suggesting that homomeric P2X2 or homomeric P2X3 receptors suffice for normal taste function [433].

Collectively, all these findings demonstrate that homomeric P2X2, homomeric P2X3, and heteromeric P2X2/3 receptors are crucial players in sensory neurotransmission.

P2X4

The first P2X4 receptor cDNAs were cloned from hippocampus, whole brain, and superior cervical ganglia cDNA libraries [87, 436, 437]. Additional P2X4 sequences were cloned from various species and tissues [328, 438–443] including *X. laevis* oocytes and zebrafish [57, 444]. A characteristic feature of the P2X4 subunit is its widespread distribution that overlaps to a large extent with the localization pattern of the P2X6 subunit [200]. An extensive and abundant P2X4 receptor expression has been demonstrated in several regions of the central and peripheral nervous systems [87, 335, 369, 374, 436, 437, 439, 445] as well as all vital and reproductive organs, skeletal and smooth muscle, epithelial and endothelial cells, and various others [87, 381, 436–438, 441, 442, 445–452]. The gene encoding the human P2X4 receptor comprises 12 exons and is located about 24 kilobases downstream of the P2X7 receptor gene. This close chromosomal localization suggests that P2X4 and P2X7 genes evolved by gene duplication. Multiple splice variants, often with distinct patterns of expression, have been found for the P2X4 receptor [441, 443, 453]. A putative minimal promoter of the human P2X4 gene was identified and shown to be regulated by the hematopoietic transcription factor GATA-2 [454].

The physiological role of P2X4 receptors has been assessed using a variety of approaches, including knockdown with either antisense oligonucleotides or small interference RNA as well as three P2X4−/− mouse lines (Fig. 4). In the first knockout strategy, a lacZ/Neo cassette was used to replace a region encompassing the first P2X4 exon (including the ATG start codon) and a short part of the first intron [455]. In a second KO model, the P2X4 genomic fragment containing exons 3, 4, and 5 was replaced with a loxP-flanked neomycin resistance cassette [456]. In the third P2X4 knockout mouse line, a short fragment within exon 2 was replaced by an IRES-lacZ/Neo cassette [457].

Numerous studies have shown the involvement of the P2X4 receptor in the pathogenesis of chronic neuropathic

and inflammatory pain. P2X4 expression is increased in microglia of the dorsal horn following spinal nerve ligation, a model of neuropathic pain [190], and intrathecal administration of P2X4 antisense oligodeoxynucleotides into rats significantly attenuated both spinal cord-induced tactile hypersensitivity as well as the increase in P2X4 receptor levels [190]. Likewise, tactile allodynia was reversed by spinal administration of TNP-ATP [190]. The increase of P2X4 receptor expression was essentially restricted to hyperactive microglia within the spinal dorsal horn and injection of ATP-stimulated microglia into normal rats resulted in P2X4-dependent tactile allodynia. In agreement with these data, spinal cord injury or formalin injection into the hindpaw of rats resulted in an increase in P2X4 receptor expression on microglial cells [458, 459]. P2X4−/− mice lines [455, 460, 461] showed a marked decrease in tactile allodynia caused by nerve injury and a significant reduction in peripheral inflammation-induced pain while behavioral responses to acute thermal, mechanical, and chemically induced pain appeared normal. It was demonstrated in two P2X4−/− lines that P2X4 receptors control the release of BDNF from activated microglia, which is promoting allodynia [191, 460]. Further analysis demonstrated that the reduction of inflammatory pain behavior in P2X4-deficient mice was due to impaired synthesis of prostaglandin E2 (PGE2), a central mediator of inflammation involved in pain hypersensitivity [461]. Accordingly, wild-type, but not P2X4−/−macrophages, exhibited elevated levels of PGE2 after ATP-mediated activation of the P2X4 receptor. For further information, see Jakobsson [462].

In both KO lines [460, 461], no differences to wt animals were observed in the formalin test of injury-induced pain, when standard amounts of formalin were used. However, injection of a lower amount of formalin into a mouse paw revealed that the second phase of the formalin test, which is attributed to chronic inflammatory response, was markedly attenuated in P2X4-deficient mice [461]. In contrast, the first phase of the formalin test, representing acute nociception in response to primary afferent activity, was unaffected in P2X4 null mice. This finding indicates that P2X4 channels are not involved in acute nociception but most likely play a role in chronic inflammatory pain that is determined by both peripheral and central sensitization. Also, a third P2X4−/− model [457] revealed the presence of functionally active P2X4 receptors in native peritoneal macrophages. Downregulation of the P2X4 receptor by knockdown approaches [463, 464] as well as its deletion [456] have also resulted in abnormal endothelial cell responses to changes in blood flow, including a lack of flow-induced Ca^{2+} influx and diminished production of the endogenous nitrovasodilator, nitric oxide (NO). Accordingly, P2X4−/− animals exhibited an elevated blood pressure, a lack of vascular remodelling, and decreased flow-induced release of NO, suggesting a crucial role of P2X4 channel in endothelial cell-mediated control of the vascular tone. Involvement of the P2X4 receptor in control of Ca^{2+} entry in vascular endothelial cells exposed to shear stress was shown [465]. A P2X4- or P2X6- mediated Ca^{2+} influx in response to extracellular Zn^{2+} and ATP has later been shown to restore Cl^- secretion across cystic fibrosis airway epithelia, suggesting P2X4 and/or P2X6 receptors as a potential therapeutic targets for the treatment of cystic fibrosis [466, 467].

P2X4 receptors have been immunohistochemically identified in perisynaptic locations on hippocampal CA1 and cerebellar Purkinje cells [335], and analysis of P2X4−/− mice revealed a significant decrease in LTP at Schaffer collateral synapses. Moreover, ivermectin had no effect on P2X4−/− animals, whereas it enhanced LTP in wild-type controls, thus indicating involvement of P2X4 receptor-mediated Ca^{2+} influx in regulation of hippocampal synaptic plasticity [455].

The role of the P2X4 receptor in the heart was studied in a transgenic mouse model overexpressing the human P2X4 subunit under the control of the cardiac-specific α-myosin heavy chain promoter [468, 469]. Although no apparent histopathological abnormalities were observed under normal physiological conditions, the hP2X4 transgenic mice exhibited increased contractility of the cardiomyocytes and greater global contraction performance in intact heart as compared with wt mice [468]. Therefore, it was hypothesized that P2X4 receptor activation may be beneficial in pathophysiological conditions, such as cardiomyopathy and ischemic heart disease. In order to test this possibility, binary P2X4 receptor (P2X4)/calsequestrin (CSQ) transgenic mice were generated by crossing hP2X4 transgenic mice with a CSQ transgenic mouse model of cardiomyopathy [470]. Interestingly, overexpression of the P2X4 receptor in the P2X4/CSQ mutant mice resulted in significant delay of heart failure progression and a more than twofold increase in life expectancy [470–472]. Similarly, chronic in vivo administration of the P2 receptor agonist MRS-2339 was found to not only reduce cardiac hypertrophy and prolong survival of the CSQ single transgenic mice but also to improve cardiac function in dogs with tachycardia-induced cardiomyopathy, thus emphasizing an important role of cardiac P2X channels in heart physiology [471, 473]. Further evidence for a rescue effect of overexpressed hP2X4 receptors in ischemic heart failure was provided in an analysis of hP2X4 receptor overexpressing transgenic mice after myocardial infarction [474], suggesting that the P2X4 channel represents a therapeutic target for the treatment of heart failure resulting from ischemia.

In zebrafish, two P2X4 genes, designated zP2rx4a (zP2rx4.1, chromosome 21) and zP2rx4b (zP2rx4.2, chromosome 8), have been identified, and the zP2rx4b transcript has been localized in the embryonic nervous system,

including dorsal and ventral neurons of spinal cord and the trigeminal nerve [429]. While no information is available concerning the expression pattern of the zP2rx4a receptor, its successful crystallization has greatly furthered our understanding of the molecular structure of the P2X channel family [48].

P2X5

A cDNA clone encoding the P2X5 receptor was originally isolated from rat celiac cervical ganglia and shortly afterwards also from a rat heart cDNA library [200, 201]. Corresponding sequences were later obtained from human brain, chicken embryo skeletal muscle (initially named cP2X8), brain and heart cDNA libraries, a mouse BAC library, bullfrog tadpole skin, and zebrafish [56, 57, 59–61, 124, 202, 429, 475].

The gene encoding the P2X5 subunit consist of 12 (chicken, human) to 13 (mouse, rat) exons and shares its chromosomal localization with the P2X1 gene (see section "P2X1") [56].

The murine P2X5 channel appears to be widely distributed in the central and enteric nervous systems [476, 477]. In addition, it has been detected in cardiac and skeletal muscle, adrenal gland, kidney, and testis [56, 61, 201, 478]. In humans, P2X5 receptor expression has been found to be predominant in the immune and central nervous systems [60]. In contrast to other vertebrate species, two alternative splice variants of the P2X5 receptor have been reported in humans, P2X5a, that lacks exon 10, and P2X5b, missing exons 3 and 10 [19, 60]. The P2X5a transcript results from a single nucleotide polymorphism (SNP) at the 3' splice site of exon 10 and thus encodes a truncated, non-functional P2X5 protein lacking a portion of both the putative ATP binding site and TM2 [55, 479]. The allele encoding this non-functional P2X5 isoform is the most prevalent variant in different human populations [479].

Interestingly, several reports have linked P2X5 receptor expression with differentiation and turnover of various cell types, such as skeletal muscle cells, osteoblasts, and epithelial cells from different tissues (nasal mucosa, skin, vagina, gut, bladder, ureter, thymus) [381, 383, 447, 480–485]. Ryten et al. provided evidence that ATP-mediated activation of P2X5 receptors suppresses proliferation and promotes differentiation of skeletal muscle progenitor cells (known as satellite cells) into muscle fibers [485]. Therefore, the P2X5 channel has been postulated to play a role in skeletal muscle development or regeneration [485, 486]. Consistent with this, developmentally regulated expression of the P2X5 receptor has been reported in rat and chick skeletal muscle [383, 484]. In addition, relatively high P2X5 receptor expression has been found in different cancer tissues, including basal and squamous cell carcinomas as well as prostate cancers, indicating that activation of P2X5 receptor may also regulate growth and differentiation of cancer cells [487–489].

P2X5 knockout or transgenic animals have not been described so far. However, data from siRNA-mediated knockdown of P2X5 receptors in human bronchial epithelial cells and the fact that most humans express the non-functional P2X5 isoform indicate that at least, in humans, it does not fulfil an essential physiological function [465, 479].

In zebrafish, two paralogous P2X5 genes have been reported. zP2rx5.1 was mapped on chromosome 5, and zP2rx5.2 (also known as zP2rx514 or zP2rx8) is located on chromosome 15 [57, 429, 490]. The zP2rx5.1 receptor is the only zP2X receptor for which mRNA has been detected in embryonic skeletal muscle, whereas zP2rx5.2 mRNA, like other zP2X receptor mRNAs, was found in the nervous system [429, 490, 491]. Using morpholino-mediated zP2rx5.1 gene knockdown, it was demonstrated that the zP2rx5.1 receptor is necessary for the muscle responsiveness to ATP but is not essential for myogenesis during zebrafish embryonic development [490].

P2X6

The P2X6 receptor sequence was first cloned from the rat superior cervical ganglia and shortly afterwards from a rat brain cDNA library [200, 205]. Subsequently, human and murine P2X6 receptor counterparts (originally designated as P2XM) have been identified in a search for novel p53-regulated genes [206, 492]. Interestingly, no P2X6 ortholog has so far been found in zebrafish or any other non-mammalian species [490].

Northern blot analyses have demonstrated a predominant expression of murine and human P2X6 transcripts in skeletal muscle [206, 492]. Further studies have shown that expression of the P2X6 receptor in chick, rat, and mouse skeletal muscle, similarly to the P2X5 receptor, is regulated during embryonic development [382, 383, 484]. In addition, widespread distribution of rat P2X6 receptors, overlapping to a large extent with the expression pattern of P2X4 and P2X2 subunits, has been reported in both the central and the peripheral nervous systems [200, 335, 493, 494]. Furthermore, the P2X6 subunit has been found, often together with the P2X4 subunit, in epithelial cells of various organs (renal tubule, bronchi, thymus, umbilical vein) but also in gland cells of the uterus and granulosa cells of the ovary [200, 270, 336, 381]. It is well established that the P2X6 subunit is unable to homo-oligomerize effectively, and the frequent co-localization of P2X6 with P2X4 or P2X2 subunits suggests the formation of heteromeric P2X2/6 and P2X4/6 channels [45, 203, 204, 495].

The P2X6 gene comprises 12 exons and lies on chromosome 16 in mouse, chromosome 11 in rat, and chromosome

22 in humans [492]. Four alternatively spliced transcripts, partially showing different expression patterns, have been described for the human P2X6 receptor [465, 492, 496]. In the first splice form, designated AL1, a portion of exon 1, encoding a part of the TM1, is eliminated. The second splice variant, AL2, lacks exon 10, whereas the third splice form, AL3, misses exons 10–11, resulting in truncated P2X6 proteins lacking the TM2 [492, 496]. A fourth splice variant of the P2X6 receptor, lacking exon 4, has later been found together with the full-length transcript in human CF and non-CF airway epithelial cell lines [465]. Interestingly, aberrant splicing patterns or even no P2X6 mRNA expression have been demonstrated in various soft tissues sarcomas, suggesting a role for P2X6 receptor in tumorigenesis [496].

An alternatively spliced P2X6 variant has also been identified in mouse. This splicing product misses exon 8 and most probably gives rise to a non-functional protein, which lacks a portion of the extracellular loop, the entire TM2, and the intracellular domain [382, 497]. Although both the full-length P2X6 transcript and the alternatively spliced form are present during mouse postnatal development and in adult brain, the expression level of the full-length form is much higher. In contrast, the splice variant appears to be the predominant form expressed during neuronal differentiation of P19 embryonal carcinoma cells [497]. The functional significance of this alternative splice product in mice is not clear, but it might regulate P2X6 receptor activity during the process of neuronal differentiation [497].

Since neither P2X6 knockout nor transgenic animals have been generated so far, the in vivo function of the P2X6 receptor remains poorly understood. Nevertheless, in human airway epithelial cells, siRNA-mediated knockdown of P2X6 subunits resulted in significant inhibition of zinc-induced Ca^{2+} entry [465]. As mentioned previously, the same phenotype has been obtained with P2X4-specific siRNA, thus indicating that both subunits may coassemble and function as heteromeric P2X4/P2X6 receptors [465]. Moreover, the re-appearance of P2X6 expression in regenerating muscle fibers from both Duchenne muscular dystrophy patients and dystrophin-deficient mice (mdx) was observed, and an involvement of P2X6 receptors in the regeneration of dystrophic muscles has been suggested [382]. In addition, marked upregulation of the P2X6 subunit in myocardial tissue from chronic heart failure patients was reported, indicating that P2X6 receptors may contribute to the progression of this disease [498]. A role for P2X6 subunits in the differentiation of mesenchymal stem cells has recently been proposed [499].

P2X7

The cDNA encoding the P2X7 receptor (originally termed P2Z) was first cloned from a rat brain cDNA library and subsequently from different tissues of various species, including human monocytes, mouse microglial cells, *X. laevis* stomach, and more recently, guinea pig brain [125, 133, 294, 500, 501]. The P2X7 channel is mainly expressed on cells of hematopoietic origin (monocytes, macrophages, lymphocytes, dendritic cells, mast cells) as well as on different types of glial cells present in the central (microglia, astrocytes, oligodendrocytes, ependymal cells) and the peripheral (Schwann cells, satellite cells, enteric glial cells) nervous systems [125, 332, 369, 449, 502–509]. In addition, it is widely distributed on various epithelial and endothelial cells [381, 510–513]. Although P2X7 mRNA has been detected in neurons and several groups have reported P2X7 antibody staining in neurons [514–520], the presence of P2X7 protein and its function in neurons remains a matter of controversial debate. This is partly due to the poor selectivity of the used antibodies and lack of selective ligands [312, 339, 521]. Experiments on P2X7−/− mouse lines suggest that the antibodies detect an unspecific or "P2X7-like" protein in neurons that could be clearly differentiated from the P2X7 protein by molecular size comparison. The currently identified rodent splice variants or potential rodent orthologues of the identified human splice variants cannot account for these observations.

The gene encoding the P2X7 receptor consists of 13 exons and lies in tandem with the P2X4 gene on human and rat chromosome 12 and murine chromosome 5 [522]. In humans, nine different splice variants, P2X7B-J, have been identified so far [308, 523, 524]. Four of these, P2X7B, P2X7E, P2X7G, and P2X7I, contain a retained intron 10 with a premature stop codon leading to C-terminally truncated P2X7 forms. P2X7G and P2X7H contain an alternative exon N3 with a new start codon that leads to the translation of non-functional P2X7 proteins lacking TM1 [523]. In addition, removal of one or more of the 13 P2X7A exons occurs in some variants [523]. The P2X7J variant is truncated downstream of exon 7 (encoding part of the extracellular loop) and non-functional on its own [524]. In heterooligomeric complexes with P2X7A, it displays dominant negative properties [524] and has been reported to inhibit P2X7A-induced apoptosis, thus contributing to uncontrolled growth of cancer cells. In contrast, heterooligomerization of P2X7B with P2X7A has been demonstrated to potentiate P2X7 receptor responses and exert trophic effects [308]. In rodents, three different splice variants have been identified [300, 525]. The fully functional P2X7K variant is derived from an alternative exon 1', within intron 1 of the rodent P2X7 gene and contains an alternative N terminus and TM1 (Fig. 4). The P2X7K channel shows higher agonist sensitivity, slower deactivation kinetics, and increased pore formation activity [300]. More recently, two novel splice variants in mouse were reported that utilize alternative exons 13b or 13c and encode different

C-terminally truncated P2X7 isoforms. The expression pattern of both splice products, but especially P2X713b, has been shown to overlap to a large extent with P2X7A. Moreover, both isoforms exhibited relatively small current responses and poor plasma membrane delivery. The P2X713b isoform was shown to form heteromeric complexes with P2X7A and downregulate its function [525].

Besides alternatively spliced forms, over 650 SNPs have been reported in the human P2X7 gene [526]. Some of these were found to confer loss- or gain-of-function phenotypes, and several of these P2X7 genetic variants have been associated with higher susceptibility to diseases, including infections with intracellular pathogens (e.g., tuberculosis, toxoplasmosis), chronic lymphocytic leukemia, diabetes, and mood disorders [527–538]. However, these genetic associations could not always be replicated across different populations [539–543].

The physiological function of P2X7 receptors has been investigated in three independently generated P2X7−/− mouse models [323, 339, 544, 545]. In the mouse line established by Glaxo, the P2X7 gene was disrupted by targeted insertion of a lacZ/Neo reporter cassette into exon 1, 2 bp downstream of the ATG start codon (Fig. 4) [339, 544, 546]. Later analysis, however, demonstrated that this knockout strategy does not result in complete inactivation of the P2X7 gene since translation of the P2X7K splice variant is not prevented (Fig. 4) [300]. This is in agreement with the observation that T cells from Glaxo P2X7 KO mice exhibit fully functional P2X7 responses [546]. In the mouse line produced by Pfizer, a portion of exon 13, encoding Cys506 to Pro532, has been deleted and replaced with a neomycin resistance cassette (Fig. 4) [323]. Also about this mouse model, concerns have been raised because a C-terminally deleted P2X7 receptor could theoretically still be expressed. Expression of a "P2X7-like" protein has been reported in the brain of these mice and could represent either an unknown P2X7 splice variant or a novel protein, with a similar antibody epitope [547, 548]. In addition, the possibility that the recently described C-terminally truncated variants of the P2X7 receptor (P2X713b, P2X713c) escape the inactivation strategy cannot be excluded either [525].

Despite the described limitations, the existing P2X7 receptor knockout mouse models greatly contributed to our understanding of the physiological function of the P2X7 receptor. The Pfizer P2X7-deficient mice have established an important role for P2X7 receptor-mediated signalling in cytokine production and inflammation [322, 323]. It was demonstrated that LPS-activated macrophages from P2X7−/− mice failed to process pro-IL-1β and consequently did not release mature IL-1β in response to ATP treatment [323]. The same effect was found with ATP-challenged and LPS-primed leukocytes derived from P2X7−/− blood samples [322]. Using a monoclonal antibody-induced arthritis model, it was further demonstrated that P2X7-deficient mice exhibited diminished inflammatory responses and reduced cartilage destruction [322].

In agreement with an important role in inflammation, analysis of the P2X7 KO mice developed by Glaxo [321] showed a complete elimination of hypersensitivity to both inflammatory (intraplantar injection of Freund's complete adjuvant) and neuropathic (partial ligation of the sciatic nerve) chronic pain states and a marked reduction in mature IL-1β production. A similar effect was also observed for LPS-activated microglia in the dorsal horn of rats [549]. The relevance of P2X7 receptors in the development of neuropathic pain was supported by experiments demonstrating an increase in P2X7 receptor expression in injured nerves from patients suffering from neuropathic pain [321]. Importantly, administration of P2X7-specific antagonists was shown to mimic the knockout phenotype in rodents and thus further established the P2X7 receptor as a therapeutic target [152, 158, 160–162].

In contrast to the above findings, it was recently shown that the P2X7 receptor does not play a role in bone cancer pain. In fact, the Pfizer P2X7 KO mice used in this study exhibited a more severe pain phenotype. This unexpected result can be explained by the different nociceptive mechanism in bone cancer pain that, in contrast to inflammatory or neuropathic pain, does not involve immune cell activation. The results thus underline the important role of microglia activation in neuropathic pain [550, 551].

The Pfizer P2X7−/− mice also developed skeletal abnormalities and revealed the involvement of P2X7 receptors in periosteal bone formation and trabecular bone remodeling [552]. Further studies demonstrated a reduction of sensitivity to mechanical loading in these mice, suggesting a role for P2X7 channels in bone mechanotransduction [553]. Regulation of osteoclast homeostasis by P2X7 receptors has also been demonstrated in vitro. For instance, blocking of the P2X7 receptor with either oxidized ATP or monoclonal antibodies in cultured human osteoclasts resulted in marked inhibition of the mononuclear preosteoclast fusion and their differentiation into multinucleated osteoclasts [554]. In contrast, no bone phenotype has been observed in the Glaxo P2X7 KO animals, suggesting that, in bone tissue, the P2X7K isoform may compensate for the lack of the P2X7A receptor [555].

Analysis of the Pfizer P2X7−/− mice further demonstrated that elimination of P2X7 receptors, in males but not in females, leads to reduced fluid secretion in salivary gland and pancreas but increased secretion in lacrimal glands [556, 557]. Hence, it has been suggested that P2X7 channel might also play a role in the regulation of exocrine gland secretion.

Several studies suggest that P2X7-mediated signalling contributes to neurodegenerative processes observed in CNS

diseases, including multiple sclerosis, Alzheimer's, and Parkinson's diseases [558–561]. Upregulation of P2X7 receptor expression has frequently been reported in animal models and patients suffering from neurodegenerative conditions [560, 562–564]. An increased expression of P2X7 receptors, altered calcium-signalling, and increased susceptibility to apoptosis was reported in neurons from a mice model of Huntington's disease [565]. Inhibition of P2X7 receptors in rats was shown to reduce neuronal degeneration and improve recovery after spinal cord injury [566]. Analysis of Glaxo P2X7−/− mice in an induced model of multiple sclerosis demonstrated a markedly reduced incident of disease and highlighted a role for astroglial P2X7 receptors in disease progression [561]. Furthermore, antagonist-mediated P2X7 receptor inhibition resulted in suppression of symptoms associated with the disease [560]. Contrary to these findings, Pfizer P2X7−/− mice were reported to be more susceptible to the disease and exhibited enhanced inflammation in CNS when compared with wild-type controls [567]. Also, in studies on the role of P2X7 receptors in Parkinson's disease, conflicting results have been obtained [559, 568]. Further studies are needed to validate the existing findings and to confirm the potential of the P2X7 receptor as a therapeutic target for the treatment of neurodegenerative diseases.

Genetic studies showed an association between the Q460R P2X7 polymorphism with mood disorders. [527, 534, 535]. A possible link to P2X7 receptor function is provided by the implication of pro-inflammatory cytokines, including IL-1β in the etiopathogenesis of "sickness behavior" in mice and depression in humans [569–572]. To further test a role of P2X receptors in mood disorders, P2X7−/− mice were assayed in different behavioral models of depression [545, 573]. Using a novel mouse line generated at Lexicon Genetics Inc. (Fig. 4), an anti-depressant-like phenotype was found [545], and in the Pfizer−/− mouse line, an impaired adaptive coping response to repeated stress was observed [573].

It was also reported that P2X7 receptors, by affecting neurotransmitters release, modulate synaptic activity and neuron-glia signalling in the brain. P2X7 receptors on murine cortical astrocytes were shown to contribute to the release of excitatory amino acids, such as glutamate and aspartate [574], and in hippocampal slices from P2X7−/− mice, the ATP-induced efflux of GABA and glutamate was found to be attenuated in comparison to wild-type animals [575]. A more recent study showed induction of IL-1β mRNA expression in the hippocampus after a spatial memory task in wt but not P2X7−/− mice [576]. Another study on P2X7-/- mice suggested the involvement of P2X7 receptors in sleep via release of cytokines and other sleep regulatory substances [577].

P2X7 receptor-mediated signalling has also been postulated to play a role in cancer physiology. Although unusually high levels of P2X7 receptor have been found in diverse tumors and the P2X7 receptor has been proposed as a novel cancer biomarker, its link to cancer remains unclear [488, 509, 578–583]. Nevertheless, some recent studies have demonstrated a positive correlation between P2X7 receptor expression/activation and tumor metastasis [269, 584], and activation of P2X7 receptors was shown to promote invasiveness of aggressive human breast cancer cells [584]. In agreement with this finding, both short hairpin RNA-mediated silencing of P2X7 mRNA or block of P2X7 receptors with antibodies resulted in attenuated metastasis of murine lymphoid neoplasm P388D1 cells [585].

An ortholog of the P2X7 gene has been identified on zebrafish chromosome 8 [429] and two transcript variants, containing 14 and 16 exons, respectively, have been deposited at the Ensembl database. A widespread distribution of the zP2X7 receptor in non-nervous tissues of zebrafish embryo has been reported [429].

Despite such a broad utility of used genetic technologies, the interspecies differences have to be taken into account when translating phenotypic data from animals to humans [80, 526]. Importantly, marked differences in P2X7 receptor function exist between rodents and human and moreover even between different mouse strains [125, 133, 526]. For instance, the naturally occurring allelic polymorphism P451L located in the P2X7 cytoplasmic domain has been shown to significantly impair function of the channel [305]. It has been reported that human, rat, and the BALB/c and 129/Sv mouse strains carry the high-activity variant P451, whereas the C57BL/6 and DBA/2 mouse strains have the low-activity allele L451 [305, 546]. This finding is essential for the interpretation of phenotypic changes observed for example in lymphocytes from P2X7 KO mice [546]. For more detailed information about pharmacological differences between various mammalian P2X7 receptors, see also the section "Pharmacological characteristics of P2X receptors" and refer to Donnelly-Roberts et al. [79].

Concluding remarks

Following the postulation of a class of ATP-gated ion channels about 20 years ago [3], P2X receptors have repeatedly amazed us as a surprisingly unusual class of ligand-gated ion channels. Cloning of the first subtypes and subsequent biochemical and functional analysis of heterologously expressed wt and mutant receptors has revealed a completely novel ion channel structure with various unexpected properties. The long-awaited first crystal structure of a P2X receptor confirmed many of the predictions based on mutagenesis studies and started a new era in which mutations can be planned and results can be explained based on this structure or subtype-specific homology models. Despite

this great progress, the movements during channel opening and desensitization are still unclear, and additional crystal structures in the ligand-bound and open states as well as studies investigating the receptor dynamics, such as voltage clamp fluorometry, would greatly help to understand these processes. In particular, the molecular mechanisms underlying subtype-specific molecular functions such as pore dilation and plasma membrane morphology changes remain absolutely enigmatic, and the elucidation of these and other receptor functions that most likely involve interactions with additional and so far mostly unidentified proteins might constitute even more challenging tasks.

Also, the phylogenetic origin of this channel family remains a mystery. Another exciting discovery was that P2X-receptors in evolutionary old organisms serve intracellular functions raising the intriguing and so far hardly addressed possibility of intracellular functions in vertebrates. After many basic principles of P2X receptor function and consequences of their activation have been worked out on the cellular level, the generation of genetically modified animal models now opens opportunities for in vivo studies. For five of the seven P2X receptor subtypes, KO mice have been generated and have confirmed the involvement of these receptors in pathological conditions such as neuropathic pain, inflammation, and thrombosis, to only name a few. P2X transgenic animal models are currently being created. Thus, multiple tools are now emerging that help to decipher the physiological functions of these receptors and their validation as drug targets. Both the advances in understanding the molecular structure and function of these receptors as well as the increasing availability of animal models will greatly accelerate the processes of drug development. However, species differences in the physiology and pharmacology of these receptors as well as the presence of receptor isoforms must be considered and might turn out to be of particular relevance for this puzzling receptor class.

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References

1. Yamagata Y (1999) Prebiotic formation of ADP and ATP from AMP, calcium phosphates and cyanate in aqueous solution. Orig Life Evol Biosph 29(5):511–520
2. Burnstock G (1972) Purinergic nerves. Pharmacol Rev 24(3):509–581
3. Abbracchio MP, Burnstock G (1994) Purinoceptors: are there families of P2X and P2Y purinoceptors? Pharmacol Ther 64 (3):445–475
4. Burnstock G, Kennedy C (1985) Is there a basis for distinguishing two types of P2-purinoceptor? Gen Pharmacol 16(5):433–440
5. Fredholm BB, Abbracchio MP, Burnstock G, Daly JW, Harden TK, Jacobson KA, Leff P, Williams M (1994) Nomenclature and classification of purinoceptors. Pharmacol Rev 46(2):143–156
6. Burnstock G, Fredholm BB, North RA, Verkhratsky A (2010) The birth and postnatal development of purinergic signalling. Acta Physiologica 199(2):93–147
7. Burnstock G (2004) Introduction: P2 receptors. Curr Top Med Chem 4(8):793–803
8. Burnstock G (2008) Purinergic signalling and disorders of the central nervous system. Nat Rev Drug Discov 7(7):575–590
9. Burnstock G (2008) Unresolved issues and controversies in purinergic signalling. J Physiol 586(14):3307–3312
10. Burnstock G, Kennedy C (2011) P2X receptors in health and disease. Adv Pharmacol 61:333–372
11. Coddou C, Stojilkovic SS, Huidobro-Toro JP (2011) Allosteric modulation of ATP-gated P2X receptor channels. Rev Neurosci 22(3):335–354
12. Coddou C, Yan Z, Obsil T, Huidobro-Toro JP, Stojilkovic SS (2011) Activation and regulation of purinergic P2X receptor channels. Pharmacol Rev 63(3):641–683
13. Egan T, Samways D, Li Z (2006) Biophysics of P2X receptors. Pflügers Archiv Eur J Physiol 452(5):501–512
14. Egan TM, Cox JA, Voigt MM (2004) Molecular structure of P2X receptors. Curr Top Med Chem 4(8):821–829
15. Evans RJ (2009) Orthosteric and allosteric binding sites of P2X receptors. Eur Biophys J 38(3):319–327
16. Evans RJ (2010) Structural interpretation of P2X receptor mutagenesis studies on drug action. Br J Pharmacol 161(5):961–971
17. Jarvis MF, Khakh BS (2009) ATP-gated P2X cation-channels. Neuropharmacology 56(1):208–215
18. Khakh BS (2001) Molecular physiology of P2X receptors and ATP signalling at synapses. Nat Rev Neurosci 2(3):165–174
19. North RA (2002) Molecular physiology of P2X receptors. Physiol Rev 82(4):1013–1067
20. Roberts J, Vial C, Digby H, Agboh K, Wen H, Atterbury-Thomas A, Evans R (2006) Molecular properties of P2X receptors. Pflügers Archiv Eur J Physiol 452(5):486–500
21. Surprenant A, North RA (2009) Signaling at purinergic P2X receptors. Annu Rev Physiol 71:333–359
22. Brake AJ, Wagenbach MJ, Julius D (1994) New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. Nature 371(6497):519–523
23. Valera S, Hussy N, Evans RJ, Adami N, North RA, Surprenant A, Buell G (1994) A new class of ligand-gated ion channel defined by P2x receptor for extracellular ATP. Nature 371(6497):516–519
24. Fountain SJ, Burnstock G (2009) An evolutionary history of P2X receptors. Purinergic Signal 5(3):269–272
25. Agboh KC, Webb TE, Evans RJ, Ennion SJ (2004) Functional characterization of a P2X receptor from *Schistosoma mansoni*. J Biol Chem 279(40):41650–41657
26. Fountain SJ, Cao L, Young MT, North RA (2008) Permeation properties of a P2X receptor in the green algae *Ostreococcus tauri*. J Biol Chem 283(22):15122–15126
27. Fountain SJ, Parkinson K, Young MT, Cao L, Thompson CR, North RA (2007) An intracellular P2X receptor required for osmoregulation in *Dictyostelium discoideum*. Nature 448 (7150):200–203
28. Courties C, Vaquer A, Troussellier M, Lautier J, Chrétiennot-Dinet M (1994) Smallest eukaryotic organism. Nature 370:255–255

29. Derelle E, Ferraz C, Rombauts S, Rouze P, Worden AZ, Robbens S, Partensky Fdr, Degroeve S, Echeyni S, Cooke R, Saeys Y, Wuyts J, Jabbari K, Bowler C, Panaud O, Piegu BÆ, Ball SG, Ral J-P, Bouget Fo-Y, Piganeau G, De Baets B, Picard A, Delseny M, Demaille J, Van de Peer Y, Moreau H (2006) Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. Proc Natl Acad Sci U S A 103(31):11647–11652
30. Ludlow MJ, Durai L, Ennion SJ (2009) Functional characterization of intracellular *Dictyostelium discoideum* P2X receptors. J Biol Chem 284(50):35227–35239
31. Burnstock G, Verkhratsky A (2009) Evolutionary origins of the purinergic signalling system. Acta Physiologica 195(4):415–447
32. Harte R, Ouzounis CA (2002) Genome-wide detection and family clustering of ion channels. FEBS Lett 514(2–3):129–134
33. Kim SY, Sivaguru M, Stacey G (2006) Extracellular ATP in plants. Visualization, localization, and analysis of physiological significance in growth and signaling. Plant Physiol 142 (3):984–992
34. Tanaka K, Gilroy S, Jones AM, Stacey G (2010) Extracellular ATP signaling in plants. Trends Cell Biol 20(10):601–608
35. King N, Westbrook MJ, Young SL, Kuo A, Abedin M, Chapman J, Fairclough S, Hellsten U, Isogai Y, Letunic I, Marr M, Pincus D, Putnam N, Rokas A, Wright KJ, Zuzow R, Dirks W, Good M, Goodstein D, Lemons D, Li W, Lyons JB, Morris A, Nichols S, Richter DJ, Salamov A, Sequencing JG, Bork P, Lim WA, Manning G, Miller WT, McGinnis W, Shapiro H, Tjian R, Grigoriev IV, Rokhsar D (2008) The genome of the choanoflagellate *Monosiga brevicollis* and the origin of metazoans. Nature 451(7180):783–788
36. Bavan S, Straub V, Blaxter M, Ennion S (2009) (A P2X receptor from the tardigrade species *Hypsibius dujardini* with fast kinetics and sensitivity to zinc and copper. BMC Evol Biol 9:17
37. Bavan S, Farmer L, Singh SK, Straub VA, Guerrero FD, Ennion SJ (2011) The penultimate arginine of the carboxyl terminus determines slow desensitization in a P2X receptor from the cattle tick *Boophilus microplus*. MolPharmacol 79(4):776–785
38. Bavan S, Straub VA, Ennion SJ (2008) Pharmacological characterisation of a P2X receptor cloned from the central nervous system of *Lymnaea stagnalis*. Purinergic Signal 4(Suppl1):1–210
39. Moroz LL, Edwards JR, Puthanveettil SV, Kohn AB, Ha T, Heyland A, Knudsen B, Sahni A, Yu F, Liu L, Jezzini S, Lovell P, Iannucculli W, Chen M, Nguyen T, Sheng H, Shaw R, Kalachikov S, Panchin YV, Farmerie W, Russo JJ, Ju J, Kandel ER (2006) Neuronal transcriptome of aplysia: neuronal compartments and circuitry. Cell 127 (7):1453–1467
40. Clyne JD, Wang LF, Hume RI (2002) Mutational analysis of the conserved cysteines of the rat P2X2 purinoceptor. J Neurosci 22 (10):3873–3880
41. Boue-Grabot E, Archambault V, Seguela P (2000) A protein kinase C site highly conserved in P2X subunits controls the desensitization kinetics of P2X2 ATP-gated channels. J Biol Chem 275(14):10190–10195
42. Bean BP (1990) ATP-activated channels in rat and bullfrog sensory neurons: concentration dependence and kinetics. J Neurosci 10(1):1–10
43. Ding S, Sachs F (1999) Single channel properties of P2X2 purinoceptors. J Gen Physiol 113(5):695–720
44. Nicke A, Baumert HG, Rettinger J, Eichele A, Lambrecht G, Mutschler E, Schmalzing G (1998) P2X1 and P2X3 receptors form stable trimers: a novel structural motif of ligand-gated ion channels. EMBO J 17(11):3016–3028
45. Barrera NP, Ormond SJ, Henderson RM, Murrell-Lagnado RD, Edwardson JM (2005) Atomic force microscopy imaging demonstrates that P2X2 receptors are trimers but that P2X6 receptor subunits do not oligomerize. J Biol Chem 280(11):10759–10765
46. Mio K, Kubo Y, Ogura T, Yamamoto T, Sato C (2005) Visualization of the trimeric P2X2 receptor with a crown-capped extracellular domain. Biochem Biophys Res Commun 337 (3):998–1005
47. Young MT, Fisher JA, Fountain SJ, Ford RC, North RA, Khakh BS (2008) Molecular shape, architecture, and size of P2X4 receptors determined using fluorescence resonance energy transfer and electron microscopy. J Biol Chem 283(38):26241–26251
48. Kawate T, Michel JC, Birdsong WT, Gouaux E (2009) Crystal structure of the ATP-gated P2X(4) ion channel in the closed state. Nature 460(7255):592–598
49. Gonzales EB, Kawate T, Gouaux E (2009) Pore architecture and ion sites in acid-sensing ion channels and P2X receptors. Nature 460(7255):599–604
50. Jasti J, Furukawa H, Gonzales EB, Gouaux E (2007) Structure of acid-sensing ion channel 1 at 1.9A resolution and low pH. Nature 449(7160):316–323
51. Nicke A, King BF (2006) Heteromerization of P2X receptors. In: Arias HR (ed) Biological and biophysical aspects of ligand-gated ion channel receptor superfamilies. Research Signpost, Kerala, pp 383–417
52. Boumechache M, Masin M, Edwardson JM, Gorecki DC, Murrell-Lagnado R (2009) Analysis of assembly and trafficking of native P2X4 and P2X7 receptor complexes in rodent immune cells. J Biol Chem 284(20):13446–13454
53. Torres GE, Egan TM, Voigt MM (1999) Hetero-oligomeric assembly of P2X receptor subunits. J Biol Chem 274(10):6653–6659
54. Aschrafi A, Sadtler S, Niculescu C, Rettinger J, Schmalzing G (2004) Trimeric architecture of homomeric P2X2 and heteromeric P2X1+2 receptor subtypes. J Mol Biol 342(1):333–343
55. Bo X, Jiang L-H, Wilson HL, Kim M, Burnstock G, Surprenant A, North RA (2003) Pharmacological and biophysical properties of the human P2X5 receptor. Mol Pharmacol 63(6):1407–1416
56. Cox JA, Barmina O, Voigt MM (2001) Gene structure, chromosomal localization, cDNA cloning and expression of the mouse ATP-gated ionotropic receptor P2X5 subunit. Gene 270(1–2):145–152
57. Diaz-Hernandez M, Cox JA, Migita K, Haines W, Egan TM, Voigt MM (2002) Cloning and characterization of two novel zebrafish P2X receptor subunits. Biochem Biophys Res Commun 295(4):849–853
58. Duckwitz W, Hausmann R, Aschrafi A, Schmalzing G (2006) P2X5 subunit assembly requires scaffolding by the second transmembrane domain and a conserved aspartate. J Biol Chem 281 (51):39561–39572
59. Jensik PJ, Holbird D, Collard MW, Cox TC (2001) Cloning and characterization of a functional P2X receptor from larval bullfrog skin. Am J Physiol Cell Physiol 281(3):C954–C962
60. Le KT, Paquet M, Nouel D, Babinski K, Seguela P (1997) Primary structure and expression of a naturally truncated human P2X ATP receptor subunit from brain and immune system. FEBS Lett 418(1–2):195–199
61. Ruppelt A, Ma W, Borchardt K, Silberberg SD, Soto F (2001) Genomic structure, developmental distribution and functional properties of the chicken P2X(5) receptor. J Neurochem 77 (5):1256–1265
62. Wildman SS, Brown SG, Rahman M, Noel CA, Churchill L, Burnstock G, Unwin RJ, King BF (2002) Sensitization by extracellular Ca2+ of rat P2X5 receptor and its pharmacological properties compared with rat P2X1. Mol Pharmacol 62(4):957–966
63. Jiang L-H, Kim M, Spelta V, Bo X, Surprenant A, North RA (2003) Subunit arrangement in P2X receptors. J Neurosci 23 (26):8903–8910
64. Wilkinson WJ, Jiang L-H, Surprenant A, North RA (2006) Role of ectodomain lysines in the subunits of the heteromeric P2X2/3 receptor. Mol Pharmacol 70(4):1159–1163

65. Cockayne DA, Dunn PM, Zhong Y, Rong W, Hamilton SG, Knight GE, Ruan H-Z, Ma B, Yip P, Nunn P, McMahon SB, Burnstock G, Ford APDW (2005) P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. J Physiol 567(2):621–639
66. Gever J, Cockayne D, Dillon M, Burnstock G, Ford A (2006) Pharmacology of P2X channels. Pflugers Arch 452(5):513–537
67. Lalo U, Pankratov Y, Wichert SP, Rossner MJ, North RA, Kirchhoff F, Verkhratsky A (2008) P2X1 and P2X5 subunits form the functional P2X receptor in mouse cortical astrocytes. J Neurosci 28(21):5473–5480
68. Ennion SJ, Evans RJ (2002) Conserved cysteine residues in the extracellular loop of the human P2X(1) receptor form disulfide bonds and are involved in receptor trafficking to the cell surface. Mol Pharmacol 61(2):303–311
69. Bodnar M, Wang H, Riedel T, Hintze S, Kato E, Fallah G, Groger-Arndt H, Giniatullin R, Grohmann M, Hausmann R, Schmalzing Gn, Illes P, Rubini P (2011) Amino acid residues constituting the agonist binding site of the human P2X3 receptor. J Biol Chem 286(4):2739–2749
70. Fischer W, Zadori Z, Kullnick Y, Grˆger-Arndt H, Franke H, Wirkner K, Illes P, Mager PP (2007) Conserved lysin and arginin residues in the extracellular loop of P2X3 receptors are involved in agonist binding. Eur J Pharmacol 576(1–3):7–17
71. Jiang L-H, Fo R, Surprenant A, North RA (2000) Identification of amino acid residues contributing to the ATP-binding site of a purinergic P2X receptor. J Biol Chem 275(44):34190–34196
72. Roberts JA, Digby HR, Kara M, Ajouz SE, Sutcliffe MJ, Evans RJ (2008) Cysteine substitution mutagenesis and the effects of methanethiosulfonate reagents at P2X2 and P2X4 receptors support a core common mode of ATP action at P2X receptors. J Biol Chem 283(29):20126–20136
73. Yan Z, Liang Z, Tomic M, Obsil T, Stojilkovic SS (2005) Molecular determinants of the agonist binding domain of a P2X receptor channel. Mol Pharmacol 67(4):1078–1088
74. Zemkova H, Yan Z, Liang Z, Jelinkova I, Tomic M, Stojilkovic SS (2007) Role of aromatic and charged ectodomain residues in the P2X(4) receptor functions. J Neurochem 102(4):1139–1150
75. Ennion S, Hagan S, Evans RJ (2000) The role of positively charged amino acids in ATP recognition by human P2X(1) receptors. J Biol Chem 275(38):29361–29367
76. Roberts JA, Evans RJ (2004) ATP binding at human P2X1 receptors. Contribution of aromatic and basic amino acids revealed using mutagenesis and partial agonists. J Biol Chem 279(10):9043–9055
77. Roberts JA, Evans RJ (2006) Contribution of conserved polar glutamine, asparagine and threonine residues and glycosylation to agonist action at human P2X1 receptors for ATP. J Neurochem 96 (3):843–852
78. Roberts JA, Valente M, Allsopp RC, Watt D, Evans RJ (2009) Contribution of the region Glu181 to Val200 of the extracellular loop of the human P2X1 receptor to agonist binding and gating revealed using cysteine scanning mutagenesis1. J Neurochem 109(4):1042–1052
79. Donnelly-Roberts DL, Namovic MT, Han P, Jarvis MF (2009) Mammalian P2X7 receptor pharmacology: comparison of recombinant mouse, rat and human P2X7 receptors. Br J Pharmacol 157 (7):1203–1214
80. Hibell AD, Kidd EJ, Chessell IP, Humphrey PPA, Michel AD (2000) Apparent species differences in the kinetic properties of P2X7 receptors. Br J Pharmacol 130(1):167–173
81. Hibell AD, Thompson KM, Xing M, Humphrey PP, Michel AD (2001) Complexities of measuring antagonist potency at P2X(7) receptor orthologs. J Pharmacol Exp Ther 296(3):947–957
82. Young MT, Pelegrin P, Surprenant A (2006) Identification of Thr283 as a key determinant of P2X7 receptor function. Br J Pharmacol 149(3):261–268
83. Marquez-Klaka B, Rettinger J, Bhargava Y, Eisele T, Nicke A (2007) Identification of an intersubunit cross-link between substituted cysteine residues located in the putative ATP binding site of the P2X1 receptor. J Neurosci 27(6):1456–1466
84. Browne LE, Jiang LH, North RA (2010) New structure enlivens interest in P2X receptors. Trends PharmacolSci 31(5):229–237
85. Allsopp RC, El Ajouz S, Schmid R, Evans RJ (2011) Cysteine scanning mutagenesis (residues E52-G96) of the human P2X1 receptor for ATP; mapping agonist binding and channel gating. J Biol Chem 286(33):29207–29217
86. Jiang R, Lemoine D, Martz A, Taly A, Gonin S, Prado de Carvalho L, Specht A, Grutter T (2011) Agonist trapped in ATP-binding sites of the P2X2 receptor. Proc Natl Acad Sci U S A 108: 9066-9071
87. Buell G, Lewis C, Collo G, North RA, Surprenant A (1996) An antagonist-insensitive P2X receptor expressed in epithelia and brain. EMBO J 15(1):55–62
88. Garcia-Guzman M, Stuhmer W, Soto F (1997) Molecular characterization and pharmacological properties of the human P2X3 purinoceptor. Brain Res Mol Brain Res 47(1–2):59–66
89. Jones CA, Chessell IP, Simon J, Barnard EA, Miller KJ, Michel AD, Humphrey PP (2000) Functional characterization of the P2X (4) receptor orthologues. Br J Pharmacol 129(2):388–394
90. North RA, Surprenant A (2000) Pharmacology of cloned P2X receptors. Annu Rev Pharmacol Toxicol 40:563–580
91. Sim JA, Broomhead HE, North RA (2008) Ectodomain lysines and suramin block of P2X1 receptors. J Biol Chem 283(44):29841–29846
92. El-Ajouz S, Ray D, Allsopp RC, Evans RJ (2012) Molecular basis of selective antagonism of the P2X1 receptor for ATP by NF449 and suramin; contribution of basic amino acids in the cysteine rich loop. Br J Pharmacol 165:390-400
93. Wolf C, Rosefort C, Fallah G, Kassack MU, Hamacher A, Bodnar M, Wang H, Illes P, Kless A, Bahrenberg G, Schmalzing G, Hausmann R (2011) Molecular Determinants of Potent P2X2 Antagonism Identified by Functional Analysis, Mutagenesis, and Homology Docking. Mol Pharmacol 79(4):649–661
94. Egan TM, Haines WR, Voigt MM (1998) A domain contributing to the ion channel of ATP-gated P2X2 receptors identified by the substituted cysteine accessibility method. J Neurosci 18(7):2350–2359
95. Jelinkova I, Vavra V, Jindrichova M, Obsil T, Zemkova HW, Zemkova H, Stojilkovic SS (2008) Identification of P2X(4) receptor transmembrane residues contributing to channel gating and interaction with ivermectin. Pflugers Arch 456(5):939–950
96. Migita K, Haines WR, Voigt MM, Egan TM (2001) Polar residues of the second transmembrane domain influence cation permeability of the ATP-gated P2X(2) receptor. J Biol Chem 276 (33):30934–30941
97. Rassendren F, Buell G, Newbolt A, North RA, Surprenant A (1997) Identification of amino acid residues contributing to the pore of a P2X receptor. EMBO J 16(12):3446–3454
98. Samways DSK, Migita K, Li Z, Egan TM (2008) On the role of the first transmembrane domain in cation permeability and flux of the ATP-gated P2X2 receptor. J Biol Chem 283(8):5110–5117
99. Cao L, Broomhead HE, Young MT, North RA (2009) Polar residues in the second transmembrane domain of the rat P2X2 receptor that affect spontaneous gating, unitary conductance, and rectification. J Neurosci 29(45):14257–14264
100. Keceli B, Kubo Y (2009) Functional and structural identification of amino acid residues of the P2X2 receptor channel critical for the voltage- and [ATP]-dependent gating. J Physiol 587(24):5801–5818

101. Kracun S, Chaptal V, Abramson J, Khakh BS (2010) Gated access to the pore of a P2X receptor. J Biol Chem 285(13):10110–10121
102. Li M, Chang TH, Silberberg SD, Swartz KJ (2008) Gating the pore of P2X receptor channels. Nat Neurosci 11(8):883–887
103. Kawate T, Robertson JL, Li M, Silberberg SD, Swartz KJ (2011) Ion access pathway to the transmembrane pore in P2X receptor channels. J Gen Physiol 137(6):579–590
104. Samways DSK, Khakh BS, Dutertre S, Egan TM (2011) Preferential use of unobstructed lateral portals as the access route to the pore of human ATP-gated ion channels (P2X receptors). Proc Natl Acad Sci U S A 108(33):13800–13805
105. Jiang L-H, Rassendren F, Spelta V, Surprenant A, North RA (2001) Amino acid residues involved in gating identified in the first membrane-spanning domain of the rat P2X2 receptor. J Biol Chem 276(18):14902–14908
106. Li M, Kawate T, Silberberg SD, Swartz KJ (2010) Pore-opening mechanism in trimeric P2X receptor channels. Nat Commun 1:44
107. Jiang R, Martz A, Gonin S, Taly A, de Carvalho LP, Grutter T (2011) A putative extracellular salt bridge at the subunit interface contributes to the ion channel function of the ATP-gated P2X2 receptor. J Biol Chem 285(21):15805–15815
108. Rettinger J, Schmalzing G (2003) Activation and desensitization of the recombinant P2X1 receptor at nanomolar ATP concentrations. J Gen Physiol 121(5):451–461
109. Rettinger J, Schmalzing G (2004) Desensitization masks nanomolar potency of ATP for the P2X1 receptor. J Biol Chem 279 (8):6426–6433
110. Pratt EB, Brink TS, Bergson P, Voigt MM, Cook SP (2005) Use-dependent inhibition of P2X3 receptors by nanomolar agonist. J Neurosci 25(32):7359–7365
111. Stojilkovic SS, TomiĆ M, He M-L, Yan Z, Koshimizu T-A, Zemkova H (2005) Molecular dissection of purinergic P2X receptor channels. Ann NY Acad Sci 1048(1):116–130
112. Werner P, Seward EP, Buell GN, North RA (1996) Domains of P2X receptors involved in desensitization. Proc Natl Acad Sci U S A 93(26):15485–15490
113. Neelands TR, Burgard EC, Uchic ME, McDonald HA, Niforatos W, Faltynek CR, Lynch KJ, Jarvis MF (2003) 2′, 3′-*O*-(2,4,6, Trinitrophenyl)-ATP and A-317491 are competitive antagonists at a slowly desensitizing chimeric human P2X3 receptor. Br J Pharmacol 140(1):202–210
114. Zemkova H, He ML, Koshimizu TA, Stojilkovic SS (2004) Identification of ectodomain regions contributing to gating, deactivation, and resensitization of purinergic P2X receptors. J Neurosci 24(31):6968–6978
115. Braendle U, Spielmanns P, Osteroth R, Sim J, Surprenant A, Buell G, Ruppersberg JP, Plinkert PK, Zenner HP, Glowatzki E (1997) Desensitization of the P2X2 receptor controlled by alternative splicing. FEBS Lett 404(2–3):294–298
116. Koshimizu T, Koshimizu M, Stojilkovic SS (1999) Contributions of the C-terminal domain to the control of P2X receptor desensitization. J Biol Chem 274(53):37651–37657
117. Fountain SJ, North RA (2006) A C-terminal lysine that controls human P2X4 receptor desensitization. J Biol Chem 281(22):15044–15049
118. Fujiwara Y, Kubo Y (2006) Regulation of the desensitization and ion selectivity of ATP-gated P2X2 channels by phosphoinositides. J Physiol 576(Pt 1):135–149
119. He ML, Koshimizu TA, Tomic M, Stojilkovic SS (2002) Purinergic P2X(2) receptor desensitization depends on coupling between ectodomain and C-terminal domain. Mol Pharmacol 62 (5):1187–1197
120. Boue-Grabot E, Akimenko MA, Seguela P (2000) Unique functional properties of a sensory neuronal P2X ATP-gated channel from zebrafish. J Neurochem 75(4):1600–1607
121. Franklin C, Braam U, Eisele T, Schmalzing G, Hausmann R (2007) Lack of evidence for direct phosphorylation of recombinantly expressed P2X(2) and P2X(3) receptors by protein kinase C. Purinergic Signal 3(4):377–388
122. Ennion SJ, Evans RJ (2002) P2X(1) receptor subunit contribution to gating revealed by a dominant negative PKC mutant. Biochem Biophys Res Commun 291(3):611–616
123. Ding S, Sachs F (2000) Inactivation of P2X2 purinoceptors by divalent cations. J Physiol 522(2):199–214
124. Soto F, Garcia-Guzman M, Stühmer W (1997) Cloned ligand-gated channels activated by extracellular ATP (P2X receptors). J Membr Biol 160(2):91–100
125. Rassendren F, Buell GN, Virginio C, Collo G, North RA, Surprenant A (1997) The permeabilizing ATP receptor, P2X7. Cloning and expression of a human cDNA. J Biol Chem 272 (9):5482–5486
126. Klapperstück M, Büttner C, Schmalzing G, Markwardt F (2001) Functional evidence of distinct ATP activation sites at the human P2X7 receptor. J Physiol 534(1):25–35
127. Khakh BS, Burnstock G, Kennedy C, King BF, North RA, Seguela P, Voigt M, Humphrey PP (2001) International union of pharmacology. XXIV. Current status of the nomenclature and properties of P2X receptors and their subunits. Pharmacol Rev 53 (1):107–118
128. Adriouch S, Bannas P, Schwarz N, Fliegert R, Guse AH, Seman M, Haag F, Koch-Nolte F (2008) ADP-ribosylation at R125 gates the P2X7 ion channel by presenting a covalent ligand to its nucleotide binding site. FASEB J 22(3):861–869
129. Schwarz N, Fliegert R, Adriouch S, Seman M, Guse A, Haag F, Koch-Nolte F (2009) Activation of the P2X7 ion channel by soluble and covalently bound ligands. Purinergic Signal 5(2):139–149
130. Jacobson K, Costanzi S, Joshi B, Besada P, Shin D, Ko H, Ivanov A, Mamedova L (2006) Agonists and antagonists for P2 receptors. Novartis Found Symp 276:58–68
131. Jacobson K, Costanzi S, Ohno M, Joshi B, Besada P, Xu B, Tchilibon S (2004) Molecular recognition at purine and pyrimidine nucleotide (P2) receptors. Curr Top Med Chem 4:805–819
132. Virginio C, Robertson G, Surprenant A, North RA (1998) Trinitrophenyl-substituted nucleotides are potent antagonists selective for P2X1, P2X3, and heteromeric P2X2/3 receptors. Mol Pharmacol 53(6):969–973
133. Chessell IP, Simon J, Hibell AD, Michel AD, Barnard EA, Humphrey PP (1998) Cloning and functional characterisation of the mouse P2X7 receptor. FEBS Lett 439(1–2):26–30
134. Gargett CE, Wiley JS (1997) The isoquinoline derivative KN-62 a potent antagonist of the P2Z-receptor of human lymphocytes. Br J Pharmacol 120(8):1483–1490
135. Humphreys BD, Virginio C, Surprenant A, Rice J, Dubyak GR (1998) Isoquinolines as antagonists of the P2X7 nucleotide receptor: high selectivity for the human versus rat receptor homologues. Mol Pharmacol 54(1):22–32
136. Hausmann R, Rettinger J, Gerevich Z, Meis S, Kassack MU, Illes P, Lambrecht G, Schmalzing G (2006) The suramin analog 4,4',4",4"'-(carbonylbis(imino-5,1,3-benzenetriylbis (carbonylimino)))tetra-kis-benzenesulfonic acid (NF110) potently blocks P2X3 receptors: subtype selectivity is determined by location of sulfonic acid groups. Mol Pharmacol 69(6):2058–2067
137. Rettinger J, Braun K, Hochmann H, Kassack MU, Ullmann H, Nickel P, Schmalzing G, Lambrecht G (2005) Profiling at recombinant homomeric and heteromeric rat P2X receptors identifies the suramin analogue NF449 as a highly potent P2X1 receptor antagonist. Neuropharmacology 48(3):461–468
138. Rettinger J, Schmalzing G, Damer S, Muller G, Nickel P, Lambrecht G (2000) The suramin analogue NF279 is a novel and potent

antagonist selective for the P2X(1) receptor. Neuropharmacology 39 (11):2044–2053
139. Braun K, Rettinger J, Ganso M, Kassack M, Hildebrandt C, Ullmann H, Nickel P, Schmalzing G, Lambrecht G (2001) NF449: a subnanomolar potency antagonist at recombinant rat P2X1 receptors. Naunyn Schmiedebergs Arch Pharmacol 364 (3):285–290
140. Baqi Y, Hausmann R, Rosefort C, Rettinger J, Schmalzing G, Mueller CE (2011) Discovery of potent competitive antagonists and positive modulators of the P2X2 receptor. J Med Chem 54 (3):817–830
141. Jarvis MF, Burgard EC, McGaraughty S, Honore P, Lynch K, Brennan TJ, Subieta A, van Biesen T, Cartmell J, Bianchi B, Niforatos W, Kage K, Yu H, Mikusa J, Wismer CT, Zhu CZ, Chu K, Lee CH, Stewart AO, Polakowski J, Cox BF, Kowaluk E, Williams M, Sullivan J, Faltynek C (2002) A-317491, a novel potent and selective non-nucleotide antagonist of P2X 3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat. Proc Natl Acad Sci U S A 99(26):17179–17184
142. Gever JR, Soto R, Henningsen RA, Martin RS, Hackos DH, Panicker S, Rubas W, Oglesby IB, Dillon MP, Milla ME, Burnstock G, Ford APDW (2010) AF-353, a novel, potent and orally bioavailable P2X3/P2X2/3 receptor antagonist. Br J Pharmacol 160(6):1387–1398
143. Carter DS, Alam M, Cai H, Dillon MP, Ford APDW, Gever JR, Jahangir A, Lin C, Moore AG, Wagner PJ, Zhai Y (2009) Identification and SAR of novel diaminopyrimidines. Part 1: the discovery of RO-4, a dual P2X3/P2X2/3 antagonist for the treatment of pain. Bioorg Med Chem Lett 19(6): 1628–1631
144. Jahangir A, Alam M, Carter DS, Dillon MP, Bois DJD, Ford APDW, Gever JR, Lin C, Wagner PJ, Zhai Y, Zira J (2009) Identification and SAR of novel diaminopyrimidines. Part 2: the discovery of RO-51, a potent and selective, dual P2X3/P2X2/3 antagonist for the treatment of pain. Bioorg Med Chem Lett 19(6):1632–1635
145. Kaan TKY, Yip PK, Patel S, Davies M, Marchand F, Cockayne DA, Nunn PA, Dickenson AH, Ford APDW, Zhong Y, Malcangio M, McMahon SB (2010) Systemic blockade of P2X3 and P2X2/3 receptors attenuates bone cancer pain behaviour in rats. Brain 133 (9):2549–2564
146. Brotherton-Pleiss CE, Dillon MP, Ford APDW, Gever JR, Carter DS, Gleason SK, Lin CJ, Moore AG, Thompson AW, Villa M, Zhai Y (2010) Discovery and optimization of RO-85, a novel drug-like, potent, and selective P2X3 receptor antagonist. Bioorg Med Chem Lett 20(3):1031–1036
147. Ballini E, Virginio C, Medhurst SJ, Summerfield SG, Aldegheri L, Buson A, Carignani C, Chen YH, Giacometti A, Lago I, Powell AJ, Jarolimek W (2011) Characterization of three diaminopyrimidines as potent and selective antagonists of P2X3 and P2X2/3 receptors with in vivo efficacy in a pain model. Br J Pharmacol 163(6):1315–1325
148. Donnelly-Roberts D, McGaraughty S, Shieh CC, Honore P, Jarvis MF (2008) Painful purinergic receptors. J Pharmacol Exp Ther 324(2):409–415
149. Jarvis MF (2010) The neural-glial purinergic receptor ensemble in chronic pain states. Trends Neurosci 33(1):48–57
150. Donnelly-Roberts DL, Namovic MT, Surber B, Vaidyanathan SX, Perez-Medrano A, Wang Y, Carroll WA, Jarvis MF (2009) [3H]A-804598 ([3H]2-Cyano-1-[(1S)-1-phenylethyl]-3-quinolin-5-ylguanidine) is a novel, potent, and selective antagonist radioligand for P2X7 receptors. Neuropharmacology 56 (1):223–229
151. Perez-Medrano A, Donnelly-Roberts DL, Florjancic AS, Nelson DW, Li T, Namovic MT, Peddi S, Faltynek CR, Jarvis MF, Carroll WA (2011) Synthesis and in vitro activity of *N*-benzyl-1-(2,3-dichlorophenyl)-1 H-tetrazol-5-amine P2X7 antagonists. Bioorg Med Chem Lett 21(11):3297–3300
152. Perez-Medrano A, Donnelly-Roberts DL, Honore P, Hsieh GC, Namovic MT, Peddi S, Shuai Q, Wang Y, Faltynek CR, Jarvis MF, Carroll WA (2009) Discovery and biological evaluation of novel cyanoguanidine P2X(7) antagonists with analgesic activity in a rat model of neuropathic pain. J Med Chem 52(10):3366–3376
153. Stokes L, Jiang LH, Alcaraz L, Bent J, Bowers K, Fagura M, Furber M, Mortimore M, Lawson M, Theaker J, Laurent C, Braddock M, Surprenant A (2006) Characterization of a selective and potent antagonist of human P2X(7) receptors, AZ11645373. Br J Pharmacol 149(7):880–887
154. Abberley L, Bebius A, Beswick PJ, Billinton A, Collis KL, Dean DK, Fonfria E, Gleave RJ, Medhurst SJ, Michel AD, Moses AP, Patel S, Roman SA, Scoccitti T, Smith B, Steadman JGA, Walter DS (2010) Identification of 2-oxo-*N*-(phenylmethyl)-4-imidazolidinecarboxamide antagonists of the P2X7 receptor. Bioorg Med Chem Lett 20(22):6370–6374
155. Chambers LJ, Stevens AJ, Moses AP, Michel AD, Walter DS, Davies DJ, Livermore DG, Fonfria E, Demont EH, Vimal M, Theobald PJ, Beswick PJ, Gleave RJ, Roman SA, Senger S (2010) Synthesis and structure-activity relationships of a series of (1 H-pyrazol-4-yl)acetamide antagonists of the P2X7 receptor. Bioorg Med Chem Lett 20(10):3161–3164
156. Gleave RJ, Walter DS, Beswick PJ, Fonfria E, Michel AD, Roman SA, Tang S-P (2010) Synthesis and biological activity of a series of tetrasubstituted-imidazoles as P2X7 antagonists. Bioorg Med Chem Lett 20(16):4951–4954
157. Chen X, Pierce B, Naing W, Grapperhaus ML, Phillion DP (2010) Discovery of 2-chloro-*N*-((4,4-difluoro-1-hydroxycyclohexyl)methyl)-5-(5-fluoropyrimidin-2-yl)benzamide as a potent and CNS penetrable P2X7 receptor antagonist. Bioorg Med Chem Lett 20(10):3107–3111
158. Abdi MH, Beswick PJ, Billinton A, Chambers LJ, Charlton A, Collins SD, Collis KL, Dean DK, Fonfria E, Gleave RJ, Lejeune CL, Livermore DG, Medhurst SJ, Michel AD, Moses AP, Page L, Patel S, Roman SA, Senger S, Slingsby B, Steadman JG, Stevens AJ, Walter DS (2010) Discovery and structure-activity relationships of a series of pyroglutamic acid amide antagonists of the P2X7 receptor. Bioorg Med Chem Lett 20(17):5080–5084
159. Broom DC, Matson DJ, Bradshaw E, Buck ME, Meade R, Coombs S, Matchett M, Ford KK, Yu W, Yuan J, Sun SH, Ochoa R, Krause JE, Wustrow DJ, Cortright DN (2008) Characterization of *N*-(adamantan-1-ylmethyl)-5-[(3R-aminopyrrolidin-1-yl)methyl]-2-chloro-benzamide, a P2X7 antagonist in animal models of pain and inflammation. J Pharmacol Exp Ther 327(3):620–633
160. Honore P, Donnelly-Roberts D, Namovic M, Zhong C, Wade C, Chandran P, Zhu C, Carroll W, Perez-Medrano A, Iwakura Y, Jarvis MF (2009) The antihyperalgesic activity of a selective P2X7 receptor antagonist, A-839977, is lost in IL-1alphabeta knockout mice. Behav Brain Res 204(1):77–81
161. Honore P, Donnelly-Roberts D, Namovic MT, Hsieh G, Zhu CZ, Mikusa JP, Hernandez G, Zhong C, Gauvin DM, Chandran P, Harris R, Medrano AP, Carroll W, Marsh K, Sullivan JP, Faltynek CR, Jarvis MF (2006) A-740003 [*N*-(1-{[(Cyanoimino)(5-quinolinylamino) methyl]amino}-2,2-dimethylpropyl)-2-(3,4-dimethoxyphenyl)acetamide], a novel and selective P2X7 receptor antagonist, dose-dependently reduces neuropathic pain in the rat. J Pharmacol Exp Ther 319(3):1376–1385
162. McGaraughty S, Chu KL, Namovic MT, Donnelly-Roberts DL, Harris RR, Zhang XF, Shieh CC, Wismer CT, Zhu CZ, Gauvin DM, Fabiyi AC, Honore P, Gregg RJ, Kort ME, Nelson DW, Carroll WA, Marsh K, Faltynek CR, Jarvis MF (2007) P2X7-related modulation of pathological nociception in rats. Neuroscience 146 (4):1817–1828
163. Beswick P, Billinton A, Chambers L, Dean D, Fonfria E, Gleave R, Medhurst S, Michel A, Moses A, Patel S, Roman S, Roomans

S, Senger S, Stevens A, Walter D (2010) Structure-activity relationships and in vivo activity of (1 H-pyrazol-4-yl)acetamide antagonists of the P2X(7) receptor. Bioorg Med Chem Lett 20:4653–4656
164. Nelson D, Gregg R, Kort M, Perez-Medrano A, Voight E, Wang Y, Grayson G, Namovic M, Donnelly-Roberts D, Niforatos W, Honore P, Jarvis M, Faltynek C, Carroll W (2006) Structure-activity relationship studies on a series of novel, substituted 1-benzyl-5-phenyltetrazole P2X7 antagonists. J Med Chem 49:3659–3666
165. Guile SD, Alcaraz L, Birkinshaw TN, Bowers KC, Ebden MR, Furber M, Stocks MJ (2009) Antagonists of the P2X(7) receptor. From lead identification to drug development. J MedChem 52 (10):3123–3141
166. Keystone EC, Wang MM, Layton M, Hollis S, McInnes IB, on behalf of the DCST (2012) Clinical evaluation of the efficacy of the P2X7 purinergic receptor antagonist AZD9056 on the signs and symptoms of rheumatoid arthritis in patients with active disease despite treatment with methotrexate or sulphasalazine. Annals of the Rheumatic Diseases (in press)
167. Duplantier AJ, Dombroski MA, Subramanyam C, Beaulieu AM, Chang S-P, Gabel CA, Jordan C, Kalgutkar AS, Kraus KG, Labasi JM, Mussari C, Perregaux DG, Shepard R, Taylor TJ, Trevena KA, Whitney-Pickett C, Yoon K (2011) Optimization of the physicochemical and pharmacokinetic attributes in a 6-azauracil series of P2X7 receptor antagonists leading to the discovery of the clinical candidate CE-224,535. Bioorg Med Chem Lett 21(12):3708–3711
168. Bongartz E, Rettinger J, Hausmann R (2010) Aminoglycoside block of P2X2 receptors heterologously expressed in *Xenopus laevis* oocytes. Purinergic Signal 6(4):393–403
169. Nagata K, Imai T, Yamashita T, Tsuda M, Tozaki-Saitoh H, Inoue K (2009) Antidepressants inhibit P2X4 receptor function: a possible involvement in neuropathic pain relief. Mol Pain 5:20
170. Sim JA, North RA (2010) Amitriptyline does not block the action of ATP at human P2X4 receptor. Br J Pharmacol 160(1):88–92
171. Toulme E, Garcia A, Samways D, Egan TM, Carson MJ, Khakh BS (2010) P2X4 receptors in activated C8-B4 cells of cerebellar microglial origin. J Gen Physiol 135(4):333–353
172. Nörenberg W, Hempel C, Urban N, Sobottka H, Illes P, Schaefer M (2011) Clemastine potentiates the human P2X7 receptor by sensitizing it to lower ATP concentrations. J Biol Chem 286(13): 11067–11081
173. Royle SJ, Lagnado L (2003) Endocytosis at the synaptic terminal. J Physiol 553(Pt 2):345–355
174. Rettinger J, Aschrafi A, Schmalzing G (2000) Roles of individual N-glycans for ATP potency and expression of the rat P2X1 receptor. J Biol Chem 275(43):33542–33547
175. Torres GE, Egan TM, Voigt MM (1998) N-Linked glycosylation is essential for the functional expression of the recombinant P2X2 receptor. Biochemistry 37(42):14845–14851
176. Vacca F, D'Ambrosi N, Nestola V, Amadio S, Giustizieri M, Cucchiaroni ML, Tozzi A, Velluz MC, Mercuri NB, Volonte C (2011) N-Glycans mutations rule oligomeric assembly and functional expression of P2X3 receptor for extracellular ATP. Glycobiology 21(5):634–643
177. Lenertz LY, Wang Z, Guadarrama A, Hill LM, Gavala ML, Bertics PJ (2010) Mutation of putative N-linked glycosylation sites on the human nucleotide receptor P2X7 reveals a key residue important for receptor function. Biochemistry 49(22):4611–4619
178. Chaumont S, Jiang L-H, Penna A, North RA, Rassendren F (2004) Identification of a trafficking motif involved in the stabilization and polarization of P2X receptors. J Biol Chem 279 (28):29628–29638
179. Dutton JL, Poronnik P, Li GH, Holding CA, Worthington RA, Vandenberg RJ, Cook DI, Barden JA, Bennett MR (2000) P2X(1) receptor membrane redistribution and down-regulation visualized by using receptor-coupled green fluorescent protein chimeras. Neuropharmacology 39(11):2054–2066
180. Li GH, Lee EM, Blair D, Holding C, Poronnik P, Cook DI, Barden JA, Bennett MR (2000) The distribution of P2X receptor clusters on individual neurons in sympathetic ganglia and their redistribution on agonist activation. J Biol Chem 275(37):29107–29112
181. Ennion SJ, Evans RJ (2001) Agonist-stimulated internalisation of the ligand-gated ion channel P2X(1) in rat vas deferens. FEBS Lett 489(2–3):154–158
182. Lalo U, Allsopp RC, Mahaut-Smith MP, Evans RJ (2010) P2X1 receptor mobility and trafficking; regulation by receptor insertion and activation. J Neurochem 113(5):1177–1187
183. Khakh BS, Smith WB, Chiu CS, Ju D, Davidson N, Lester HA (2001) Activation-dependent changes in receptor distribution and dendritic morphology in hippocampal neurons expressing P2X2-green fluorescent protein receptors. Proc Natl Acad Sci U S A 98 (9):5288–5293
184. Shrivastava AN, Triller A, Sieghart W, Sarto-Jackson I (2011) Regulation of GABA(A) receptor dynamics by interaction with purinergic P2X(2) receptors. J Biol Chem 286(16):14455–14468
185. Xu GY, Huang LY (2002) Peripheral inflammation sensitizes P2X receptor-mediated responses in rat dorsal root ganglion neurons. J Neurosci 22(1):93–102
186. Chen Y, Li G-W, Wang C, Gu Y, Huang L-YM (2005) Mechanisms underlying enhanced P2X receptor-mediated responses in the neuropathic pain state. Pain 119(1–3):38–48
187. Xu GY, Huang LY (2004) Ca2+/calmodulin-dependent protein kinase II potentiates ATP responses by promoting trafficking of P2X receptors. Proc Natl Acad Sci U S A 101(32):11868–11873
188. Vacca F, Giustizieri M, Ciotti MT, Mercuri NB, Volonte C (2009) Rapid constitutive and ligand-activated endocytic trafficking of P2X receptor. J Neurochem 109(4):1031–1041
189. Giniatullin R, Nistri A, Fabbretti E (2008) Molecular mechanisms of sensitization of pain-transducing P2X3 receptors by the migraine mediators CGRP and NGF. Mol Neurobiol 37(1):83–90
190. Tsuda M, Shigemoto-Mogami Y, Koizumi S, Mizokoshi A, Kohsaka S, Salter MW, Inoue K (2003) P2X4 receptors induced in spinal microglia gate tactile allodynia after nerve injury. Nature 424(6950):778–783
191. Ulmann L, Hatcher JP, Hughes JP, Chaumont S, Green PJ, Conquet F, Buell GN, Reeve AJ, Chessell IP, Rassendren F (2008) Up-regulation of P2X4 receptors in spinal microglia after peripheral nerve injury mediates BDNF release and neuropathic pain. J Neurosci 28(44):11263–11268
192. Bobanovic LK, Royle SJ, Murrell-Lagnado RD (2002) P2X receptor trafficking in neurons is subunit specific. J Neurosci 22 (12):4814–4824
193. Royle SJ, Bobanovic LK, Murrell-Lagnado RD (2002) Identification of a non-canonical tyrosine-based endocytic motif in an ionotropic receptor. J Biol Chem 277(38):35378–35385
194. Brown DA, Yule DI (2010) Protein kinase A regulation of P2X(4) receptors: requirement for a specific motif in the C-terminus. Biochim Biophys Acta 1803(2):275–287
195. Toulme E, Soto F, Garret M, Boue-Grabot E (2006) Functional properties of internalization-deficient P2X4 receptors reveal a novel mechanism of ligand-gated channel facilitation by ivermectin. Mol Pharmacol 69(2):576–587
196. Silberberg SD, Li M, Swartz KJ (2007) Ivermectin interaction with transmembrane helices reveals widespread rearrangements during opening of P2X receptor channels. Neuron 54(2):263–274
197. Qureshi OS, Paramasivam A, Yu JC, Murrell-Lagnado RD (2007) Regulation of P2X4 receptors by lysosomal targeting, glycan protection and exocytosis. J Cell Sci 120(Pt 21):3838–3849

198. Stokes L, Surprenant A (2009) Dynamic regulation of the P2X4 receptor in alveolar macrophages by phagocytosis and classical activation. Eur J Immunol 39(4):986–995
199. Kuehnel MP, Rybin V, Anand PK, Anes E, Griffiths G (2009) Lipids regulate P2X7-receptor-dependent actin assembly by phagosomes via ADP translocation and ATP synthesis in the phagosome lumen. J Cell Sci 122(Pt 4):499–504
200. Collo G, North RA, Kawashima E, Merlo-Pich E, Neidhart S, Surprenant A, Buell G (1996) Cloning OF P2X5 and P2X6 receptors and the distribution and properties of an extended family of ATP-gated ion channels. J Neurosci 16(8):2495–2507
201. Garcia-Guzman M, Soto F, Laube B, Stuhmer W (1996) Molecular cloning and functional expression of a novel rat heart P2X purinoceptor. FEBS Lett 388(2–3):123–127
202. Bo X, Schoepfer R, Burnstock G (2000) Molecular cloning and characterization of a novel ATP P2X receptor subtype from embryonic chick skeletal muscle. J Biol Chem 275(19):14401–14407
203. Jones CA, Vial C, Sellers LA, Humphrey PP, Evans RJ, Chessell IP (2004) Functional regulation of P2X6 receptors by N-linked glycosylation: identification of a novel alpha beta-methylene ATP-sensitive phenotype. Mol Pharmacol 65(4):979–985
204. King BF, Townsend-Nicholson A, Wildman SS, Thomas T, Spyer KM, Burnstock G (2000) Coexpression of rat P2X2 and P2X6 subunits in *Xenopus* oocytes. J Neurosci 20(13):4871–4877
205. Soto F, Garcia-Guzman M, Karschin C, Stuhmer W (1996) Cloning and tissue distribution of a novel P2X receptor from rat brain. Biochem Biophys Res Commun 223(2):456–460
206. Nawa G, Urano T, Tokino T, Ochi T, Miyoshi Y (1998) Cloning and characterization of the murine P2XM receptor gene. J Hum Genet 43(4):262–267
207. Ormond SJ, Barrera NP, Qureshi OS, Henderson RM, Edwardson JM, Murrell-Lagnado RD (2006) An uncharged region within the N terminus of the P2X6 receptor inhibits its assembly and exit from the endoplasmic reticulum. Mol Pharmacol 69(5):1692–1700
208. Gu BJ, Zhang WY, Bendall LJ, Chessell IP, Buell GN, Wiley JS (2000) Expression of P2X(7) purinoceptors on human lymphocytes and monocytes: evidence for nonfunctional P2X(7) receptors. Am J Physiol Cell Physiol 279(4):C1189–1197
209. Gudipaty L, Humphreys BD, Buell G, Dubyak GR (2001) Regulation of P2X(7) nucleotide receptor function in human monocytes by extracellular ions and receptor density. Am J Physiol Cell Physiol 280(4):C943–953
210. Hickman SE, Khoury J, Greenberg S, Schieren I, Silverstein SC (1994) P2Z adenosine triphosphate receptor activity in cultured human monocyte-derived macrophages. Blood 84(8):2452–2456
211. Denlinger LC, Sommer JA, Parker K, Gudipaty L, Fisette PL, Watters JW, Proctor RA, Dubyak GR, Bertics PJ (2003) Mutation of a dibasic amino acid motif within the C terminus of the P2X7 nucleotide receptor results in trafficking defects and impaired function. J Immunol 171(3):1304–1311
212. Denlinger LC, Fisette PL, Sommer JA, Watters JJ, Prabhu U, Dubyak GR, Proctor RA, Bertics PJ (2001) Cutting edge: the nucleotide receptor P2X7 contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. J Immunol 167(4):1871–1876
213. Smart ML, Gu B, Panchal RG, Wiley J, Cromer B, Williams DA, Petrou S (2003) P2X7 receptor cell surface expression and cytolytic pore formation are regulated by a distal C-terminal region. J Biol Chem 278(10):8853–8860
214. Wiley JS, Dao-Ung LP, Li C, Shemon AN, Gu BJ, Smart ML, Fuller SJ, Barden JA, Petrou S, Sluyter R (2003) An Ile-568 to Asn polymorphism prevents normal trafficking and function of the human P2X7 receptor. J Biol Chem 278(19):17108–17113
215. Garcia-Marcos M, Pérez-Andrés E, Tandel S, Fontanils U, Kumps A, Kabré E, Gómez-Muñoz A, Marino A, Dehaye JP, Pochet S (2006) Coupling of two pools of P2X7 receptors to distinct intracellular signaling pathways in rat submandibular gland. J Lipid Res 47(4):705–714
216. Barth K, Weinhold K, Guenther A, Young MT, Schnittler H, Kasper M (2007) Caveolin-1 influences P2X7 receptor expression and localization in mouse lung alveolar epithelial cells. FEBS J 274(12):3021–3033
217. Gonnord P, Delarasse C, Auger R, Benihoud K, Prigent M, Cuif MH, Lamaze C, Kanellopoulos JM (2009) Palmitoylation of the P2X7 receptor, an ATP-gated channel, controls its expression and association with lipid rafts. FASEB J 23(3):795–805
218. Hiken JF, Steinberg TH (2004) ATP downregulates P2X7 and inhibits osteoclast formation in RAW cells. Am J Physiol Cell Physiol 287(2):C403–C412
219. Vial C, Tobin AB, Evans RJ (2004) G-protein-coupled receptor regulation of P2X1 receptors does not involve direct channel phosphorylation. Biochem J 382(Pt 1):101–110
220. Wen H, Evans RJ (2009) Regions of the amino terminus of the P2X1 receptor required for modification by phorbol ester and mGluR1α receptors. J Neurochem 108(2):331–340
221. Brown DA, Yule DI (2007) Protein kinase C regulation of P2X3 receptors is unlikely to involve direct receptor phosphorylation. Biochim Biophys Acta 1773(2):166–175
222. Paukert M, Osteroth R, Geisler H-S, Braendle U, Glowatzki E, Ruppersberg JP, Gruender S (2001) Inflammatory mediators potentiate ATP-gated channels through the P2X3 subunit. J Biol Chem 276(24):21077–21082
223. Stanchev D, Flehmig G, Gerevich Z, Noerenberg W, Dihazi H, Fuerst S, Eschrich K, Illes P, Wirkner K (2006) Decrease of current responses at human recombinant P2X3 receptors after substitution by Asp of Ser/Thr residues in protein kinase C phosphorylation sites of their ecto-domains. Neurosci Lett 393(1):78–83
224. Wirkner K, Stanchev D, Koles L, Klebingat M, Dihazi H, Flehmig G, Vial C, Evans RJ, Fuerst S, Mager PP, Eschrich K, Illes P (2005) Regulation of human recombinant P2X3 receptors by ecto-protein kinase C. J Neurosci 25(34):7734–7742
225. Kim M, Jiang LH, Wilson HL, North RA, Surprenant A (2001) Proteomic and functional evidence for a P2X7 receptor signalling complex. EMBO J 20(22):6347–6358
226. Ding S, Sachs F (2002) Evidence for non-independent gating of P2X2 receptors expressed in *Xenopus* oocytes. BMC Neurosci 3:17
227. Clyne JD, Brown TC, Hume RI (2003) Expression level dependent changes in the properties of P2X2 receptors. Neuropharmacology 44(3):403–412
228. Fujiwara Y, Kubo Y (2004) Density-dependent changes of the pore properties of the P2X2 receptor channel. J Physiol 558(Pt 1):31–43
229. Casas-Pruneda G, Reyes JP, Perez-Flores G, Perez-Cornejo P, Arreola J (2009) Functional interactions between P2X4 and P2X7 receptors from mouse salivary epithelia. J Physiol 587(Pt 12):2887–2901
230. Guo C, Masin M, Qureshi OS, Murrell-Lagnado RD (2007) Evidence for functional P2X4/P2X7 heteromeric receptors. Mol Pharmacol 72(6):1447–1456
231. Weinhold K, Krause-Buchholz U, Rodel G, Kasper M, Barth K (2010) Interaction and interrelation of P2X7 and P2X4 receptor complexes in mouse lung epithelial cells. Cell Mol Life Sci 67(15):2631–2642
232. Antonio LS, Stewart AP, Xu XJ, Varanda WA, Murrell-Lagnado RD, Edwardson JM (2011) P2X4 receptors interact with both P2X2 and P2X7 receptors in the form of homotrimers. Br J Pharmacol 163(5):1069–1077
233. Nicke A (2008) Homotrimeric complexes are the dominant assembly state of native P2X7 subunits. Biochem Biophys Res Commun 377(3):803–808

234. Babelova A, Moreth K, Tsalastra-Greul W, Zeng-Brouwers J, Eickelberg O, Young MF, Bruckner P, Pfeilschifter J, Schaefer RM, Grone HJ, Schaefer L (2009) Biglycan, a danger signal that activates the NLRP3 inflammasome via toll-like and P2X receptors. J Biol Chem 284(36):24035–24048
235. Boue-Grabot E, Toulme E, Emerit MB, Garret M (2004) Subunit-specific coupling between gamma-aminobutyric acid type A and P2X2 receptor channels. J Biol Chem 279(50):52517–52525
236. Karanjia R, Garcia-Hernandez LM, Miranda-Morales M, Somani N, Espinosa-Luna R, Montano LM, Barajas-Lopez C (2006) Cross-inhibitory interactions between GABAA and P2X channels in myenteric neurones. Eur J Neurosci 23(12):3259–3268
237. Sokolova E, Nistri A, Giniatullin R (2001) Negative cross talk between anionic GABAA and cationic P2X ionotropic receptors of rat dorsal root ganglion neurons. J Neurosci 21(14):4958–4968
238. Boue-Grabot E, Emerit MB, Toulme E, Seguela P, Garret M (2004) Cross-talk and co-trafficking between rho1/GABA receptors and ATP-gated channels. J Biol Chem 279(8):6967–6975
239. Barajas-Lopez C, Espinosa-Luna R, Zhu Y (1998) Functional interactions between nicotinic and P2X channels in short-term cultures of guinea-pig submucosal neurons. J Physiol 513(Pt 3):671–683
240. Khakh BS, Henderson G (2000) Modulation of fast synaptic transmission by presynaptic ligand-gated cation channels. J Auton Nerv Syst 81(1–3):110–121
241. Nakazawa K (1994) ATP-activated current and its interaction with acetylcholine-activated current in rat sympathetic neurons. J Neurosci 14(2):740–750
242. Rodrigues RJ, Almeida T, de Mendonca A, Cunha RA (2006) Interaction between P2X and nicotinic acetylcholine receptors in glutamate nerve terminals of the rat hippocampus. J Mol Neurosci 30(1–2):173–176
243. Searl TJ, Redman RS, Silinsky EM (1998) Mutual occlusion of P2X ATP receptors and nicotinic receptors on sympathetic neurons of the guinea-pig. J Physiol 510(Pt 3):783–791
244. Zhou X, Galligan JJ (1998) Non-additive interaction between nicotinic cholinergic and P2X purine receptors in guinea-pig enteric neurons in culture. J Physiol 513(Pt 3):685–697
245. Barajas-Lopez C, Montano LM, Espinosa-Luna R (2002) Inhibitory interactions between 5-HT3 and P2X channels in submucosal neurons. Am J Physiol Gastrointest Liver Physiol 283(6): G1238–1248
246. Boue-Grabot E, Barajas-Lopez C, Chakfe Y, Blais D, Belanger D, Emerit MB, Seguela P (2003) Intracellular cross talk and physical interaction between two classes of neurotransmitter-gated channels. J Neurosci 23(4):1246–1253
247. Toulme E, Blais D, Leger C, Landry M, Garret M, Seguela P, Boue-Grabot E (2007) An intracellular motif of P2X(3) receptors is required for functional cross-talk with GABA(A) receptors in nociceptive DRG neurons. J Neurochem 102(4):1357–1368
248. Jo YH, Donier E, Martinez A, Garret M, Toulme E, Boue-Grabot E (2011) Cross-talk between P2X4 and gamma-aminobutyric acid, type A receptors determines synaptic efficacy at a central synapse. J Biol Chem 286(22):19993–20004
249. Khakh BS, Fisher JA, Nashmi R, Bowser DN, Lester HA (2005) An angstrom scale interaction between plasma membrane ATP-gated P2X2 and alpha4beta2 nicotinic channels measured with fluorescence resonance energy transfer and total internal reflection fluorescence microscopy. J Neurosci 25(29):6911–6920
250. Wildman SS, Marks J, Churchill LJ, Peppiatt CM, Chraibi A, Shirley DG, Horisberger JD, King BF, Unwin RJ (2005) Regulatory interdependence of cloned epithelial Na+channels and P2X receptors. J Am Soc Nephrol 16(9):2586–2597
251. Birdsong WT, Fierro L, Williams FG, Spelta V, Naves LA, Knowles M, Marsh-Haffner J, Adelman JP, Almers W, Elde RP, McCleskey EW (2010) Sensing muscle ischemia: coincident detection of acid and ATP via interplay of two ion channels. Neuron 68(4):739–749
252. Pankratov Y, Lalo U, Krishtal OA, Verkhratsky A (2009) P2X receptors and synaptic plasticity. Neuroscience 158(1):137–148
253. Pankratov YV, Lalo UV, Krishtal OA (2002) Role for P2X receptors in long-term potentiation. J Neurosci 22(19):8363–8369
254. Pelegrin P, Surprenant A (2006) Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. EMBO J 25(21):5071–5082
255. Chaumont S, Khakh BS (2008) Patch-clamp coordinated spectroscopy shows P2X2 receptor permeability dynamics require cytosolic domain rearrangements but not Panx-1 channels. Proc Natl Acad Sci U S A 105(33):12063–12068
256. Ma W, Hui H, Pelegrin P, Surprenant A (2009) Pharmacological characterization of pannexin-1 currents expressed in mammalian cells. J Pharmacol Exp Ther 328(2):409–418
257. Yan Z, Li S, Liang Z, Tomic M, Stojilkovic SS (2008) The P2X7 receptor channel pore dilates under physiological ion conditions. J Gen Physiol 132(5):563–573
258. Yan Z, Khadra A, Li S, Tomic M, Sherman A, Stojilkovic SS (2010) Experimental characterization and mathematical modeling of P2X7 receptor channel gating. J Neurosci 30(42):14213–14224
259. Gendreau S, Schirmer J, Schmalzing G (2003) Identification of a tubulin binding motif on the P2X2 receptor. J Chromatogr B 786 (1–2):311–318
260. Chaumont S, Compan V, Toulme E, Richler E, Housley GD, Rassendren F, Khakh BS (2008) Regulation of P2X2 receptors by the neuronal calcium sensor VILIP1. Sci Signal 1(41):ra8
261. Masin M, Kerschensteiner D, Dumke K, Rubio ME, Soto F (2006) Fe65 interacts with P2X2 subunits at excitatory synapses and modulates receptor function. J Biol Chem 281(7):4100–4108
262. Guenette S, Chang Y, Hiesberger T, Richardson JA, Eckman CB, Eckman EA, Hammer RE, Herz J (2006) Essential roles for the FE65 amyloid precursor protein-interacting proteins in brain development. EMBO J 25(2):420–431
263. Braunewell K-H, Szanto A (2009) Visinin-like proteins (VSNLs): interaction partners and emerging functions in signal transduction of a subfamily of neuronal Ca2 + sensor proteins. Cell Tissue Res 335(2):301–316
264. Roger S, Gillet L, Baroja-Mazo A, Surprenant A, Pelegrin P (2010) C-terminal calmodulin-binding motif differentially controls human and rat P2X7 receptor current facilitation. J Biol Chem 285(23):17514–17524
265. Roger S, Pelegrin P, Surprenant A (2008) Facilitation of P2X7 receptor currents and membrane blebbing via constitutive and dynamic calmodulin binding. J Neurosci 28(25):6393–6401
266. Adinolfi E, Kim M, Young MT, Di Virgilio F, Surprenant A (2003) Tyrosine phosphorylation of HSP90 within the P2X7 receptor complex negatively regulates P2X7 receptors. J Biol Chem 278(39):37344–37351
267. Wilson HL, Wilson SA, Surprenant A, North RA (2002) Epithelial membrane proteins induce membrane blebbing and interact with the P2X7 receptor C terminus. J Biol Chem 277(37):34017–34023
268. Gu BJ, Rathsam C, Stokes L, McGeachie AB, Wiley JS (2009) Extracellular ATP dissociates nonmuscle myosin from P2X(7) complex: this dissociation regulates P2X(7) pore formation. Am J Physiol Cell Physiol 297(2):C430–439
269. Gu BJ, Saunders BM, Jursik C, Wiley JS (2010) The P2X7-nonmuscle myosin membrane complex regulates phagocytosis of nonopsonized particles and bacteria by a pathway attenuated by extracellular ATP. Blood 115(8):1621–1631
270. Glass R, Loesch A, Bodin P, Burnstock G (2002) P2X4 and P2X6 receptors associate with VE-cadherin in human endothelial cells. Cell Mol Life Sci 59(5):870–881

271. Lalo U, Roberts JA, Evans RJ (2011) Identification of human P2X1 receptor-interacting proteins reveals a role of the cytoskeleton in receptor regulation. J Biol Chem 286(35):30591–30599
272. Koeles L, Gerevich Z, Jo O, Zadori Z, Wirkner K, Illes P (2008) Interaction of P2 purinergic receptors with cellular macromolecules. Naunyn Schmiedeberg's Arch Pharmacol 377(1):1–33
273. Nakatsuka T, Gu JG (2001) ATP P2X receptor-mediated enhancement of glutamate release and evoked EPSCs in dorsal horn neurons of the rat spinal cord. J Neurosci 21(17):6522–6531
274. Schicker K, Dorostkar M, Boehm S (2008) Modulation of transmitter release via presynaptic ligand-gated ion channels. Curr Mol Pharmacol J 1(2):106–129
275. Khakh BS, Gittermann D, Cockayne DA, Jones A (2003) ATP modulation of excitatory synapses onto interneurons. J Neurosci 23(19):7426–7437
276. Khakh BS, Henderson G (1998) ATP receptor-mediated enhancement of fast excitatory neurotransmitter release in the brain. Mol Pharmacol 54(2):372–378
277. Sperlagh B, Heinrich A, Csolle C (2007) P2 receptor-mediated modulation of neurotransmitter release-an update. Purinergic Signal 3(4):269–284
278. Nörenberg W, Illes P (2000) Neuronal P2X receptors: localisation and functional properties. Naunyn Schmiedeberg's Arch Pharmacol 362(4):324–339
279. Khakh BS, North RA (2006) P2X receptors as cell-surface ATP sensors in health and disease. Nature 442(7102):527–532
280. Shigetomi E, Kato F (2004) Action Potential-Independent Release of Glutamate by Ca^{2+} Entry through Presynaptic P2X Receptors Elicits Postsynaptic Firing in the Brainstem Autonomic Network. J Neurosci 24(12):3125–3135
281. Koshimizu TA, Van Goor F, Tomic M, Wong AO, Tanoue A, Tsujimoto G, Stojilkovic SS (2000) Characterization of calcium signaling by purinergic receptor-channels expressed in excitable cells. Mol Pharmacol 58(5):936–945
282. Egan TM, Khakh BS (2004) Contribution of calcium ions to P2X channel responses. J Neurosci 24(13):3413–3420
283. Khakh BS, Bao XR, Labarca C, Lester HA (1999) Neuronal P2X transmitter-gated cation channels change their ion selectivity in seconds. Nat Neurosci 2(4):322–330
284. Chung MK, Guler AD, Caterina MJ (2008) TRPV1 shows dynamic ionic selectivity during agonist stimulation. Nat Neurosci 11(5):555–564
285. Virginio C, MacKenzie A, Rassendren FA, North RA, Surprenant A (1999) Pore dilation of neuronal P2X receptor channels. Nat Neurosci 2(4):315–321
286. Virginio C, MacKenzie A, North RA, Surprenant A (1999) Kinetics of cell lysis, dye uptake and permeability changes in cells expressing the rat P2X7 receptor. J Physiol 519(Pt 2):335–346
287. Eickhorst AN, Berson A, Cockayne D, Lester HA, Khakh BS (2002) Control of P2X(2) channel permeability by the cytosolic domain. J Gen Physiol 120(2):119–131
288. Khakh BS, Egan TM (2005) Contribution of transmembrane regions to ATP-gated P2X2 channel permeability dynamics. J Biol Chem 280(7):6118–6129
289. Spelta V, Jiang LH, Bailey RJ, Surprenant A, North RA (2003) Interaction between cysteines introduced into each transmembrane domain of the rat P2X2 receptor. Br J Pharmacol 138(1): 131–136
290. Doyle D (2004) Molecular insights into ion channel function (review). Mol Membr Biol 21(4):221–225
291. Fisher JA, Girdler G, Khakh BS (2004) Time-resolved measurement of state-specific P2X2 ion channel cytosolic gating motions. J Neurosci 24(46):10475–10487
292. Marques-da-Silva C, Chaves MM, Castro NG, Coutinho-Silva R, Guimaraes MZP (2011) Colchicine inhibits cationic dye uptake induced by ATP in P2X2 and P2X7 receptor-expressing cells: implications for its therapeutic action. Br J Pharmacol 163 (5):912–926
293. Mackenzie AB, Young MT, Adinolfi E, Surprenant A (2005) Pseudoapoptosis induced by brief activation of ATP-gated P2X7 receptors. J Biol Chem 280(40):33968–33976
294. Surprenant A, Rassendren F, Kawashima E, North RA, Buell G (1996) The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7). Science 272(5262):735–738
295. Schilling WP, Wasylyna T, Dubyak GR, Humphreys BD, Sinkins WG (1999) Maitotoxin and P2Z/P2X(7) purinergic receptor stimulation activate a common cytolytic pore. Am J Physiol 277(4 Pt 1):C766–776
296. Donnelly-Roberts DL, Namovic MT, Faltynek CR, Jarvis MF (2004) Mitogen-activated protein kinase and caspase signaling pathways are required for P2X7 receptor (P2X7R)-induced pore formation in human THP-1 cells. J Pharmacol Exp Ther 308 (3):1053–1061
297. Faria RX, Defarias FP, Alves LA (2005) Are second messengers crucial for opening the pore associated with P2X7 receptor? Am J Physiol Cell Physiol 288(2):C260–271
298. Jiang LH, Rassendren F, Mackenzie A, Zhang YH, Surprenant A, North RA (2005) *N*-methyl-D-glucamine and propidium dyes utilize different permeation pathways at rat P2X(7) receptors. Am J Physiol Cell Physiol 289(5):C1295–1302
299. Cankurtaran-Sayar S, Sayar K, Ugur M (2009) P2X7 receptor activates multiple selective dye-permeation pathways in RAW 264.7 and human embryonic kidney 293 cells. Mol Pharmacol 76(6):1323–1332
300. Nicke A, Kuan Y-H, Masin M, Rettinger J, Marquez-Klaka B, Bender O, Górecki DC, Murrell-Lagnado RD, Soto F (2009) A functional P2X7 splice variant with an alternative transmembrane domain 1 escapes gene inactivation in P2X7 knock-out mice. J Biol Chem 284(38):25813–25822
301. Pelegrin P, Surprenant A (2009) The P2X(7) receptor-pannexin connection to dye uptake and IL-1beta release. Purinergic Signal 5(2):129–137
302. Riedel T, Lozinsky I, Schmalzing G, Markwardt F (2007) Kinetics of P2X7 receptor-operated single channels currents. Biophys J 92(7):2377–2391
303. Gu BJ, Zhang W, Worthington RA, Sluyter R, Dao-Ung P, Petrou S, Barden JA, Wiley JS (2001) A Glu-496 to Ala polymorphism leads to loss of function of the human P2X7 receptor. J Biol Chem 276(14):11135–11142
304. Boldt W, Klapperstuck M, Buttner C, Sadtler S, Schmalzing G, Markwardt F (2003) Glu496Ala polymorphism of human P2X7 receptor does not affect its electrophysiological phenotype. Am J Physiol Cell Physiol 284(3):C749–756
305. Adriouch S, Dox C, Welge V, Seman M, Koch-Nolte F, Haag F (2002) Cutting edge: a natural P451L mutation in the cytoplasmic domain impairs the function of the mouse P2X7 receptor. J Immunol 169(8):4108–4112
306. Le Stunff H, Auger R, Kanellopoulos J, Raymond MN (2004) The Pro-451 to Leu polymorphism within the C-terminal tail of P2X7 receptor impairs cell death but not phospholipase D activation in murine thymocytes. J Biol Chem 279(17):16918–16926
307. Auger R, Motta I, Benihoud K, Ojcius DM, Kanellopoulos JM (2005) A role for mitogen-activated protein kinaseErk1/2 activation and non-selective pore formation in P2X7 receptor-mediated thymocyte death. J Biol Chem 280(30):28142–28151
308. Adinolfi E, Cirillo M, Woltersdorf R, Falzoni S, Chiozzi P, Pellegatti P, Callegari MG, Sandona D, Markwardt F, Schmalzing G, Di Virgilio F (2010) Trophic activity of a naturally occurring truncated isoform of the P2X7 receptor. FASEB J 24(9):3393–3404
309. Monif M, Burnstock G, Williams DA (2010) Microglia: proliferation and activation driven by the P2X7 receptor. Int J Biochem Cell Biol 42(11):1753–1756

310. Kettenmann H, Hanisch UK, Noda M, Verkhratsky A (2011) Physiology of microglia. Physiol Rev 91 (2):461–553
311. Erb L, Liao Z, Seye C, Weisman G (2006) P2 receptors: intracellular signaling. Pflugers Arch 452(5):552–562
312. Duan S, Neary JT (2006) P2X(7) receptors: properties and relevance to CNS function. GLIA 54(7):738–746
313. Lenertz LY, Gavala ML, Zhu Y, Bertics PJ (2011) Transcriptional control mechanisms associated with the nucleotide receptor P2X7, a critical regulator of immunologic, osteogenic, and neurologic functions. Immunol Res 50(1):22–38
314. van de Veerdonk FL, Netea MG, Dinarello CA, Joosten LA (2011) Inflammasome activation and IL-1beta and IL-18 processing during infection. Trends Immunol 32(3):110–116
315. Mariathasan S, Weiss DS, Newton K, McBride J, O'Rourke K, Roose-Girma M, Lee WP, Weinrauch Y, Monack DM, Dixit VM (2006) Cryopyrin activates the inflammasome in response to toxins and ATP. Nature 440(7081):228–232
316. McIntire C, Yeretssian G, Saleh M (2009) Inflammasomes in infection and inflammation. Apoptosis 14(4):522–535
317. Ferrari D, Chiozzi P, Falzoni S, Dal Susino M, Melchiorri L, Baricordi OR, Di Virgilio F (1997) Extracellular ATP triggers IL-1 beta release by activating the purinergic P2Z receptor of human macrophages. J Immunol 159(3):1451–1458
318. Griffiths RJ, Stam EJ, Downs JT, Otterness IG (1995) ATP induces the release of IL-1 from LPS-primed cells in vivo. J Immunol 154(6):2821–2828
319. Hogquist KA, Unanue ER, Chaplin DD (1991) Release of IL-1 from mononuclear phagocytes. J Immunol 147(7):2181–2186
320. Perregaux D, Gabel CA (1994) Interleukin-1 beta maturation and release in response to ATP and nigericin. Evidence that potassium depletion mediated by these agents is a necessary and common feature of their activity. J Biol Chem 269(21):15195–15203
321. Chessell IP, Hatcher JP, Bountra C, Michel AD, Hughes JP, Green P, Egerton J, Murfin M, Richardson J, Peck WL, Grahames CB, Casula MA, Yiangou Y, Birch R, Anand P, Buell GN (2005) Disruption of the P2X7 purinoceptor gene abolishes chronic inflammatory and neuropathic pain. Pain 114(3):386–396
322. Labasi JM, Petrushova N, Donovan C, McCurdy S, Lira P, Payette MM, Brissette W, Wicks JR, Audoly L, Gabel CA (2002) Absence of the P2X7 receptor alters leukocyte function and attenuates an inflammatory response. J Immunol 168 (12):6436–6445
323. Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH, Griffiths RJ, Gabel CA (2001) Altered cytokine production in mice lacking P2X(7) receptors. J Biol Chem 276(1):125–132
324. Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M, Panther E, Di Virgilio F (2006) The P2X7 receptor: a key player in IL-1 processing and release. J Immunol 176(7):3877–3883
325. Di Virgilio F (2007) Liaisons dangereuses: P2X(7) and the inflammasome. Trends Pharmacol Sci 28(9):465–472
326. MacKenzie A, Wilson HL, Kiss-Toth E, Dower SK, North RA, Surprenant A (2001) Rapid secretion of interleukin-1beta by microvesicle shedding. Immunity 15(5):825–835
327. Qu Y, Dubyak GR (2009) P2X7 receptors regulate multiple types of membrane trafficking responses and non-classical secretion pathways. Purinergic Signal 5(2):163–173
328. Bo X, Liu M, Schoepfer R, Burnstock G (2001) Characterization and expression of ATP P2X4 receptor from embryonic chick skeletal muscle. Drug Dev Res 53:22–28
329. Tsuzuki K, Kondo E, Fukuoka T, Yi D, Tsujino H, Sakagami M, Noguchi K (2001) Differential regulation of P2X(3) mRNA expression by peripheral nerve injury in intact and injured neurons in the rat sensory ganglia. Pain 91(3):351–360
330. Wareham K, Vial C, Wykes RC, Bradding P, Seward EP (2009) Functional evidence for the expression of P2X1, P2X4 and P2X7 receptors in human lung mast cells. Br J Pharmacol 157(7):1215–1224
331. Chan CM, Unwin RJ, Bardini M, Oglesby IB, Ford AP, Townsend-Nicholson A, Burnstock G (1998) Localization of P2X1 purinoceptors by autoradiography and immunohistochemistry in rat kidneys. Am J Physiol 274(4 Pt 2):F799–804
332. Collo G, Neidhart S, Kawashima E, Kosco-Vilbois M, North RA, Buell G (1997) Tissue distribution of the P2X7 receptor. Neuropharmacology 36(9):1277–1283
333. Cook SP, Vulchanova L, Hargreaves KM, Elde R, McCleskey EW (1997) Distinct ATP receptors on pain-sensing and stretch-sensing neurons. Nature 387(6632):505–508
334. Oglesby IB, Lachnit WG, Burnstock G, Ford AP (1999) Subunit specificity of polyclonal antisera to the carboxy terminal regions of P2X receptors, P2X1 through P2X7. Drug Dev Res 47:189–195
335. Rubio ME, Soto F (2001) Distinct localization of P2X receptors at excitatory postsynaptic specializations. J Neurosci 21(2):641–653
336. Turner CM, Vonend O, Chan C, Burnstock G, Unwin RJ (2003) The pattern of distribution of selected ATP-sensitive P2 receptor subtypes in normal rat kidney: an immunohistological study. Cells Tissues Organs 175(2):105–117
337. Vulchanova L, Arvidsson U, Riedl M, Wang J, Buell G, Surprenant A, North RA, Elde R (1996) Differential distribution of two ATP-gated channels (P2X receptors) determined by immunocytochemistry. Proc Natl Acad Sci U S A 93(15):8063–8067
338. Ashour F, Atterbury-Thomas M, Deuchars J, Evans RJ (2006) An evaluation of antibody detection of the P2X1 receptor subunit in the CNS of wild type and P2X1-knockout mice. Neurosci Lett 397(1–2):120–125
339. Sim JA, Young MT, Sung HY, North RA, Surprenant A (2004) Reanalysis of P2X7 receptor expression in rodent brain. J Neurosci 24(28):6307–6314
340. Liang SX, Jenkins NA, Gilbert DJ, Copeland NG, Phillips WD (2001) Structure and chromosome location of the mouse P2X(1) purinoceptor gene (P2rx1). Cytogenet Cell Genet 92(3–4):333–336
341. Longhurst PA, Schwegel T, Folander K, Swanson R (1996) The human P2x1 receptor: molecular cloning, tissue distribution, and localization to chromosome 17. Biochim Biophys Acta 1308 (3):185–188
342. Sun B, Li J, Okahara K, Kambayashi J (1998) P2X1 purinoceptor in human platelets. Molecular cloning and functional characterization after heterologous expression. J Biol Chem 273 (19):11544–11547
343. Valera S, Talabot F, Evans RJ, Gos A, Antonarakis SE, Morris MA, Buell GN (1995) Characterization and chromosomal localization of a human P2X receptor from the urinary bladder. Receptors Channels 3(4):283–289
344. Mulryan K, Gitterman DP, Lewis CJ, Vial C, Leckie BJ, Cobb AL, Brown JE, Conley EC, Buell G, Pritchard CA, Evans RJ (2000) Reduced vas deferens contraction and male infertility in mice lacking P2X1 receptors. Nature 403(6765):86–89
345. Vial C, Evans RJ (2000) P2X receptor expression in mouse urinary bladder and the requirement of P2X(1) receptors for functional P2X receptor responses in the mouse urinary bladder smooth muscle. Br J Pharmacol 131(7):1489–1495
346. Vial C, Evans RJ (2001) Smooth muscle does not have a common P2x receptor phenotype: expression, ontogeny and function of P2x1 receptors in mouse ileum, bladder and reproductive systems. Auton Neurosci 92(1–2):56–64
347. Vial C, Evans RJ (2002) P2X(1) receptor-deficient mice establish the native P2X receptor and a P2Y6-like receptor in arteries. Mol Pharmacol 62(6):1438–1445
348. Vial C, Hechler B, Leon C, Cazenave JP, Gachet C (1997) Presence of P2X1 purinoceptors in human platelets and megakaryoblastic cell lines. Thromb Haemost 78(6):1500–1504

349. Chvatchko Y, Valera S, Aubry J-P, Renno T, Buell G, Bonnefoy J-Y (1996) The involvement of an ATP-gated ion channel, P2X1, in thymocyte apoptosis. Immunity 5(3):275–283
350. Palygin O, Lalo U, Verkhratsky A, Pankratov Y (2010) Ionotropic NMDA and P2X1/5 receptors mediate synaptically induced Ca2+ signalling in cortical astrocytes. Cell Calcium 48 (4):225–231
351. Cavaliere F, Amadio S, Dinkel K, Reymann KG, Volonte C (2007) P2 receptor antagonist trinitrophenyl-adenosine-triphosphate protects hippocampus from oxygen and glucose deprivation cell death. J Pharmacol Exp Ther 323(1):70–77. doi:10.1124/jpet.106.119024
352. Sim JA, Park CK, Oh SB, Evans RJ, North RA (2007) P2X1 and P2X4 receptor currents in mouse macrophages. Br J Pharmacol 152(8):1283–1290
353. Banks FC, Knight GE, Calvert RC, Thompson CS, Morgan RJ, Burnstock G (2006) The purinergic component of human vas deferens contraction. Fertil Steril 85(4):932–939
354. Burnstock G, Verkhratsky A (2010) Vas deferens—a model used to establish sympathetic cotransmission. Trends Pharmacol Sci 31 (3):131–139
355. Guan Z, Osmond DA, Inscho EW (2007) P2X receptors as regulators of the renal microvasculature. Trends Pharmacol Sci 28(12):646–652
356. Inscho EW, Cook AK, Imig JD, Vial C, Evans RJ (2003) Physiological role for P2X1 receptors in renal microvascular autoregulatory behavior. J Clin Invest 112(12):1895–1905
357. Inscho EW, Cook AK, Imig JD, Vial C, Evans RJ (2004) Renal autoregulation in P2X1 knockout mice. Acta Physiol Scand 181 (4):445–453
358. Bell PD, Komlosi P, Zhang ZR (2009) ATP as a mediator of macula densa cell signalling. Purinergic Signal 5(4):461–471
359. Osmond DA, Inscho EW (2010) P2X1 receptor blockade inhibits whole kidney autoregulation of renal blood flow in vivo. Am J Physiol Renal Physiol 298(6):F1360–F1368. doi:10.1152/ajprenal.00016.2010
360. Schnermann J (2011) Maintained tubuloglomerular feedback responses during acute inhibition of P2 purinergic receptors in mice. Am J Physiol Renal Physiol 300 (2):F339-F344
361. Hechler B, Lenain N, Marchese P, Vial C, Heim V, Freund M, Cazenave JP, Cattaneo M, Ruggeri ZM, Evans R, Gachet C (2003) A role of the fast ATP-gated P2X1 cation channel in thrombosis of small arteries in vivo. J Exp Med 198(4):661–667
362. Oury C, Kuijpers MJ, Toth-Zsamboki E, Bonnefoy A, Danloy S, Vreys I, Feijge MA, De Vos R, Vermylen J, Heemskerk JW, Hoylaerts MF (2003) Overexpression of the platelet P2X1 ion channel in transgenic mice generates a novel prothrombotic phenotype. Blood 101(10):3969–3976
363. Hu H, Hoylaerts MF (2010) The P2X1 ion channel in platelet function. Platelets 21(3):153–166
364. Mahaut-Smith MP, Jones S, Evans RJ (2011) The P2X1 receptor and platelet function. Purinergic Signal 7(3):341–56, Epub 2011 Mar 22
365. Burnstock G, Knight G (2004) Cellular distribution and functions of P2 receptor subtypes in different systems. Intl Rev Cytol 240:31–304
366. Kanjhan R, Housley GD, Burton LD, Christie DL, Kippenberger A, Thorne PR, Luo L, Ryan AF (1999) Distribution of the P2X2 receptor subunit of the ATP-gated ion channels in the rat central nervous system. J Comp Neurol 407(1):11–32
367. Kidd EJ, Grahames CB, Simon J, Michel AD, Barnard EA, Humphrey PP (1995) Localization of P2X purinoceptor transcripts in the rat nervous system. Mol Pharmacol 48(4):569–573
368. Simon J, Kidd EJ, Smith FM, Chessell IP, Murrell-Lagnado R, Humphrey PP, Barnard EA (1997) Localization and functional expression of splice variants of the P2X2 receptor. Mol Pharmacol 52(2):237–248
369. Xiang Z, Burnstock G (2005) Changes in expression of P2X purinoceptors in rat cerebellum during postnatal development. Brain Res Dev Brain Res 156(2):147–157
370. Calvert JA, Evans RJ (2004) Heterogeneity of P2X receptors in sympathetic neurons: contribution of neuronal P2X1 receptors revealed using knockout mice. Mol Pharmacol 65(1):139–148
371. Ma B, Ruan HZ, Burnstock G, Dunn PM (2005) Differential expression of P2X receptors on neurons from different parasympathetic ganglia. Neuropharmacology 48(5):766–777
372. Ma B, Ruan HZ, Cockayne DA, Ford AP, Burnstock G, Dunn PM (2004) Identification of P2X receptors in cultured mouse and rat parasympathetic otic ganglion neurones including P2X knockout studies. Neuropharmacology 46(7):1039–1048
373. Vulchanova L, Riedl MS, Shuster SJ, Buell G, Surprenant A, North RA, Elde R (1997) Immunohistochemical study of the P2X2 and P2X3 receptor subunits in rat and monkey sensory neurons and their central terminals. Neuropharmacology 36(9):1229–1242
374. Xiang Z, Bo X, Burnstock G (1998) Localization of ATP-gated P2X receptor immunoreactivity in rat sensory and sympathetic ganglia. Neurosci Lett 256(2):105–108
375. Zhong Y, Dunn PM, Bardini M, Ford AP, Cockayne DA, Burnstock G (2001) Changes in P2X receptor responses of sensory neurons from P2X3-deficient mice. Eur J Neurosci 14(11):1784–1792
376. Zhong Y, Dunn PM, Burnstock G (2000) Guinea-pig sympathetic neurons express varying proportions of two distinct P2X receptors. J Physiol 523(Pt 2):391–402
377. Zhong Y, Dunn PM, Burnstock G (2000) Pharmacological comparison of P2X receptors on rat coeliac, mouse coeliac and mouse pelvic ganglion neurons. Neuropharmacology 39(2):172–180
378. Zhong Y, Dunn PM, Xiang Z, Bo X, Burnstock G (1998) Pharmacological and molecular characterization of P2X receptors in rat pelvic ganglion neurons. Br J Pharmacol 125(4):771–781
379. Burton LD, Housley GD, Salih SG, Jarlebark L, Christie DL, Greenwood D (2000) P2X2 receptor expression by interstitial cells of Cajal in vas deferens implicated in semen emission. Auton Neurosci 84(3):147–161
380. Cheung KK, Burnstock G (2002) Localization of P2X3 receptors and coexpression with P2X2 receptors during rat embryonic neurogenesis. J Comp Neurol 443(4):368–382
381. Glass R, Townsend-Nicholson A, Burnstock G (2000) P2 receptors in the thymus: expression of P2X and P2Y receptors in adult rats, an immunohistochemical and in situ hybridisation study. Cell Tissue Res 300(2):295–306
382. Jiang T, Yeung D, Lien CF, Gorecki DC (2005) Localized expression of specific P2X receptors in dystrophin-deficient DMD and mdx muscle. Neuromuscul Disord 15(3):225–236
383. Ryten M, Hoebertz A, Burnstock G (2001) Sequential expression of three receptor subtypes for extracellular ATP in developing rat skeletal muscle. Dev Dyn 221(3):331–341
384. Ryten M, Yang SY, Dunn PM, Goldspink G, Burnstock G (2004) Purinoceptor expression in regenerating skeletal muscle in the mdx mouse model of muscular dystrophy and in satellite cell cultures. FASEB J 18(12):1404–1406
385. Studeny S, Torabi A, Vizzard MA (2005) P2X2 and P2X3 receptor expression in postnatal and adult rat urinary bladder and lumbosacral spinal cord. Am J Physiol Regul Integr Comp Physiol 289(4):R1155–1168
386. Housley GD, Greenwood D, Bennett T, Ryan AF (1995) Identification of a short form of the P2xR1-purinoceptor subunit produced by alternative splicing in the pituitary and cochlea. Biochem Biophys Res Commun 212(2):501–508
387. Koshimizu T, Tomic M, Van Goor F, Stojilkovic SS (1998) Functional role of alternative splicing in pituitary P2X2 receptor-channel activation and desensitization. Mol Endocrinol 12(7):901–913

388. Parker MS, Larroque ML, Campbell JM, Bobbin RP, Deininger PL (1998) Novel variant of the P2X2 ATP receptor from the guinea pig organ of Corti. Hear Res 121(1–2):62–70
389. Troyanovskaya M, Wackym PA (1998) Evidence for three additional P2X2 purinoceptor isoforms produced by alternative splicing in the adult rat vestibular end-organs. Hear Res 126(1–2):201–209
390. Chen C, Parker MS, Barnes AP, Deininger P, Bobbin RP (2000) Functional expression of three P2X(2) receptor splice variants from guinea pig cochlea. J Neurophysiol 83(3):1502–1509
391. Koshimizu TA, Kretschmannova K, He ML, Ueno S, Tanoue A, Yanagihara N, Stojilkovic SS, Tsujimoto G (2006) Carboxyl-terminal splicing enhances physical interactions between the cytoplasmic tails of purinergic P2X receptors. Mol Pharmacol 69 (5):1588–1598
392. Koshimizu TA, Tsujimoto G (2006) Functional role of spliced cytoplasmic tails in P2X2-receptor-mediated cellular signaling. J Pharmacol Sci 101(4):261–266
393. Lynch KJ, Touma E, Niforatos W, Kage KL, Burgard EC, van Biesen T, Kowaluk EA, Jarvis MF (1999) Molecular and functional characterization of human P2X(2) receptors. Mol Pharmacol 56(6):1171–1181
394. Ren J, Bian X, DeVries M, Schnegelsberg B, Cockayne DA, Ford AP, Galligan JJ (2003) P2X2 subunits contribute to fast synaptic excitation in myenteric neurons of the mouse small intestine. J Physiol 552(Pt 3):809–821
395. Devries MP, Vessalo M, Galligan JJ (2010) Deletion of P2X2 and P2X3 receptor subunits does not alter motility of the mouse colon. Front Neurosci 4:22
396. Rong W, Gourine AV, Cockayne DA, Xiang Z, Ford AP, Spyer KM, Burnstock G (2003) Pivotal role of nucleotide P2X2 receptor subunit of the ATP-gated ion channel mediating ventilatory responses to hypoxia. J Neurosci 23(36):11315–11321
397. Burnstock G (2000) P2X receptors in sensory neurones. Br J Anaesth 84(4):476–488
398. Khakh BS, Surprenant A, Humphrey PP (1995) A study on P2X purinoceptors mediating the electrophysiological and contractile effects of purine nucleotides in rat vas deferens. Br J Pharmacol 115(1):177–185
399. Ryten M, Koshi R, Knight GE, Turmaine M, Dunn P, Cockayne DA, Ford AP, Burnstock G (2007) Abnormalities in neuromuscular junction structure and skeletal muscle function in mice lacking the P2X2 nucleotide receptor. Neuroscience 148(3):700–711
400. Chen CC, Akopian AN, Sivilotti L, Colquhoun D, Burnstock G, Wood JN (1995) A P2X purinoceptor expressed by a subset of sensory neurons. Nature 377(6548):428–431
401. Lewis C, Neidhart S, Holy C, North RA, Buell G, Surprenant A (1995) Coexpression of P2X2 and P2X3 receptor subunits can account for ATP-gated currents in sensory neurons. Nature 377 (6548):432–435
402. Souslova V, Ravenall S, Fox M, Wells D, Wood JN, Akopian AN (1997) Structure and chromosomal mapping of the mouse P2X3 gene. Gene 195(1):101–111
403. Barden JA, Bennett MR (2000) Distribution of P2X purinoceptor clusters on individual rat dorsal root ganglion cells. Neurosci Lett 287(3):183–186
404. Bradbury EJ, Burnstock G, McMahon SB (1998) The expression of P2X3 purinoreceptors in sensory neurons: effects of axotomy and glial-derived neurotrophic factor. Mol Cell Neurosci 12(4–5):256–268
405. Novakovic SD, Kassotakis LC, Oglesby IB, Smith JA, Eglen RM, Ford AP, Hunter JC (1999) Immunocytochemical localization of P2X3 purinoceptors in sensory neurons in naive rats and following neuropathic injury. Pain 80(1–2):273–282
406. Ruan HZ, Moules E, Burnstock G (2004) Changes in P2X3 purinoceptors in sensory ganglia of the mouse during embryonic and postnatal development. Histochem Cell Biol 122(6):539–551
407. Staikopoulos V, Sessle BJ, Furness JB, Jennings EA (2007) Localization of P2X2 and P2X3 receptors in rat trigeminal ganglion neurons. Neuroscience 144(1):208–216
408. Vulchanova L, Riedl MS, Shuster SJ, Stone LS, Hargreaves KM, Buell G, Surprenant A, North RA, Elde R (1998) P2X3 is expressed by DRG neurons that terminate in inner lamina II. Eur J Neurosci 10(11):3470–3478
409. North RA (2004) P2X3 receptors and peripheral pain mechanisms. J Physiol 554(Pt 2):301–308. doi:10.1113/jphysiol.2003.048587
410. Elneil S, Skepper JN, Kidd EJ, Williamson JG, Ferguson DR (2001) Distribution of P2X(1) and P2X(3) receptors in the rat and human urinary bladder. Pharmacology 63(2):120–128
411. Alavi AM, Dubyak GR, Burnstock G (2001) Immunohistochemical evidence for ATP receptors in human dental pulp. J Dent Res 80(2):476–483
412. Liu F, Takahashi N, Yamaguchi O (2009) Expression of P2X3 purinoceptors in suburothelial myofibroblasts of the normal human urinary bladder. Int J Urol 16(6):570–575
413. Tempest HV, Dixon AK, Turner WH, Elneil S, Sellers LA, Ferguson DR (2004) P2X and P2X receptor expression in human bladder urothelium and changes in interstitial cystitis. BJU Int 93 (9):1344–8
414. Yiangou Y, Facer P, Baecker PA, Ford AP, Knowles CH, Chan CL, Williams NS, Anand P (2001) ATP-gated ion channel P2X (3) is increased in human inflammatory bowel disease. Neurogastroenterol Motil 13(4):365–369
415. Yiangou Y, Facer P, Birch R, Sangameswaran L, Eglen R, Anand P (2000) P2X3 receptor in injured human sensory neurons. Neuroreport 11(5):993–996
416. Cockayne DA, Hamilton SG, Zhu QM, Dunn PM, Zhong Y, Novakovic S, Malmberg AB, Cain G, Berson A, Kassotakis L, Hedley L, Lachnit WG, Burnstock G, McMahon SB, Ford AP (2000) Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407(6807):1011–1015
417. Souslova V, Cesare P, Ding Y, Akopian AN, Stanfa L, Suzuki R, Carpenter K, Dickenson A, Boyce S, Hill R, Nebenuis-Oosthuizen D, Smith AJ, Kidd EJ, Wood JN (2000) Warm-coding deficits and aberrant inflammatory pain in mice lacking P2X3 receptors. Nature 407(6807):1015–1017
418. Dorn G, Patel S, Wotherspoon G, Hemmings-Mieszczak M, Barclay J, Natt FJ, Martin P, Bevan S, Fox A, Ganju P, Wishart W, Hall J (2004) siRNA relieves chronic neuropathic pain. Nucleic Acids Res 32(5):e49
419. Hemmings-Mieszczak M, Dorn G, Natt FJ, Hall J, Wishart WL (2003) Independent combinatorial effect of antisense oligonucleotides and RNAi-mediated specific inhibition of the recombinant rat P2X3 receptor. Nucleic Acids Res 31(8):2117–2126
420. Barclay J, Patel S, Dorn G, Wotherspoon G, Moffatt S, Eunson L, Abdel'al S, Natt F, Hall J, Winter J, Bevan S, Wishart W, Fox A, Ganju P (2002) Functional downregulation of P2X3 receptor subunit in rat sensory neurons reveals a significant role in chronic neuropathic and inflammatory pain. J Neurosci 22(18):8139–8147
421. Vlaskovska M, Kasakov L, Rong W, Bodin P, Bardini M, Cockayne DA, Ford AP, Burnstock G (2001) P2X3 knock-out mice reveal a major sensory role for urothelially released ATP. J Neurosci 21 (15):5670–5677
422. Cook SP, McCleskey EW (2000) ATP, pain and a full bladder. Nature 407(6807):951–952
423. Bian X, Ren J, DeVries M, Schnegelsberg B, Cockayne DA, Ford AP, Galligan JJ (2003) Peristalsis is impaired in the small intestine of mice lacking the P2X3 subunit. J Physiol 551(Pt 1):309–322

424. McIlwrath SL, Davis BM, Bielefeldt K (2009) Deletion of P2X3 receptors blunts gastro-oesophageal sensation in mice. Neurogastroenterol Motil 21(8):890–e866
425. Shimizu I, Iida T, Guan Y, Zhao C, Raja SN, Jarvis MF, Cockayne DA, Caterina MJ (2005) Enhanced thermal avoidance in mice lacking the ATP receptor P2X3. Pain 116(1–2):96–108
426. Wang Y, Mackes J, Chan S, Haughey NJ, Guo Z, Ouyang X, Furukawa K, Ingram DK, Mattson MP (2006) Impaired long-term depression in P2X3 deficient mice is not associated with a spatial learning deficit. J Neurochem 99(5):1425–1434
427. Egan TM, Cox JA, Voigt MM (2000) Molecular cloning and functional characterization of the zebrafish ATP-gated ionotropic receptor P2X(3) subunit. FEBS Lett 475(3):287–290
428. Norton WH, Rohr KB, Burnstock G (2000) Embryonic expression of a P2X(3) receptor encoding gene in zebrafish. Mech Dev 99(1–2):149–152
429. Kucenas S, Li Z, Cox JA, Egan TM, Voigt MM (2003) Molecular characterization of the zebrafish P2X receptor subunit gene family. Neuroscience 121(4):935–945
430. Kucenas S, Cox JA, Soto F, Lamora A, Voigt MM (2009) Ectodermal P2X receptor function plays a pivotal role in craniofacial development of the zebrafish. Purinergic Signal 5(3):395–407
431. Kucenas S, Soto F, Cox JA, Voigt MM (2006) Selective labeling of central and peripheral sensory neurons in the developing zebrafish using P2X(3) receptor subunit transgenes. Neuroscience 138 (2):641–652
432. Ma B, Wynn G, Dunn PM, Burnstock G (2006) Increased 5-HT (3)-mediated signalling in pelvic afferent neurons from mice deficient in P2X(2) and/or P2X (3) receptor subunits. Purinergic Signal 2(3):481–489
433. Finger TE, Danilova V, Barrows J, Bartel DL, Vigers AJ, Stone L, Hellekant G, Kinnamon SC (2005) ATP signaling is crucial for communication from taste buds to gustatory nerves. Science 310 (5753):1495–1499
434. Eddy MC, Eschle BK, Barrows J, Hallock RM, Finger TE, Delay ER (2009) Double P2X2/P2X3 purinergic receptor knockout mice do not taste NaCl or the artificial sweetener SC45647. Chem Senses 34(9):789–797
435. Hallock RM, Tatangelo M, Barrows J, Finger TE (2009) Residual chemosensory capabilities in double P2X2/P2X3 purinergic receptor null mice: intraoral or postingestive detection? Chem Senses 34(9):799–808
436. Bo X, Zhang Y, Nassar M, Burnstock G, Schoepfer R (1995) A P2X purinoceptor cDNA conferring a novel pharmacological profile. FEBS Lett 375(1–2):129–133
437. Soto F, Garcia-Guzman M, Gomez-Hernandez JM, Hollmann M, Karschin C, Stuhmer W (1996) P2X4: an ATP-activated ionotropic receptor cloned from rat brain. Proc Natl Acad Sci U S A 93(8):3684–3688
438. Garcia-Guzman M, Soto F, Gomez-Hernandez JM, Lund P-E, Stühmer W (1997) Characterization of recombinant human P2X4 receptor reveals pharmacological differences to the rat homologue. Mol Pharmacol 51(1):109–118
439. Seguela P, Haghighi A, Soghomonian JJ, Cooper E (1996) A novel neuronal P2x ATP receptor ion channel with widespread distribution in the brain. J Neurosci 16(2):448–455
440. Wang CZ, Namba N, Gonoi T, Inagaki N, Seino S (1996) Cloning and pharmacological characterization of a fourth P2X receptor subtype widely expressed in brain and peripheral tissues including various endocrine tissues. Biochem Biophys Res Commun 220(1):196–202
441. Dhulipala PD, Wang YX, Kotlikoff MI (1998) The human P2X4 receptor gene is alternatively spliced. Gene 207(2):259–266
442. Naemsch LN, Weidema AF, Sims SM, Underhill TM, Dixon SJ (1999) P2X(4) purinoceptors mediate an ATP-activated, non-selective cation current in rabbit osteoclasts. J Cell Sci 112(Pt 23):4425–4435
443. Townsend-Nicholson A, King BF, Wildman SS, Burnstock G (1999) Molecular cloning, functional characterization and possible cooperativity between the murine P2X4 and P2X4a receptors. Brain Res Mol Brain Res 64(2):246–254
444. Juranka PF, Haghighi AP, Gaertner T, Cooper E, Morris CE (2001) Molecular cloning and functional expression of *Xenopus laevis* oocyte ATP-activated P2X4 channels. Biochim Biophys Acta 1512(1):111–124
445. Bo X, Kim M, Nori SL, Schoepfer R, Burnstock G, North RA (2003) Tissue distribution of P2X4 receptors studied with an ectodomain antibody. Cell Tissue Res 313(2):159–165
446. Hodges RR, Vrouvlianis J, Scott R, Dartt DA (2011) Identification of P2X3 and P2X7 purinergic receptors activated by ATP in rat lacrimal gland. Invest Ophthalmol Vis Sci 52(6):3254–3263
447. Hoebertz A, Townsend-Nicholson A, Glass R, Burnstock G, Arnett TR (2000) Expression of P2 receptors in bone and cultured bone cells. Bone 27(4):503–510
448. Ohata Y, Ogata S, Nakanishi K, Kanazawa F, Uenoyama M, Hiroi S, Tominaga S, Kawai T (2011) Expression of P2X4R mRNA and protein in rats with hypobaric hypoxia-induced pulmonary hypertension. Circ J 75 (4):945–954
449. Sluyter R, Barden JA, Wiley JS (2001) Detection of P2X purinergic receptors on human B lymphocytes. Cell Tissue Res 304 (2):231–236
450. Tanaka J, Murate M, Wang CZ, Seino S, Iwanaga T (1996) Cellular distribution of the P2X4 ATP receptor mRNA in the brain and non-neuronal organs of rats. Arch Histol Cytol 59 (5):485–490
451. Tenneti L, Gibbons SJ, Talamo BR (1998) Expression and transsynaptic regulation of P2X4 and P2Z receptors for extracellular ATP in parotid acinar cells. Effects of parasympathetic denervation. J Biol Chem 273(41):26799–26808
452. Wu T, Dai M, Shi XR, Jiang ZG, Nuttall AL (2011) Functional expression of P2X4 receptor in capillary endothelial cells of the cochlear spiral ligament and its role in regulating the capillary diameter. Am J Physiol Heart Circ Physiol 301 (1):H69-H78
453. Carpenter D, Meadows HJ, Brough S, Chapman G, Clarke C, Coldwell M, Davis R, Harrison D, Meakin J, McHale M, Rice SQ, Tomlinson WJ, Wood M, Sanger GJ (1999) Site-specific splice variation of the human P2X4 receptor. Neurosci Lett 273 (3):183–186
454. Gu BJ, Sun C, Valova VA, Skarratt KK, Wiley JS (2010) Identification of the promoter region of the P2RX4 gene. Mol Biol Rep 37(7):3369–3376
455. Sim JA, Chaumont S, Jo J, Ulmann L, Young MT, Cho K, Buell G, North RA, Rassendren F (2006) Altered hippocampal synaptic potentiation in P2X4 knock-out mice. J Neurosci 26(35):9006–9009
456. Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, Ohura N, Fukuda T, Sato T, Sekine K, Kato S, Isshiki M, Fujita T, Kobayashi M, Kawamura K, Masuda H, Kamiya A, Ando J (2006) Impaired flow-dependent control of vascular tone and remodeling in P2X4-deficient mice. Nat Med 12(1):133–137
457. Brone B, Moechars D, Marrannes R, Mercken M, Meert T (2007) P2X currents in peritoneal macrophages of wild type and P2X4−/− mice. Immunol Lett 113(2):83–89
458. Schwab JM, Guo L, Schluesener HJ (2005) Spinal cord injury induces early and persistent lesional P2X4 receptor expression. J Neuroimmunol 163(1–2):185–189
459. Guo LH, Trautmann K, Schluesener HJ (2005) Expression of P2X4 receptor by lesional activated microglia during formalin-induced inflammatory pain. J Neuroimmunol 163(1–2):120–127

460. Tsuda M, Kuboyama K, Inoue T, Nagata K, Tozaki-Saitoh H, Inoue K (2009) Behavioral phenotypes of mice lacking purinergic P2X4 receptors in acute and chronic pain assays. Mol Pain 5:28
461. Ulmann L, Hirbec H, Rassendren F (2010) P2X4 receptors mediate PGE2 release by tissue-resident macrophages and initiate inflammatory pain. EMBO J 29(14):2290–2300
462. Jakobsson PJ (2010) Pain: how macrophages mediate inflammatory pain via ATP signaling. Nat Rev Rheumatol 6(12):679–681
463. Yamamoto K, Korenaga R, Kamiya A, Ando J (2000) Fluid shear stress activates Ca(2+) influx into human endothelial cells via P2X4 purinoceptors. Circ Res 87(5):385–391
464. Yamamoto K, Korenaga R, Kamiya A, Qi Z, Sokabe M, Ando J (2000) P2X(4) receptors mediate ATP-induced calcium influx in human vascular endothelial cells. Am J Physiol Heart Circ Physiol 279(1):H285–292
465. Liang L, Zsembery A, Schwiebert EM (2005) RNA interference targeted to multiple P2X receptor subtypes attenuates zinc-induced calcium entry. Am J Physiol Cell Physiol 289(2):C388–396
466. Zsembery A, Boyce AT, Liang L, Peti-Peterdi J, Bell PD, Schwiebert EM (2003) Sustained calcium entry through P2X nucleotide receptor channels in human airway epithelial cells. J Biol Chem 278(15):13398–13408
467. Zsembery A, Fortenberry JA, Liang L, Bebok Z, Tucker TA, Boyce AT, Braunstein GM, Welty E, Bell PD, Sorscher EJ, Clancy JP, Schwiebert EM (2004) Extracellular zinc and ATP restore chloride secretion across cystic fibrosis airway epithelia by triggering calcium entry. J Biol Chem 279(11):10720–10729
468. Hu B, Mei QB, Yao XJ, Smith E, Barry WH, Liang BT (2001) A novel contractile phenotype with cardiac transgenic expression of the human P2X4 receptor. FASEB J 15(14):2739–2741
469. Shen JB, Pappano AJ, Liang BT (2006) Extracellular ATP-stimulated current in wild-type and P2X4 receptor transgenic mouse ventricular myocytes: implications for a cardiac physiologic role of P2X4 receptors. FASEB J 20(2):277–284
470. Yang A, Sonin D, Jones L, Barry WH, Liang BT (2004) A beneficial role of cardiac P2X4 receptors in heart failure: rescue of the calsequestrin overexpression model of cardiomyopathy. Am J Physiol Heart Circ Physiol 287(3):H1096–1103
471. Shen JB, Cronin C, Sonin D, Joshi BV, Gongora Nieto M, Harrison D, Jacobson KA, Liang BT (2007) P2X purinergic receptor-mediated ionic current in cardiac myocytes of calsequestrin model of cardiomyopathy: implications for the treatment of heart failure. Am J Physiol Heart Circ Physiol 292(2):H1077–1084
472. Shen JB, Shutt R, Agosto M, Pappano A, Liang BT (2009) Reversal of cardiac myocyte dysfunction as a unique mechanism of rescue by P2X4 receptors in cardiomyopathy. Am J Physiol Heart Circ Physiol 296(4):H1089–1095
473. Zhou SY, Mamdani M, Qanud K, Shen JB, Pappano AJ, Kumar TS, Jacobson KA, Hintze T, Recchia FA, Liang BT (2010) Treatment of heart failure by a methanocarba derivative of adenosine monophosphate: implication for a role of cardiac purinergic P2X receptors. J Pharmacol Exp Ther 333(3):920–928
474. Sonin D, Zhou SY, Cronin C, Sonina T, Wu J, Jacobson KA, Pappano A, Liang BT (2008) Role of P2X purinergic receptors in the rescue of ischemic heart failure. Am J Physiol Heart Circ Physiol 295(3):H1191–H1197
475. Barnard EA, Simon J, Webb TE (1997) Nucleotide receptors in the nervous system. An abundant component using diverse transduction mechanisms. Mol Neurobiol 15(2):103–129
476. Guo W, Xu X, Gao X, Burnstock G, He C, Xiang Z (2008) Expression of P2X5 receptors in the mouse CNS. Neuroscience 156(3):673–692
477. Ruan HZ, Burnstock G (2005) The distribution of P2X5 purinergic receptors in the enteric nervous system of mouse. Cell Tissue Res 319(2):191–200
478. Glass R, Bardini M, Robson T, Burnstock G (2001) Expression of nucleotide P2X receptor subtypes during spermatogenesis in the adult rat testis. Cells Tissues Organs 169(4):377–387
479. Kotnis S, Bingham B, Vasilyev DV, Miller SW, Bai Y, Yeola S, Chanda PK, Bowlby MR, Kaftan EJ, Samad TA, Whiteside GT (2010) Genetic and functional analysis of human P2X5 reveals a distinct pattern of exon 10 polymorphism with predominant expression of the nonfunctional receptor isoform. Mol Pharmacol 77(6):953–960
480. Gayle S, Burnstock G (2005) Immunolocalisation of P2X and P2Y nucleotide receptors in the rat nasal mucosa. Cell Tissue Res 319(1):27–36
481. Groschel-Stewart U, Bardini M, Robson T, Burnstock G (1999) Localisation of P2X5 and P2X7 receptors by immunohistochemistry in rat stratified squamous epithelia. Cell Tissue Res 296(3):599–605
482. Groschel-Stewart U, Bardini M, Robson T, Burnstock G (1999) P2X receptors in the rat duodenal villus. Cell Tissue Res 297(1):111–117
483. Lee HY, Bardini M, Burnstock G (2000) Distribution of P2X receptors in the urinary bladder and the ureter of the rat. J Urol 163(6):2002–2007
484. Meyer MP, Groschel-Stewart U, Robson T, Burnstock G (1999) Expression of two ATP-gated ion channels, P2X5 and P2X6, in developing chick skeletal muscle. Dev Dyn 216(4–5):442–449
485. Ryten M, Dunn PM, Neary JT, Burnstock G (2002) ATP regulates the differentiation of mammalian skeletal muscle by activation of a P2X5 receptor on satellite cells. J Cell Biol 158(2):345–355
486. Banachewicz W, Suplat D, Krzeminski P, Pomorski P, Baranska J (2005) P2 nucleotide receptors on C2C12 satellite cells. Purinergic Signal 1(3):249–257
487. Calvert RC, Shabbir M, Thompson CS, Mikhailidis DP, Morgan RJ, Burnstock G (2004) Immunocytochemical and pharmacological characterisation of P2-purinoceptor-mediated cell growth and death in PC-3 hormone refractory prostate cancer cells. Anticancer Res 24(5A):2853–2859
488. Deli T, Varga N, Adam A, Kenessey I, Raso E, Puskas LG, Tovari J, Fodor J, Feher M, Szigeti GP, Csernoch L, Timar J (2007) Functional genomics of calcium channels in human melanoma cells. Int J Cancer 121(1):55–65
489. Greig AV, Linge C, Healy V, Lim P, Clayton E, Rustin MH, McGrouther DA, Burnstock G (2003) Expression of purinergic receptors in non-melanoma skin cancers and their functional roles in A431 cells. J Invest Dermatol 121(2):315–327
490. Low SE, Kuwada JY, Hume RI (2008) Amino acid variations resulting in functional and nonfunctional zebrafish P2X(1) and P2X (5.1) receptors. Purinergic Signal 4(4):383–392
491. Appelbaum L, Skariah G, Mourrain P, Mignot E (2007) Comparative expression of p2x receptors and ecto-nucleoside triphosphate diphosphohydrolase 3 in hypocretin and sensory neurons in zebrafish. Brain Res 1174:66–75
492. Urano T, Nishimori H, H-j H, Furuhata T, Kimura Y, Nakamura Y, Tokino T (1997) Cloning of P2XM, a Novel Human P2X Receptor Gene Regulated by p53. Cancer Res 57(15):3281–3287
493. Xiang Z, Burnstock G (2005) Expression of P2X receptors on rat microglial cells during early development. GLIA 52(2):119–126
494. Yu Q, Zhao Z, Sun J, Guo W, Fu J, Burnstock G, He C, Xiang Z (2010) Expression of P2X6 receptors in the enteric nervous system of the rat gastrointestinal tract. Histochem Cell Biol 133 (2):177–188
495. Le KT, Babinski K, Seguela P (1998) Central P2X4 and P2X6 channel subunits coassemble into a novel heteromeric ATP receptor. J Neurosci 18(18):7152–7159
496. Nawa G, Miyoshi Y, Yoshikawa H, Ochi T, Nakamura Y (1999) Frequent loss of expression or aberrant alternative splicing of P2XM, a p53-inducible gene, in soft-tissue tumours. Br J Cancer 80(8):1185–1189

497. da Silva RL, Resende RR, Ulrich H (2007) Alternative splicing of P2X6 receptors in developing mouse brain and during in vitro neuronal differentiation. Exp Physiol 92(1):139–145
498. Banfi C, Ferrario S, De Vincenti O, Ceruti S, Fumagalli M, Mazzola A, DA N, Volonte C, Fratto P, Vitali E, Burnstock G, Beltrami E, Parolari A, Polvani G, Biglioli P, Tremoli E, Abbracchio MP (2005) P2 receptors in human heart: upregulation of P2X6 in patients undergoing heart transplantation, interaction with TNFalpha and potential role in myocardial cell death. J Mol Cell Cardiol 39(6):929–939
499. Zippel N, Limbach CA, Ratajski N, Urban C, Pansky A, Luparello C, Kassack MU, Tobiasch E (2012) Purinergic receptors influence the differentiation of human mesenchymal stem cells. Stem Cells Dev 21:884-900
500. Fonfria E, Clay WC, Levy DS, Goodwin JA, Roman S, Smith GD, Condreay JP, Michel AD (2008) Cloning and pharmacological characterization of the guinea pig P2X7 receptor orthologue. Br J Pharmacol 153(3):544–556
501. Paukert M, Hidayat S, Grunder S (2002) The P2X(7) receptor from *Xenopus laevis*: formation of a large pore in *Xenopus* oocytes. FEBS Lett 513(2–3):253–258
502. Berchtold S, Ogilvie AL, Bogdan C, Muhl-Zurbes P, Ogilvie A, Schuler G, Steinkasserer A (1999) Human monocyte derived dendritic cells express functional P2X and P2Y receptors as well as ecto-nucleotidases. FEBS Lett 458(3):424–428
503. Chen Y, Zhang X, Wang C, Li G, Gu Y, Huang LY (2008) Activation of P2X7 receptors in glial satellite cells reduces pain through downregulation of P2X3 receptors in nociceptive neurons. Proc Natl Acad Sci U S A 105(43):16773–16778
504. Di Virgilio F, Chiozzi P, Ferrari D, Falzoni S, Sanz JM, Morelli A, Torboli M, Bolognesi G, Baricordi OR (2001) Nucleotide receptors: an emerging family of regulatory molecules in blood cells. Blood 97(3):587–600
505. Franke H, Grosche J, Schadlich H, Krugel U, Allgaier C, Illes P (2001) P2X receptor expression on astrocytes in the nucleus accumbens of rats. Neuroscience 108(3):421–429
506. Genzen JR, Platel JC, Rubio ME, Bordey A (2009) Ependymal cells along the lateral ventricle express functional P2X(7) receptors. Purinergic Signal 5(3):299–307
507. Vanderwinden JM, Timmermans JP, Schiffmann SN (2003) Glial cells, but not interstitial cells, express P2X7, an ionotropic purinergic receptor, in rat gastrointestinal musculature. Cell Tissue Res 312(2):149–154
508. Yu Y, Ugawa S, Ueda T, Ishida Y, Inoue K, Kyaw Nyunt A, Umemura A, Mase M, Yamada K, Shimada S (2008) Cellular localization of P2X7 receptor mRNA in the rat brain. Brain Res 1194:45–55
509. Zhang XJ, Zheng GG, Ma XT, Yang YH, Li G, Rao Q, Nie K, Wu KF (2004) Expression of P2X7 in human hematopoietic cell lines and leukemia patients. Leuk Res 28(12):1313–1322
510. Garcia-Marcos M, Pochet S, Marino A, Dehaye JP (2006) P2X7 and phospholipid signalling: the search of the "missing link" in epithelial cells. Cell Signal 18(12):2098–2104
511. Ramirez AN, Kunze DL (2002) P2X purinergic receptor channel expression and function in bovine aortic endothelium. Am J Physiol Heart Circ Physiol 282(6):H2106–2116
512. Ray FR, Huang W, Slater M, Barden JA (2002) Purinergic receptor distribution in endothelial cells in blood vessels: a basis for selection of coronary artery grafts. Atherosclerosis 162(1):55–61
513. Schwiebert LM, Rice WC, Kudlow BA, Taylor AL, Schwiebert EM (2002) Extracellular ATP signaling and P2X nucleotide receptors in monolayers of primary human vascular endothelial cells. Am J Physiol Cell Physiol 282(2):C289–301
514. Armstrong JN, Brust TB, Lewis RG, MacVicar BA (2002) Activation of presynaptic P2X7-like receptors depresses mossy fiber-CA3 synaptic transmission through p38 mitogen-activated protein kinase. J Neurosci 22(14):5938–5945
515. Atkinson L, Batten TF, Moores TS, Varoqui H, Erickson JD, Deuchars J (2004) Differential co-localisation of the P2X7 receptor subunit with vesicular glutamate transporters VGLUT1 and VGLUT2 in rat CNS. Neuroscience 123(3):761–768
516. Atkinson L, Milligan CJ, Buckley NJ, Deuchars J (2002) An ATP-gated ion channel at the cell nucleus. Nature 420(6911):42
517. Deuchars SA, Atkinson L, Brooke RE, Musa H, Milligan CJ, Batten TF, Buckley NJ, Parson SH, Deuchars J (2001) Neuronal P2X7 receptors are targeted to presynaptic terminals in the central and peripheral nervous systems. J Neurosci 21(18):7143–7152
518. Ishii K, Kaneda M, Li H, Rockland KS, Hashikawa T (2003) Neuron-specific distribution of P2X7 purinergic receptors in the monkey retina. J Comp Neurol 459(3):267–277
519. Lundy PM, Hamilton MG, Mi L, Gong W, Vair C, Sawyer TW, Frew R (2002) Stimulation of Ca(2+) influx through ATP receptors on rat brain synaptosomes: identification of functional P2X (7) receptor subtypes. Br J Pharmacol 135(7):1616–1626
520. Sperlagh B, Kofalvi A, Deuchars J, Atkinson L, Milligan CJ, Buckley NJ, Vizi ES (2002) Involvement of P2X7 receptors in the regulation of neurotransmitter release in the rat hippocampus. J Neurochem 81(6):1196–1211
521. Anderson CM, Nedergaard M (2006) Emerging challenges of assigning P2X7 receptor function and immunoreactivity in neurons. Trends Neurosci 29(5):257–262
522. Buell GN, Talabot F, Gos A, Lorenz J, Lai E, Morris MA, Antonarakis SE (1998) Gene structure and chromosomal localization of the human P2X7 receptor. Receptors Channels 5(6):347–354
523. Cheewatrakoolpong B, Gilchrest H, Anthes JC, Greenfeder S (2005) Identification and characterization of splice variants of the human P2X7 ATP channel. Biochem Biophys Res Commun 332(1):17–27
524. Feng YH, Li X, Wang L, Zhou L, Gorodeski GI (2006) A truncated P2X7 receptor variant (P2X7-j) endogenously expressed in cervical cancer cells antagonizes the full-length P2X7 receptor through hetero-oligomerization. J Biol Chem 281(25):17228–17237
525. Masin M, Young C, Lim K, Barnes SJ, Xu XJ, Marschall V, Brutkowski W, Mooney ER, Gorecki DC, Murrell-Lagnado R (2012) Expression, assembly and function of novel C-terminal truncated variants of the mouse P2X7 receptor: Re-evaluation of P2X7 knockouts. Br J Pharmacol 165:978-993
526. Bradley HJ, Baldwin JM, Goli GR, Johnson B, Zou J, Sivaprasadarao A, Baldwin SA, Jiang L-H (2011) Residues 155 and 348 contribute to the determination of P2X7 receptor function via distinct mechanisms revealed by single-nucleotide polymorphisms. J Biol Chem 286 (10):8176–8187
527. Barden N, Harvey M, Gagne B, Shink E, Tremblay M, Raymond C, Labbe M, Villeneuve A, Rochette D, Bordeleau L, Stadler H, Holsboer F, Muller-Myhsok B (2006) Analysis of single nucleotide polymorphisms in genes in the chromosome 12Q24.31 region points to P2RX7 as a susceptibility gene to bipolar affective disorder. Am J Med Genet B Neuropsychiatr Genet 141B (4):374–382
528. Ben-Selma W, Ben-Kahla I, Boukadida J, Harizi H (2011) Contribution of the P2X7 1513A/C loss-of-function polymorphism to extrapulmonary tuberculosis susceptibility in Tunisian populations. FEMS Immunol Med Microbiol 63(1):65–72
529. Dao-Ung LP, Fuller SJ, Sluyter R, SkarRatt KK, Thunberg U, Tobin G, Byth K, Ban M, Rosenquist R, Stewart GJ, Wiley JS (2004) Association of the 1513 C polymorphism in the P2X7 gene with familial forms of chronic lymphocytic leukaemia. Br J Haematol 125(6):815–817
530. Elliott JI, Higgins CF (2004) Major histocompatibility complex class I shedding and programmed cell death stimulated through

the proinflammatory P2X7 receptor: a candidate susceptibility gene for NOD diabetes. Diabetes 53(8):2012–2017
531. Fernando SL, Saunders BM, Sluyter R, Skarratt KK, Goldberg H, Marks GB, Wiley JS, Britton WJ (2007) A polymorphism in the P2X7 gene increases susceptibility to extrapulmonary tuberculosis. Am J Respir Crit Care Med 175(4):360–366
532. Jamieson SE, Peixoto-Rangel AL, Hargrave AC, Roubaix LA, Mui EJ, Boulter NR, Miller EN, Fuller SJ, Wiley JS, Castellucci L, Boyer K, Peixe RG, Kirisits MJ, Elias Lde S, Coyne JJ, Correa-Oliveira R, Sautter M, Smith NC, Lees MP, Swisher CN, Heydemann P, Noble AG, Patel D, Bardo D, Burrowes D, McLone D, Roizen N, Withers S, Bahia-Oliveira LM, McLeod R, Blackwell JM (2010) Evidence for associations between the purinergic receptor P2X(7) (P2RX7) and toxoplasmosis. Genes Immun 11 (5):374–383
533. Li CM, Campbell SJ, Kumararatne DS, Bellamy R, Ruwende C, McAdam KP, Hill AV, Lammas DA (2002) Association of a polymorphism in the P2X7 gene with tuberculosis in a Gambian population. J Infect Dis 186(10):1458–1462
534. Lucae S, Salyakina D, Barden N, Harvey M, Gagne B, Labbe M, Binder EB, Uhr M, Paez-Pereda M, Sillaber I, Ising M, Bruckl T, Lieb R, Holsboer F, Muller-Myhsok B (2006) P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major depressive disorder. Hum Mol Genet 15(16):2438–2445
535. Roger S, Mei ZZ, Baldwin JM, Dong L, Bradley H, Baldwin SA, Surprenant A, Jiang LH (2010) Single nucleotide polymorphisms that were identified in affective mood disorders affect ATP-activated P2X7 receptor functions. J Psychiatr Res 44(6):347–355
536. Tekin D, Kayaalti Z, Dalgic N, Cakir E, Soylemezoglu T, Isin Kutlubay B, Aydin Kilic B (2010) Polymorphism in the p2x7 gene increases susceptibility to extrapulmonary tuberculosis in Turkish children. Pediatr Infect Dis J 29 (8):779–782
537. Wiley JS, Dao-Ung LP, Gu BJ, Sluyter R, Shemon AN, Li C, Taper J, Gallo J, Manoharan A (2002) A loss-of-function polymorphic mutation in the cytolytic P2X7 receptor gene and chronic lymphocytic leukaemia: a molecular study. Lancet 359 (9312):1114–1119
538. Xiao J, Sun L, Yan H, Jiao W, Miao Q, Feng W, Wu X, Gu Y, Jiao A, Guo Y, Peng X, Shen A (2010) Metaanalysis of P2X7 gene polymorphisms and tuberculosis susceptibility. FEMS Immunol Med Microbiol 60 (2):165–170
539. Green EK, Grozeva D, Raybould R, Elvidge G, Macgregor S, Craig I, Farmer A, McGuffin P, Forty L, Jones L, Jones I, O'Donovan MC, Owen MJ, Kirov G, Craddock N (2009) P2RX7: a bipolar and unipolar disorder candidate susceptibility gene? Am J Med Genet B Neuropsychiatr Genet 150B(8):1063–1069
540. Grigoroiu-Serbanescu M, Herms S, Muhleisen TW, Georgi A, Diaconu CC, Strohmaier J, Czerski P, Hauser J, Leszczynska-Rodziewicz A, Jamra RA, Babadjanova G, Tiganov A, Krasnov V, Kapiletti S, Neagu AI, Vollmer J, Breuer R, Rietschel M, Nothen MM, Cichon S, Propping P, Nothen MM, Cichon S (2009) Variation in P2RX7 candidate gene (rs2230912) is not associated with bipolar I disorder and unipolar major depression in four European samples. Am J Med Genet B Neuropsychiatr Genet 150B(7):1017–1021
541. Nuckel H, Frey UH, Durig J, Duhrsen U, Siffert W (2004) 1513A/C polymorphism in the P2X7 receptor gene in chronic lymphocytic leukemia: absence of correlation with clinical outcome. Eur J Haematol 72(4):259–263
542. Xiao J, Sun L, Jiao W, Li Z, Zhao S, Li H, Jin J, Jiao A, Guo Y, Jiang Z, Mokrousov I, Shen A (2009) Lack of association between polymorphisms in the P2X7 gene and tuberculosis in a Chinese Han population. FEMS Immunol Med Microbiol 55 (1):107–111
543. Zhang LY, Ibbotson RE, Orchard JA, Gardiner AC, Seear RV, Chase AJ, Oscier DG, Cross NC (2003) P2X7 polymorphism and chronic lymphocytic leukaemia: lack of correlation with incidence, survival and abnormalities of chromosome 12. Leukemia 17(11):2097–2100
544. Sikora A, Liu J, Brosnan C, Buell G, Chessel I, Bloom BR (1999) Cutting edge: purinergic signaling regulates radical-mediated bacterial killing mechanisms in macrophages through a P2X7-independent mechanism. J Immunol 163(2):558–561
545. Basso AM, Bratcher NA, Harris RR, Jarvis MF, Decker MW, Rueter LE (2009) Behavioral profile of P2X7 receptor knockout mice in animal models of depression and anxiety: relevance for neuropsychiatric disorders. Behav Brain Res 198(1):83–90
546. Taylor SR, Gonzalez-Begne M, Sojka DK, Richardson JC, Sheardown SA, Harrison SM, Pusey CD, Tam FW, Elliott JI (2009) Lymphocytes from P2X7-deficient mice exhibit enhanced P2X7 responses. J Leukoc Biol 85(6):978–986
547. Sanchez-Nogueiro J, Marin-Garcia P, Miras-Portugal MT (2005) Characterization of a functional P2X(7)-like receptor in cerebellar granule neurons from P2X(7) knockout mice. FEBS Lett 579 (17):3783–3788
548. MarÌn-GarcÌa P, Sanchez-Nogueiro J, GÛmez-Villafuertes R, LeÛn D, Miras-Portugal MT (2008) Synaptic terminals from mice midbrain exhibit functional P2X7 receptor. Neuroscience 151(2):361–373
549. Clark AK, Staniland AA, Marchand F, Kaan TK, McMahon SB, Malcangio M (2010) P2X7-dependent release of interleukin-1beta and nociception in the spinal cord following lipopolysaccharide. J Neurosci 30(2):573–582
550. Hansen RR, Nielsen CK, Nasser A, Thomsen SI, Eghorn LF, Pham Y, Schulenburg C, Syberg S, Ding M, Stojilkovic SS, Jorgensen NR, Heegaard AM (2011) P2X7 receptor-deficient mice are susceptible to bone cancer pain. Pain 152(8):1766–1776
551. Chessell I, Hatcher J, Billinton A (2011) Mechanistic differentiation of cancer pain: a pivotal role of P2X7 is absent. Pain 152 (8):1703–1704
552. Ke HZ, Qi H, Weidema AF, Zhang Q, Panupinthu N, Crawford DT, Grasser WA, Paralkar VM, Li M, Audoly LP, Gabel CA, Jee WS, Dixon SJ, Sims SM, Thompson DD (2003) Deletion of the P2X7 nucleotide receptor reveals its regulatory roles in bone formation and resorption. Mol Endocrinol 17(7):1356–1367
553. Li J, Liu D, Ke HZ, Duncan RL, Turner CH (2005) The P2X7 nucleotide receptor mediates skeletal mechanotransduction. J Biol Chem 280(52):42952–42959
554. Gartland A, Buckley KA, Bowler WB, Gallagher JA (2003) Blockade of the pore-forming P2X7 receptor inhibits formation of multinucleated human osteoclasts in vitro. Calcif Tissue Int 73 (4):361–369
555. Gartland A, Buckley KA, Hipskind RA, Perry MJ, Tobias JH, Buell G, Chessell I, Bowler WB, Gallagher JA (2003) Multinucleated osteoclast formation in vivo and in vitro by P2X7 receptor-deficient mice. Crit Rev Eukaryot Gene Expr 13(2–4):243–253
556. Nakamoto T, Brown DA, Catalan MA, Gonzalez-Begne M, Romanenko VG, Melvin JE (2009) Purinergic P2X7 receptors mediate ATP-induced saliva secretion by the mouse submandibular gland. J Biol Chem 284(8):4815–4822
557. Novak I, Jans IM, Wohlfahrt L (2010) Effect of P2X(7) receptor knockout on exocrine secretion of pancreas, salivary glands and lacrimal glands. J Physiol 588 (Pt 18):3615–3627
558. Sanz JM, Chiozzi P, Ferrari D, Colaianna M, Idzko M, Falzoni S, Fellin R, Trabace L, Di Virgilio F (2009) Activation of microglia by amyloid beta requires P2X7 receptor expression. J Immunol 182(7):4378–4385
559. Hracsko Z, Baranyi M, Csolle C, Goloncser F, Madarasz E, Kittel A, Sperlagh B (2011) Lack of neuroprotection in the absence of

P2X7 receptors in toxin-induced animal models of Parkinson's disease. Mol Neurodegener 6:28
560. Matute C, Torre I, Perez-Cerda F, Perez-Samartin A, Alberdi E, Etxebarria E, Arranz AM, Ravid R, Rodriguez-Antiguedad A, Sanchez-Gomez M, Domercq M (2007) P2X(7) receptor blockade prevents ATP excitotoxicity in oligodendrocytes and ameliorates experimental autoimmune encephalomyelitis. J Neurosci 27 (35):9525–9533
561. Sharp AJ, Polak PE, Simonini V, Lin SX, Richardson JC, Bongarzone ER, Feinstein DL (2008) P2x7 deficiency suppresses development of experimental autoimmune encephalomyelitis. J Neuroinflammation 5:33
562. McLarnon JG, Ryu JK, Walker DG, Choi HB (2006) Upregulated expression of purinergic P2X(7) receptor in Alzheimer disease and amyloid-beta peptide-treated microglia and in peptide-injected rat hippocampus. J Neuropathol Exp Neurol 65(11):1090–1097
563. Lee HG, Won SM, Gwag BJ, Lee YB (2011) Microglial P2X receptor expression is accompanied by neuronal damage in the cerebral cortex of the APPswe/PS1dE9 mouse model of Alzheimer's disease. Exp Mol Med 43 (1):7–14
564. Parvathenani LK, Tertyshnikova S, Greco CR, Roberts SB, Robertson B, Posmantur R (2003) P2X7 mediates superoxide production in primary microglia and is up-regulated in a transgenic mouse model of Alzheimer's disease. J Biol Chem 278(15):13309–13317
565. Diaz-Hernandez M, Diez-Zaera M, Sanchez-Nogueiro J, Gomez-Villafuertes R, Canals JM, Alberch J, Miras-Portugal MT, Lucas JJ (2009) Altered P2X7-receptor level and function in mouse models of Huntington's disease and therapeutic efficacy of antagonist administration. FASEB J 23(6):1893–1906
566. Peng W, Cotrina ML, Han X, Yu H, Bekar L, Blum L, Takano T, Tian GF, Goldman SA, Nedergaard M (2009) Systemic administration of an antagonist of the ATP-sensitive receptor P2X7 improves recovery after spinal cord injury. Proc Natl Acad Sci U S A 106(30):12489–12493
567. Chen L, Brosnan CF (2006) Exacerbation of experimental autoimmune encephalomyelitis in P2X7R−/− mice: evidence for loss of apoptotic activity in lymphocytes. J Immunol 176(5):3115–3126
568. Marcellino D, Suarez-Boomgaard D, Sanchez-Reina MD, Aguirre JA, Yoshitake T, Yoshitake S, Hagman B, Kehr J, Agnati LF, Fuxe K, Rivera A (2010) On the role of P2X(7) receptors in dopamine nerve cell degeneration in a rat model of Parkinson's disease: studies with the P2X(7) receptor antagonist A-438079. J Neural Transm 117 (6):681–687
569. Anisman H, Hayley S, Turrin N, Merali Z (2002) Cytokines as a stressor: implications for depressive illness. Int J Neuropsychopharmacol 5(4):357–373
570. Dantzer R (2001) Cytokine-induced sickness behavior: mechanisms and implications. Ann N Y Acad Sci 933:222–234
571. Sharpley CF, Agnew LL (2011) Cytokines and depression: findings, issues, and treatment implications. Rev Neurosci 22 (3):295–302
572. Wichers M, Maes M (2002) The psychoneuroimmunopathophysiology of cytokine-induced depression in humans. Int J Neuropsychopharmacol 5(4):375–388
573. Boucher AA, Arnold JC, Hunt GE, Spiro A, Spencer J, Brown C, McGregor IS, Bennett MR, Kassiou M (2011) Resilience and reduced c-Fos expression in P2X7 receptor knockout mice exposed to repeated forced swim test. Neuroscience 189:170–177
574. Duan S, Anderson CM, Keung EC, Chen Y, Chen Y, Swanson RA (2003) P2X7 receptor-mediated release of excitatory amino acids from astrocytes. J Neurosci 23(4):1320–1328
575. Papp L, Vizi E, Sperlágh B (2004) Lack of ATP-evoked GABA and glutamate release in the hippocampus of P2X7 receptor−/− mice. Neuroreport 15(15):2387–2391
576. Labrousse VF, Costes L, Aubert A, Darnaudery M, Ferreira G, Amedee T, Laye S (2009) Impaired interleukin-1beta and c-Fos expression in the hippocampus is associated with a spatial memory deficit in P2X(7) receptor-deficient mice. PLoS One 4(6): e6006
577. Krueger JM, Taishi P, De A, Davis CJ, Winters BD, Clinton J, Szentirmai E, Zielinski MR (2010) ATP and the purine type 2 X7 receptor affect sleep. J Appl Physiol 109 (5):1318–1327.
578. Adinolfi E, Melchiorri L, Falzoni S, Chiozzi P, Morelli A, Tieghi A, Cuneo A, Castoldi G, Di Virgilio F, Baricordi OR (2002) P2X7 receptor expression in evolutive and indolent forms of chronic B lymphocytic leukemia. Blood 99(2):706–708
579. Li X, Zhou L, Feng YH, Abdul-Karim FW, Gorodeski GI (2006) The P2X7 receptor: a novel biomarker of uterine epithelial cancers. Canc Epidemiol Biomarkers Prev 15(10):1906–1913
580. Raffaghello L, Chiozzi P, Falzoni S, Di Virgilio F, Pistoia V (2006) The P2X7 receptor sustains the growth of human neuroblastoma cells through a substance P-dependent mechanism. Cancer Res 66(2):907–914
581. Slater M, Danieletto S, Barden JA (2005) Expression of the apoptotic calcium channel P2X7 in the glandular epithelium. J Mol Histol 36(3):159–165
582. Slater M, Danieletto S, Gidley-Baird A, Teh LC, Barden JA (2004) Early prostate cancer detected using expression of non-functional cytolytic P2X7 receptors. Histopathology 44(3):206–215
583. Solini A, Cuccato S, Ferrari D, Santini E, Gulinelli S, Callegari MG, Dardano A, Faviana P, Madec S, Di Virgilio F, Monzani F (2008) Increased P2X7 receptor expression and function in thyroid papillary cancer: a new potential marker of the disease? Endocrinology 149(1):389–396
584. Jelassi B, Chantome A, Alcaraz-Perez F, Baroja-Mazo A, Cayuela ML, Pelegrin P, Surprenant A, Roger S (2011) P2X(7) receptor activation enhances SK3 channels- and cystein cathepsin-dependent cancer cells invasiveness. Oncogene 30 (18):2108–2122
585. Ren S, Zhang Y, Wang Y, Lui Y, Wei W, Huang X, Mao W, Zuo Y (2010) Targeting P2X receptor inhibits the metastasis of murine P388D1 lymphoid neoplasm cells to lymph nodes. Cell Biol Int 34 (12):1205–1211