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Allosteric modulation of ATP-gated P2X receptor channels

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Abstract

Seven mammalian purinergic receptor subunits, denoted P2X1 to P2X7, and several spliced forms of these subunits have been cloned. When heterologously expressed, these cDNAs encode ATP-gated non-selective cation channels organized as trimers. All activated receptors produce cell depolarization and promote Ca²⁺ influx through their pores and indirectly by activating voltage-gated calcium channels. However, the biophysical and pharmacological properties of these receptors differ considerably, and the majority of these subunits are also capable of forming heterotrimers with other members of the P2X receptor family, which confers further different properties. These channels have three ATP binding domains, presumably located between neighboring subunits, and occupancy of at least two binding sites is needed for their activation. In addition to the orthosteric binding sites for ATP, these receptors have additional allosteric sites that modulate the agonist action at receptors, including sites for trace metals, protons, neurosteroids, reactive oxygen species and phosphoinositides. The allosteric regulation of P2X receptors is frequently receptor-specific and could be a useful tool to identify P2X members in native tissues and their roles in signaling. The focus of this review is on common and receptor-specific allosteric modulation of P2X receptors and the molecular base accounting for allosteric binding sites.

Keywords

Purinergic receptor channels; allosteric P2X receptor regulation; reactive oxygen species; zinc; copper; neurosteroids; ivermectin; alcohol; phosphoinositides

ALLOSTERISM AS A MODE OF RECEPTOR FUNCTION REGULATION

Enzyme activity depends not only on substrate availability but also on the final pathway products and on numerous endogenous small-sized compounds, including nucleotide and nucleotide derivatives. Exogenous compounds, including toxins, may also alter enzyme activity, a concept that has clinical applications. These early findings lead to discovery that enzymes, in addition to their classical catalytic sites, have non-catalytic regulator sites. Because these sites are independent and physically separated from the catalytic domain of the enzyme, the term allosteric was coined, referring to *allos* and *stereo* (spatially separated sites). This hypothesis implies that modulators alter the tertiary and quaternary structure of enzymes, leading to increase or decrease in their activity. Allosteric regulators may therefore

be classified either as positive or negative, depending on their mode of action at the regulatory site /124/.

Soon after the description of allosterism as a mechanism of enzyme regulation, Changeaux and collaborators applied this concept to explain essential pharmacological features of the enzyme acetylcholinesterase and to the acetylcholine receptor identified from *Torpedo*'s electroplax /19–21/. They demonstrated positive cooperativeness in acetylcholine binding, a phenomenon previously observed for hemoglobin oxygen binding. Once the pentameric structure of the nicotinic receptor was disclosed, it was immediately recognized that two acetylcholine molecules are required to open the nicotinic receptor channel and was confirmed that the binding of the first ligand molecule facilitates the binding of the second molecule /69/. Several modulator sites were soon discovered, affecting the affinity of receptors for acetylcholine and channel opening /69/.

Following the seminal work with the nicotinic receptor, multiple allosteric regulatory sites were soon described for other ligand-gated receptor channels. For example, the GABA_A receptor, which shares with the nicotinic receptor a pentameric subunit composition, is regulated allosterically by a variety of endogenous and exogenous compounds/drugs /36/. Among them, perhaps the best studied are the benzodiazepines and their endogenous counterpart, the alleged endozepines /72/. Steroids, including brain-derived neurosteroids, also allosterically modulate this receptor at a site mimicked by alphaxolone, a steroid with anesthetic properties. The GABA_A receptors also have ethanol and barbiturate regulatory sites, which might or not be related to the site for non-steroidal volatile or venous anesthetic agents, such as propofol or ketamine.

In addition, the group headed by A. Christophanoulus has extensively elaborated on the role of allosteric modulators of G protein-coupled receptors as a novel approach for the treatment of brain disorders /25/. Also, several compounds previously thought to be competitive inhibitors of receptors have now been found to fit best as negative receptor modulators; inversely, compounds known to facilitate receptor activity are in fact, positive allosteric receptor modulators.

Allosteric modulation has opened exciting opportunities in pharmacology, with wide implications not only to the clinic of anesthetic drugs but also to the industry. For example, in the case of taste receptors, a positive allosteric regulator of the sweet receptors will require less sugar or its substitute, for an equivalent sweet taste. Likewise, for the salt receptor, a positive allosteric modulator will be useful for the food industry increasing the salty taste without increasing the salt concentration in food or beverages.

Like glutamate, nicotinic and GABA_A receptor-channels, the function of P2X receptors (P2XRs) is also allosterically modulated and multiple regulator allosteric sites have been identified. This review focuses on endogenous trace metals such as zinc or copper, ivermectin (IVM), neurosteroids, and other drugs that act as P2XR modulators. We also will cover the role of reactive oxygen species (ROS), the phosphoinositides, alcohols and related clinically relevant anesthetic drugs as P2XR modulators and their putative mechanism(s) of receptor activity modulation.

MOLECULAR STRUCTURE OF PURINERGIC P2X RECEPTORS

ATP was suspected to be a biosignaling molecule circa 80 years ago following the pioneer work of the Szent-Gyorgyi's laboratory in Hungary; they demonstrated cardiovascular properties of muscle extracts enriched in adenine derivatives /52/. However, the idea that purines are involved in nerve communication, as in purinergic transmission, emerged 40 years later /16/; this concept by Burnstock was received with great skepticism, until the first

purinergic receptors were cloned in the 90's /113, 180/. It is well established now that nucleotides derived from either purines or pyrimidines activate separate families of membrane receptors: the two transmembrane (TM) domain P2XRs, and the seven TM domain P2Y receptors. In addition, it was soon realized that adenosine activates a separate family of receptors (the P1 or adenosine receptors); both the P1 and the P2Y receptors signaling through heterotrimeric G proteins /147/. The first two P2XRs were cloned 15 years ago by two independent groups /13, 175/ and gained immediate attention because of their unique structural and biological properties. Subsequently five additional mammalian subunits were cloned and termed P2X1-7. These receptors are organized as trimeric homomers or heteromers /134/.

All P2XR subunits have two TM domains (TM1 and TM2), a large extracellular loop, and intracellularly located N- and C- termini /133, 170, 175/. The TM domains are involved in gating the ion channel and lining the ion pore; these amino acid sequences are predicted to adopt α -helical structures in activated P2X2Rs /54, 73, 74, 89, 97, 148/ and P2X4Rs /86, 91, 159/. Helices of different subunits move relative to each other during channel opening and closing /54/. The TM2 appears to play a dominant role in receptor functions, including the fixation of receptors in the membrane /53, 169/, channel assembly, gating, ion selectivity, and permeability for divalent ions /54, 97, 107, 153/. The TM2 residues Thr³³⁶, Thr³³⁹ and Ser³⁴⁰ were suggested to contribute to the formation of the pore, gate, and selectivity filter of P2X2Rs /55, 120, 153/.

The TM regions are separated by a large hydrophilic extracellular loop (ectodomain) containing several conserved amino acid residues, including ten conserved cysteine residues that have been predicted to make disulfide bonds in the following order (P2X4R numbering): 116–165 (SS1), 126–149 (SS2), 132–159 (SS3), 217–227 (SS4) and 261–270 (SS5) /28, 58/. These bonds provide the structural base for the tridimensional organization of P2XRs. The ectodomain contains 2–6 asparagines that may serve as N-linked glycosylation sites, as well as residues relevant for formation of orthosteric and multiple allosteric binding sites /60/. Positively charged lysine residues 67, 69, and 313 (P2X4R numbering) are probably involved in coordinating the binding of negatively charged phosphates of ATP /57, 90, 196/. The role of Lys¹⁹⁰ in ATP binding to the P2X1R and P2X4R as well as Thr¹⁸⁶ and Phe¹⁸⁸ residues in binding to P2X1R has also been suggested /150, 151, 192/. It is also possible that the AspPheArg ectodomain motif contributes to ATP binding /150/. Furthermore, a large sequence downstream of the Lys³¹³ residue of P2X4R may contribute to agonist evoked conformational changes /191/ as well as the Thr¹⁸⁶ and Phe¹⁸⁸ residues of P2X1R /151/. The cysteine residues forming SS2 and SS3 bonds may contribute to the formation of a metal ion-binding site, which together with a nearby histidine residue, contributes to Zn²⁺ binding of P2X2R /27, 28, 66/ and P2X4R /29/. The relevance of SS bonds in responsiveness of P2X4R to ethanol was also suggested /193/. These findings will be discussed in details in the following sections.

The N-termini of all P2XRs are relatively short (around 25 amino acids), whereas the length of C-termini ranges from around 30 amino acids (P2X6R) to about 215 residues (P2X7R). Both N- and C-termini serve as molecular targets for a series of posttranscriptional modifications, including RNA splicing, phosphorylation, and protein-protein interactions. The C-terminal tail of P2X2R plays important roles in determining the rate of desensitization (a decrease in the current amplitude during the sustained agonist application), as documented by characterization of functional splice forms of these receptors and side-directed mutagenesis experiments /14, 99–101/. In contrast, the C-terminal of P2X7R is involved in the sustained facilitation of receptors (an increase in the current amplitude during the sustained agonist application) /9, 152, 190/. It also appears that the interactions between N- and C-termini play a role in P2X2R gating /101/.

Recently, crystallization of the zebrafish P2X4R has fully confirmed the trimeric structure of the functional P2XRs, helical organization of TM domain, detailed structural elements of the pore and channel vestibules, as well as sharp anatomical evidences of the orthosteric ATP binding sites /96/. This work also revealed that a single P2X4 subunit resembles a “dolphin” with three disulfide bonds (SS1-3) occurring in the “head,” the SS4 bond in the “dorsal fin” and the SS5 bond located near the TM domains at the bottom of the body region. Using this tridimensional structure as a molecular sculpt it is now possible to better define and characterize the allosteric sites that we will be next discussing.

TRACE METALS AS ALLOSTERIC MODULATORS OF P2XRs

Trace metals are essential to life; these atoms are integral components of enzymes and other proteins. Metals coordinate with specific amino acid residues, such as histidines or cysteines, and amino acids with negatively charged residues, such as aspartic and glutamic acid. Among them, the main body trace metals zinc, copper and iron have particular relevance; cells have evolved unique mechanisms for their absorption, intracellular transport and elimination /114/. In addition, intracellular trafficking is tightly regulated by several specific metallo-chaperones that direct the metals to specific cellular pathways avoiding toxicity through non-specific binding to other proteins. The catalytic activity of several CNS enzymes, including superoxide dismutase, alcohol dehydrogenase, cytochrome c-oxidase and tyrosine or tryptophan hydroxylases, are zinc and/or copper-dependent /93, 127, 149/. Zinc is also important in regulation of gene expression by binding to specific DNA sequences and forming zinc-finger motifs /121/ and copper in expression of the prion protein /176/.

A turning point in the understanding of the physiology of these metals was the discovery that trace metals zinc and copper are transported intracellularly and stored in synaptic vesicles through transporters like ZnT3 and ZnT4 /135/ or Ctr1 for copper /104/ together with transmitters such as glutamate, GABA or ATP. After release to the synaptic cleft, these metals can reach concentrations as high as 250–300 μM /8, 94/. The development of molecular biology techniques in the 80's and 90's generated the possibility to explore in details the metal-modulation of receptors and channels expressed in heterologous systems leading to the identification of metal allosteric sites.

In general, the released metal may interact with several membrane channels and/or receptors, modulating the activity of these proteins and changing pre or postsynaptic responses; metal targets include voltage-gated sodium (Na_v), calcium (Ca_v), or potassium (K_v) channels; store-operated calcium channels, transient receptor potential channels and ligand-gated channels /115/. Zinc and copper also modulate the activity of most ligand-gated ionic channels, including NMDA and AMPA/kainate glutamate receptors, GABA_A, glycine, and nicotinic receptors; these metals do not gate *per-se* the channel, since they all require the ligand to open the pore /84/. Several reports have identified critical residues required for zinc and copper effects on these receptors, most of them are histidines, cysteine or aspartic acid located in the extracellular domain of the channel /84, 155/. A most direct and illustrative experimental protocol that fully supports and illuminates the relevance of metal-modulation comes from the work with the glycine receptor channel that is positively regulated by zinc. Transgenic mice expressing the D80A mutant of the α -1 subunit of this receptor, which is zinc-insensitive, exhibited hyperekplexia, a complex neurological disorder, compared to littermates expressing the wild type glycine receptor. This finding allowed to the authors to conclude that the endogenous release of zinc in the brain is decisive for normal glycinergic transmission /79/.

One of the most remarkable characteristics of P2XRs is their differential allosteric modulation by metals /2, 29, 32, 33, 84/. The effects vary among different metals and between the different P2X subunits (summarized in Table 1); several amino acid residues have been identified as crucial for metal effects and they probably form part of specific metal allosteric sites as is summarized in Fig. 1. The first allosteric modulators described for the P2XR family were trace metals zinc and copper, which were also used as additional criteria to characterize P2XRs.

P2X1R, P2X3R and P2X5R. Whereas the P2X1R function is inhibited by zinc in a concentration-dependent manner, the P2X3R function is weakly potentiated by this metal, with a maximal effect at 10 μ M /182/. Higher zinc concentrations reduce P2X3R current, suggesting the possible existence of 2 allosteric sites at this receptor, a high-affinity with a positive modulator effect and a low-affinity site with a negative modulator role. The P2X5R is also modulated in a biphasic fashion by zinc, suggesting again at least two allosteric sites for this metal /181/. A modest inhibitory effect of cadmium on P2X1R and P2X3R has also been reported /132/. There is no information about the amino acid residues involved in the putative allosteric sites for trace metals of these receptors.

P2X2R. Both zinc and copper have a biphasic effect on this receptor, a significant potentiation of the ATP-induced responses at 10–100 μ M, and an inhibition with millimolar metal concentrations /112, 129/. Because zinc and copper have similar effects on receptor function, it was hypothesized that these metals act at a common allosteric site. Directed mutagenesis showed the critical role of extracellular His¹²⁰ and His²¹³ for both the zinc and copper positive modulation /27, 112/, whereas His¹⁹², His²⁴⁵ and His³¹⁹ are partially involved, probably contributing to the stabilization of the allosteric site /112/. Using concatamers, Hume's group demonstrated that the allosteric sites for zinc and probably copper are formed by His¹²⁰ and His²¹³ of adjacent subunits /129/, a hypothesis that has been recently confirmed with the crystallization of a zebrafish P2XR /96/. More recently, the same group showed that contrary to the rat and mouse P2X2Rs, the human P2X2R is inhibited by zinc and copper /128, 166/, revealing the species-specific modes of metal modulation. A study of single-channel properties of the P2X2R has revealed complex copper effects because the metal increases the gating of the channel in a cooperative manner (Leiva-Salcedo, personal communication). A plausible interpretation of this finding suggests that copper may not only modify the channel aperture but it also may recruit subunits and induce a synergistic effect on the macro current. Other divalent metals also modulate the activity of the P2X2R; cadmium, mercury, nickel, palladium and cobalt potentiate the receptor activity, and with the exemption of mercury, all of these metals interact with the same allosteric site of zinc and mercury /112/. In the case of mercury potentiation, the use of chimeric P2X2/4Rs allowed us to determine that the primary site of action of this metal is an intracellular cysteine at position 430 /30/. We postulate that this effect is more related with the oxidative capacities of mercury than to a direct binding of the metal at a common extracellular metal allosteric site; this issue will be discussed in more detail in a next section.

P2X4R. The most exceptional characteristic of this receptor is its differential modulation by zinc and copper; whereas zinc potentiates the receptor activity, copper inhibits it /2/, making it a suitable model to identify and dissect allosteric sites. This receptor contains only 3 extracellular histidines (a common target for copper and zinc binding) in its ectodomain. Site-directed mutagenesis studies revealed that out of the three histidines, only His¹⁴⁰ was critical for the inhibitory action of copper /32/. Additionally, replacement of His¹⁴⁰ by alanine dramatically modified the pattern of zinc action, changing it from a bell-shaped curve, typical of biphasic modulation, to a sigmoid curve in which the maximal effect of zinc was dramatically increased /32/. These observations suggest the following conclusions: i) His¹⁴⁰ is part of an inhibitory allosteric site for copper; ii) zinc also binds to this inhibitory

site (with lower affinity than copper) but when this site is absent, as in the H140A mutant, zinc only binds to the positive modulator site resulting in a sigmoid concentration-response curve.

Later, Asp¹³⁸ was identified as a second component of the P2X₄R inhibitory site /29/; mutation of this residue to an alanine as in D138A, rendered similar effects to those observed with H140A, obliterating the copper-inhibition and modifying the zinc-potential from biphasic to sigmoid. On the other hand, Cys¹³² was identified to be part of the positive allosteric site; the mutation of this residue to alanine, not only obliterated the zinc-potential but in this mutant zinc also exerted an inhibitory modulation, mimicking somehow the inhibitor modulation exerted by copper. As discussed before, since zinc may interact with the 2 metal allosteric sites of the P2X₄R, in the C132A mutant the metal now only binds to the inhibitory allosteric site and in this regard zinc behaves like copper in this particular receptor mutant /29/.

The allosteric actions of metals are not limited to copper and zinc; cadmium, mercury and cobalt also modulate P2X₄R activity /31/. The effects of cadmium are similar to that of zinc. Competition studies of both metals and the use of P2X₄R mutants indicate that cadmium binds to the same positive allosteric site as zinc /2, 31/. In contrast, mercury inhibited the P2X₄R activity in a similar fashion to copper; however, these two metals do not interact at a common and unique negative allosteric site since the H140A and D138A mutants, which are totally resistant to the action of copper, were inhibited by mercury even to a larger extent than the wild type receptor /31/. The precise determination of the negative modulator site of mercury remains unknown; it is possible that this metal acts at an intracellular site as it has been observed for the P2X₂R /30/. Other metals, like manganese, lead, barium, or nickel did not affect P2X₄R activity /2, 31/, demonstrating the strict structural requirements of the modulator allosteric sites characterized.

P2X₇R. Divalent cations calcium and magnesium inhibit P2X₇R activity /178/ as well as copper and zinc /1/, indicating these trace metals exert a negative modulator role. Copper is 20-fold more potent than zinc, with an IC₅₀ of 4 μ M versus 80 μ M, respectively /1/. Mutagenesis of three of the six extracellular histidines results in an attenuated copper or zinc modulation, indicating that His²⁶⁷ is a key amino acid for trace metal modulation. An additional role for His¹³⁰ and His²⁰¹ residues in copper inhibition and for His²¹⁹ residue in zinc inhibition was deduced by Acuna-Castillo et al. /1/. More recently, a role of Asp¹⁹⁷ and His⁹² in copper and zinc inhibition was proposed; none of these mutants alone eliminated the modulation by these trace metals; however, the modulation was blunted by the combined mutation of these two amino acids /111/. Minor discrepancies between the later two studies discussed could be accounted for by differences in the P2X₇R expression system (oocytes vs HEK293 cells) or the agonist used (ATP vs BzATP). It should be kept in mind that trace metals might exert a complex modulation due to multiple allosteric sites for copper and zinc; future experiments should clarify this issue. As with the P2X₂R, species differences in metal modulation have been reported for this receptor; whilst copper or zinc attenuated ATP-gated current in the rat P2X₇R, only copper, but not zinc, inhibited the mouse receptor /125/. In contrast, zinc effects were found to be dependent on the agonist used to activate the receptor; zinc potentiated the ATP-gated currents but inhibited those elicited by BzATP /125/; we lack a molecular interpretation of these findings, although minor divergences could be attributed, at least in part, to the nature of the ligand-receptor complex.

IVERMECTIN, A SPECIFIC P2X₄R MODULATOR

The selectivity, potency, and large efficacy of IVM to potentiate the ATP-gated currents of homomeric and heteromeric P2X₄R explains its interest as a nucleotide receptor modulator;

in view of its specificity, IVM is used as a tool to support nucleotide receptor subtype classification. In addition, a search for its putative endogenous ligand is a challenging feature that will open novel insights to the evolution of this modulator site that, while selective for P2X4R, also interacts with other prominent brain transmitter receptors of the pentameric family of ionotropic channels.

IVM is a member of the avermectin family of lipophylic compounds; it is widely used as an antiparasitic agent in human and veterinary medicine /85/. IVM is a relatively bulky molecule (870 molecular weight); it spans a distance of about 20Å, as documented by nuclear magnetic resonance and X-ray crystallographic analysis /82, 163/. It has been suggested that the therapeutic effects of IVM are mediated by an interaction with a glutamate-gated chloride channel expressed by parasites, leading to muscle paralysis and starvation /38, 47/. In addition to glutamate-gated chloride channels expressed in invertebrate nerve and muscle cells /85/, IVM was found to modulate GABA_A receptors from chick /158/ and mouse /103/, recombinant glycine-activated chloride channels /157/, and neuronal $\alpha 7$ nicotinic receptors from chick and humans /102/.

IVM is a selective and specific allosteric modulator of mammalian P2X4R /98, 145/; the P2X4-like receptor cloned from the parasitic blood fluke *Schistosoma mansoni* is also sensitive to this drug /3/. Extracellularly applied IVM increases both current amplitude in response to supramaximal agonist concentration and sensitivity to ATP and α, β -meATP in rat P2X4R expressed in either *Xenopus* oocytes or the human receptor expressed in HEK293 cells. In both cell types, IVM also reduces the desensitization rate and greatly prolongs the deactivation of current after ATP removal /98, 145/. Single channel analysis showed that IVM increases the probability of channel opening and prolongs the mean channel open time without affecting channel conductance /145/.

Initial experiments indicated that IVM is effective only when applied extracellularly, suggesting that the receptor ectodomain contains the binding site for this compound /145/. However, it was recently reported that effects of IVM on the current amplitude, but not on the deactivation kinetics, are abolished in the C-terminal tail-truncated P2X4R and on the Y378A receptor mutant /171/, implying that intracellular sites might also be involved. Experiments using chimeric receptors revealed that TM domains and nearby residues of P2X4R are involved in the IVM effects on channel deactivation /87/. This conclusion was confirmed in gain-of-function experiments by making eight TM chimeras between P2X4R and P2X2R /160/. This group also searched for residues involved in IVM binding using tryptophan scanning mutagenesis. To evaluate IVM effects in such mutants, and to identify residues showing altered IVM effects, the authors used the ATP EC₅₀, a saturating concentration of IVM, and less than two-fold and larger than 5-fold increases in the amplitude of current attained in the presence of IVM. Their study revealed weakened effects of IVM at Val²⁸, Ile³⁹, Val⁴³ and Val⁴⁷ TM-1 mutants, as well as at Gly³⁴⁰, Gly³⁴², Leu³⁴⁵ and Val³⁴⁸ TM-2 mutants, and enhanced effects of IVM at Gly²⁹, Val³⁵, Leu³⁷, Leu⁴⁰, Ala⁴¹, Ser³⁴¹, Gly³⁴⁷, Ala³⁴⁹, Val³⁵¹, and Cys³⁵³ mutants. Helical net diagrams of TM domains showed a random distribution of these residues; none of these residues was P2X4R-specific /160/.

In another study, cysteine-scanning mutagenesis of the rat P2X4R TM domain was used to identify IVM-sensitive hits: Gln³⁶, Leu⁴⁰, Val⁴³, Val⁴⁷, Trp⁵⁰, Asn³³⁸, Gly³⁴², Leu³⁴⁶, Ala³⁴⁹, and Ile³⁵⁶ /86/. The pattern of these predominantly nonpolar residues, which are also present in the IVM-sensitive *Schistosoma mansoni* P2X subunit, was consistent with helical topology of both TM domains, with every third or fourth amino acid affected by substitution. Five of these residues have also been identified by tryptophan scanning mutagenesis /160/. These residues lie on the same side of their helices, whereas the IVM-

independent hits Met³¹, Tyr⁴², Gly⁴⁵, Val⁴⁹, Gly³⁴⁰, Leu³⁴³, Ala³⁴⁴, Gly³⁴⁷, Thr³⁵⁰, Asp³⁵⁴, and Val³⁵⁷ map on the opposite side of the helices. Such a model is consistent with a hypothesis that the IVM-sensitive hits indicate residues that face lipids in the open conformation state and could provide the binding pocket for IVM and that IVM-insensitive hits indicate residues that could play a role in permeation or other receptor functions /86/. The same residues in TM-1 were also identified as IVM-sensitive hits in alanine scanning mutagenesis /91/. Altogether, this data strengthens the view that IVM selectively recognizes a site in close association to the TM domain of the P2X4R.

The P2X4R sensitivity to IVM was used as an assay for further structural-functional studies. For example, it is generally accepted that mutation of residues contributing to the ligand binding results in rightward shifts of the ATP concentration-response curve. However, this hypothesis could not always be tested experimentally because many of these mutants were non-functional with up to 10 mM ATP. A leftward shift in the wild type P2X4R ATP concentration-response curve in the presence of IVM provided a valuable pharmacological assay to evaluate the response of receptor mutants /196/. Functional relevance of aromatic residues in TM-1 domain of P2X4R was also studied using the combined ATP and IVM treatments /91/. Finally, the specificity of allosteric actions of IVM among P2XRs was used to identify native P2X4Rs in different cell types /56, 126, 161, 179, 195/ and their functional interactions with other members of this family of channels /5,18/.

INDEPENDENT ACTIONS OF TRACE METALS AND IVERMECTIN

A good example of different allosteric sites that modulate the P2X4R is illustrated by the interaction of trace metals and IVM, as shown in Figure 2. As mentioned previously, zinc potentiates the activity of this receptor by binding to an extracellular allosteric site; Cys¹³² plays a critical role in the action of zinc but not copper /29/. In contrast, IVM potentiates receptor activity but its allosteric site is located in TM1 and TM2 domains of the receptor /86, 87, 160/. We reasoned therefore that if the sites for these modulators are distant and independent, the joint application of Zn²⁺ plus IVM should result in additive effects on the ATP-evoked P2X4R currents. In the wild-type P2X4R, expressed in *Xenopus* oocytes, both Zn²⁺ and IVM potentiated the ATP-evoked currents; the joint application of both Zn²⁺ plus IVM elicited a dramatic increase of the ATP-gated currents (Fig. 2), a likely indication that these modulators act at a separate site causing additive effects. In contrast, when oocytes expressing the zinc-resistant C132A mutant were examined, the joint application of these modulators did not elicit a larger response than that elicited by IVM. Consonant with these results, when the same protocol was performed in oocytes expressing the P2X2/4 chimera, a construct with the TM domain corresponding to the P2X2R, only the zinc but not the IVM-induced potentiation was attained (Fig 2), emphasizing distinct sites of modulator action. These results highlight that the metal and IVM interact at separate sites, and that likely the interaction of each of these positive modulators is independent from each other.

P2XR MODULATION BY PROTONS

Extracellular and intracellular pH is determined by proton (H⁺) concentration and it is an important regulatory mechanism of voltage and ligand-gated ionic channels /167, 172/. Likewise, P2XRs are subject to H⁺ modulation. With the exception of P2X2R, which is potentiated, all the other homomeric P2XRs are inhibited by acidic pH /26, 28, 70, 192/. A recurrent target for H⁺ binding are non-protonated carboxylic acid residues or extracellular histidines; the basic residues identified as putative pH sensors are His³¹⁹ for P2X2R, His²⁰⁶ for P2X3R and His²⁸⁶ for P2X4R (see Fig. 1). For the P2X7R, the modulation by pH seems to be more complex; mutation of His¹³⁰ confers a partial resistance to H⁺ inhibition /1/. In a

recent study it was demonstrated that Asp¹⁹⁷ is also partially involved in pH modulation / 110/.

In view that hypoxia, as in ischemia, causes tissue acidification, a local change in pH might be considered as a biological signal, which may ultimately results in altered gating of P2XRs. Taking into account that P2X2/3R heteromers are involved in the primary afferents of the pain pathway and, in addition that the P2X7Rs, have a role in inflammation, subtle changes in localized pH, where these receptors are localized, can have a profound effect in receptor function.

NEUROSTEROIDS AND FAST NON-GENOMIC P2XRs MODULATION

It is common knowledge that gonadal and adrenal steroids activate intracellular receptors, which migrate to the nucleus to modify *de novo* protein synthesis. However, the observation that the systemic administration of progesterone, but not β -estradiol, induced within minutes deep sleep, mimicking general anesthesia /156/, challenged the concept of genomic effects as the exclusive mechanism of steroid hormone action. Two decades later, the synthetic steroid alfaxolone, was introduced to the clinic as an endovenous anesthetic. The finding that betaxolone was inactive /95/, pointed to a stereo-selective mechanism, ruling out non-specific steroid lipophilic membrane interaction. Altogether, these observations set the stage to propose that steroids modify neuronal activity through rapid, non-genomic effects, independent of nuclear receptor activation and gene expression.

Consonant with this proposal, it was soon reported that neurosteroids change neuronal firing, acting on ionotropic receptors, via a fast non-genomic allosteric action /35, 173/. Ligand-gated ion channels are one of the possible targets for the rapid action of neurosteroids; in fact, the GABA_A receptor channel is apparently a main target of the anesthetic action of alfaxolone /78/. The allosteric modulation of the GABA_{A/C} receptors by neurosteroids is the most thoroughly studied model system. Compounds such as 3 α , 21-dihydroxy- 5 α - pregnan-20-one (5- α -THDOC, THDOC), a metabolite of deoxycorticosterone, and allopregnanolone, a brain progesterone metabolite, enhance within less than 1 minute and with nanomolar concentrations the GABA_A-induced currents acting as a positive receptor modulator /77, 141, 189/. At higher concentrations, allopregnanolone and related neurosteroids behave as partial GABA_A receptor agonists opening *per se* the receptor channel /78, 80, 81, 105/. The nicotinic α 4 β 2, NMDA, or glycine receptors are also allosterically modulated in a positive manner by neurosteroids, including 17 β -estradiol / 4, 92, 137, 155/, implying that steroids modulate not only the pentameric Cys-loop family of receptors but also the tetrameric glutamate-gated receptors.

Although steroids are well-recognized allosteric modulators of ligand-gated receptor channels, few studies have addressed the basis of this interaction. A recent report of Codocedo et al. /34/ elegantly showed in two independent cell systems the modulator role of neurosteroids on the P2X4R; the modulation required a preincubation of only 30 to 60-sec, because no effect was evidenced when alfaxolone or pregnanolone were co-incubated with ATP in either cell type. Allopregnanolone or THDOC (3 α , 21-dihydroxy-5 α -pregnan-20-one) also potentiated the ATP-gated currents in cells expressing P2X4Rs, but with a maximal effect much less than observed for alfaxolone. In contrast, pregnanolone, but not its sulfated derivative, inhibited the ATP-gated currents, reaching a maximal effect of 40%. In a view of the short preincubation required for the modulation to occur, we deduced that these compounds must equilibrate with a steroid-selective site, a mechanism independent of gene expression. Consonant with this interpretation, the modulation was rapidly reversible by steroid washout. In our view, the putative site(s) of steroid action is/are allosteric in nature. We cannot discard, however, that neuroactive steroids might interact with a cell membrane

steroid receptor, which directly or indirectly, modifies the ATP-gated currents. We are aware that steroids may also activate intracellular membrane receptors coupled to G proteins /122, 154/, activating cascade mechanisms that indirectly modulate growth factor receptors, as extensively discussed for an intracellular 17- β -estradiol receptor /146/. One of the reasons for choosing two different cell types was to avoid the possibility that the steroids could signal through G protein-coupled receptors that may indirectly affect P2X4R functioning.

Codocedo et al. /34/ also observed that steroid concentrations about 100-fold larger than required to modulate the receptor acted as partial P2X4R agonists, gating *per se* the channel activity. This observation was supported by the finding that suramin, a non-selective P2XR antagonist, blocked the alfaxolone and related neurosteroid-gated currents. This result highlights that, in addition to the GABA_A receptor, neurosteroids are also able to activate P2XRs by as yet undetermined molecular mechanisms.

De Roo et al. /44, 45/ concentrated on the role of progesterone as a positive modulator of P2X2R activity; they have shown that dehydroepiandrosterone (DHEA), an endogenous steroid, potentiated in a concentration-dependent manner ATP-induced membrane currents mediated by P2X2R but inhibited those mediated by P2X3R /45/. In dorsal root ganglion neurons, progesterone rapidly and reversibly potentiated submaximal ATP-gated currents but had no effect on the currents activated by α,β -methylene ATP, an agonist of homomeric or heteromeric receptors containing P2X1 or P2X3 subunits /45/. In addition, in oocytes or HEK293 cells expressing homomeric P2X2Rs, responses to submaximal ATP, were systematically potentiated by progesterone in a concentration-dependent manner with a threshold between 1 and 10 nM. However, progesterone had no effect on the ATP currents carried by homomeric P2X1R, P2X3R, or P2X4R or by heteromeric P2X1/5Rs and P2X2/3Rs, implying that progesterone selectively potentiates homomeric P2X2Rs and, in contrast with dehydroepiandrosterone (DHEA), discriminates between homomeric and heteromeric P2X2Rs /44/. This might have important physiological implications since the P2X2 subunit is the most widely distributed P2X subunit in organisms.

Although at present the P2XR steroid binding site(s) has(have) not been delineated at the molecular level, it is obvious that steroids/neurosteroid interact reversibly as positive or negative allosteric modulators rather than affecting gene expression. Systematic studies with a wide collection of steroids, from the sex to the neurosteroids need to be assayed in a same cell system prior to arriving to fine conclusions regarding the steroid binding site(s).

ROS AND CARBON MONOXIDE AS MODULATORS OF P2XRs

ROS, including oxygen ions (such as superoxide), free radicals and peroxides, are chemically unstable, highly reactive molecules well known to induce cell damage by oxidative stress. Although historically associated to cell damage and/or to the initiation of pathological processes due to their ability to chemically modify essential protein residues, there is growing evidence that ROS (and also nitrogen reactive species, NOS) are signaling molecules that may modify gene expression /51/. In this context, it is known that ROS, NOS and free radicals modify the activity of ionic channels in the central nervous system and other tissues; for example, thiol-oxidizing agents inhibit Ca_v channels in NB41A3 neuroblastoma cells, implicating cysteine residues in this reaction /59/.

Hydrogen peroxide is indirectly produced by cells as a result of the activation of several cell membrane receptors and other biochemical reactions; it is a prominent endogenous ROS that can significantly increase after several pathophysiological conditions. Stimulation of AT1 receptor by angiotensin II promotes NADH/NADHP oxidase activity, resulting in an increase of superoxide, which is avidly transformed by catalase into peroxides and may,

among other pathways, modify gene expression through MAPK activity /194/. Within the past years, it has become evident that brain and peripheral ionic channels are targets of ROS activity. For example, hydrogen peroxide increased Ca_v currents, an effect mimicked by thiol oxidizing agents /23/; in contrast, the high conductance BK type K_{Ca} channel was inhibited by H_2O_2 /48/. The K_v channels are also modulated by ROS and in the KCNH2 channel, two extracellular histidines have been identified as the ROS sensor /136/. Likewise, the ryanodine receptor channel is modulated by NOS; cysteine residues of its redox sensor site have been identified /6/. In a recent report we described modulation of the P2X2R by H_2O_2 , mercury and the mitochondrial stress inducers myxothiazol and rotenone and identified the intracellular Cys^{430} as a redox sensor of this receptor /30/. The use of receptor chimeras, composed of P2X4 ectodomain and P2X2 intracellular and TM domains, was instrumental to uncover the intracellular nature of this redox modulation, because mercury potentiates P2X2R but inhibits P2X4R activity. Using an H_2O_2 -resistant splice variant of P2X2R, termed P2X2bR, we determined that the redox sensor site was located on a 69-residue sequence absent in P2X2bR variant. This intracellular stretch, not contained in the P2X2bR, includes a single cysteine localized in position 430. Site directed mutagenesis, changing Cys^{430} for either Ala or Ser, confirmed that this residue is an intracellular redox sensor of the P2X2R. Interestingly, the ATP-evoked currents in the P2X4R were slightly inhibited by H_2O_2 , suggesting an opposite redox modulation, which remains to be identified /30/. These novel findings indicate that P2XRs can modify their activity depending on the redox state of the cell. Future experiments will be crucial for determine if other P2XRs are also modulated by ROS and whether other NOS and reactive species could also act as modulators.

Carbon monoxide (CO), the metabolic product of heme oxygenase, one of the degradation products of the heme ring in aging red blood cells, is the second gas transmitter discovered in the CNS. Together with NO, these endogenous gas signals challenged the view that transmitters need to be stored and released from nerve ending vesicles. The biochemistry and cell biology of CO has attracted attention in cell signaling following the seminal discovery NO, and its role in the CNS. CO had been considered traditionally a toxic gas because of its poisonous properties; this gas has caused innumerable deaths by asphyxia in view of its approximate 250 greater affinity for hemoglobin than oxygen. However, this molecule is also an important metabolite of anaerobic bacteria and in mammals it acts as a signaling molecule binding to heme-containing proteins /187/. As a part of the host defense system, CO prevents diverse procedures that induce cell stress, such as heavy metals, ROS, heat shock and inflammatory processes /187/. The proteins that can be modulated by CO include soluble guanylyl cyclase, nitric oxide synthase, haemoglobin, and cytochromes affecting several physiological variables like cardiac function, vascular contractibility, platelet aggregation and in the central nervous system circadian rhythm, odor sensation and hearing, between others /187/. CO modulates the activity of several ionic channels and potassium channels are one of its main targets. There is evidence that CO affects BK type K_{Ca} channels and ATP-sensitive K_{ATP} channels /62, 186/. Other channels like Ca_v , Na_v and cyclic nucleotide-gated channels are also sensitive to CO modulation /17, 106, 108/.

The hypothesis that CO modulates P2XRs was recently tested by Kemp's group /183/. They found that CO potentiates ATP-evoked currents only in cells that express the P2X2R; CO had no effect in cells expressing either the P2X3R or the heteromeric P2X2/3R, showing subunit specificity. Moreover, these authors found a small, but significant inhibition of ATP-evoked currents in P2X4R expressing cells. These results suggest that the P2X2R and P2X4R have an allosteric site for CO modulation or that this gas exerts its actions through indirect mechanisms. In this regard, the effects of CO in P2X2R and P2X4R are similar to those reported by Coddou et al. /30/ for ROS and mitochondrial stress inducers; it is therefore plausible that CO can bind to mitochondrial complexes and stimulate ROS

production, a mechanism that has been demonstrated to modulate L-type Ca_v channels /140/. As to whether NO can replace CO as a P2XR modulator, remains to be explored; NO could have ample biological significance in view of its abundance and critical role in multiple CNS neuronal networks.

MODULATION OF P2XRs BY PHOSPHOINOSITIDES

Phosphoinositides are key components of biological membranes, particularly in the inner leaflet of the plasma membrane. These phospholipids coexist with a variety of membrane proteins and signaling enzymes such as phospholipase C, receptors and ion channels. The relative abundance of the different phosphoinositides (PIP, PIP_2 , PIP_n s) change depending on the activation of phospholipases, PI kinases, and phosphatases /144/. PIPs can modulate the activity of a variety of ionic channels, including several potassium channels /39, 83, 164, 174/, Ca_v channels /185/, transient receptor potential channels /75/, and Na_v channels /142/. P2XRs are novel targets for phosphoinositide modulation and differential effects of these membrane lipids at different receptor subtypes makes this receptor family an attractive model to study the role of PIPs modulation.

The first report of PIPs modulation on P2XR activity showed an effect of these membrane lipids on P2X2R desensitization /67/. PI3K inhibitors accelerated P2X2R desensitization; two positively charged residues located in the proximal region of the cytoplasmic C-terminal domain, Lys^{365} and Lys^{369} , were identified as critical for the interaction of membrane PIPs with the P2X2R /67/. Immediately thereafter, Seguela's group showed that PIP_2 directly interacts with Lys^{364} , located in the C-terminal domain of the P2X1R; inhibition of this interaction (by either mutagenesis of Lys^{364} or PI3K/PI4K blockade, to reduce the abundance of membrane PIPs resulted in a decrease of current amplitude and recovery from desensitization /11/. The same group demonstrated similar effects of PIP_2 and PIP_3 on P2X4R activity; using an *in-vitro* binding assay they found that PIs interact with the Cys^{360} - Val^{375} sequence located in the proximal C-terminal domain of the P2X4R /10/. Similarly, P2X3R and P2X7R are inhibited by THE PIP_2 decrease induced either by PI4K inhibition or phospholipase C activation by co-expressed G protein coupled receptors /197, 198/.

Altogether, these studies indicate that the lipid and protein composition of the plasma membrane has a marked influence on P2XR activity. This might be a physiological relevant issue because ATP is one of the sympathetic co-transmitters, which operates in conjunction with noradrenaline and neuropeptide Y in the sympathetic nerve reflexes /50/. Therefore, depletion of PIs in the cell membrane following a sustained sympathetic discharge might reduce the sympathetic response due to a lesser IP_3 production following noradrenaline receptor activation, a condition that will also affect P2XR signaling as already discussed. For example, desensitization of P2X1R activity might have a component related to a decreased signaling by this receptor subtype. Therefore, the role of P2X1R desensitization and particularly following repeated α , β methylene applications, vs PIs depletion remains an issue to be further investigated.

MODULATION OF P2XRS BY ENDOGENOUS PROTEINS

Phosphorylation of serine, threonine or tyrosine residues by protein kinases is one of the most important regulatory mechanisms of enzyme and/or receptor activity. Reversible phosphorylation/dephosphorylation of proteins occur upon the action of several kinases or phosphatases; ample evidence supports not only the regulation of the activity by receptors by phosphorylation, but also its expression and trafficking. For example, ionic channel regulation by phosphorylation/dephosphorylation was shown for the K_v and BK-type K_{ca} channels and for the ionotropic receptors GABA_A , NMDA and acetylcholine receptors, among others/43, 165/.

Protein kinases. Two families of protein kinases are most relevant to biological systems, one is activated by intracellular messengers cAMP (protein kinase A-PKA) and the other by diacylglycerol/calcium (protein kinase C-PKC). The intracellular concentration of these intracellular messengers varies on a minute-to-minute basis based on the activity of many membrane receptors and ligand availability. Several studies indicate that P2XRs, as other ionotropic receptors, are modulated by phosphorylation, although some of the observed effects remain controversial. The first report on P2XR phosphorylation was done in the P2X2R and described the modulation of the receptor amplitude by PKA through the phosphorylation of the C-terminal residue Ser⁴³¹ /24/. More recently, similar studies have been reported for the P2X4R, whose activity is potentiated by cAMP through PKA activation. This mechanism involves the phosphorylation by PKA of an accessory protein that interacts with the YXXGL endocytosis motif located on the receptor's C-terminal and related to receptor internalization, that results in an augment of P2X4Rs on the plasma membrane /15/.

P2XRs share a putative PKC-binding motif (TXR/K) in the receptor N-terminal end that has been proposed as a modulator site by phosphorylation. This was first proposed for the P2X2R, because the mutation of the N-terminal Thr¹⁸ dramatically affected receptor desensitization and the addition of the PKC-activator PMA recovered the desensitization profile in chimeric receptors /12/. However, using biochemical approaches another study could not find any evidence of a direct P2X2R phosphorylation at Thr¹⁸, suggesting instead that this residue could exert an important structural role but not through phosphorylation /64/. The ATP-induced currents of P2X1R and P2X3R are potentiated by PMA, suggesting again the phosphorylation of the TXR/K motif by PKC /139, 177/. In this case, mutation of the respective threonine residues resulted in receptors with either significantly smaller amplitudes or absence of P2X1R and P2X3R-gated currents, respectively /64, 109/. Finally, biochemical experiments discarded that these threonine residues are directly phosphorylated /64, 177/.

Additional studies addressed the importance of the Thr¹⁵ of the P2X7R in the pore dilation of the channel after prolonged applications of ATP or BzATP /190/. Mutations of Thr¹⁵ as in T15E, T15K or T15W mutants, showed instantaneous permeation to large organic cations in contrast to wild-type and T15A, T15S or Thr15 mutants that showed a biphasic current that reflects the pore dilation /190/. In cells expressing P2X5R, mutation of the corresponding Thr¹⁸ resulted in non-functional channels, although they were expressed in the plasma membrane /88/. Altogether, these results indicate that the TXR/K sequence located in the N-terminal of P2XRs has important structural roles and it is necessary for channel functioning; however, it remains unclear at present whether this residue is a PKC phosphorylation site.

Recent studies proposed that the P2X3R is regulated by cyclin-dependent kinase-5 (Cdk5) and C-terminal Src kinase (Csk), and that the phosphorylation of receptor residues by these kinases results in inhibition of the ATP or α , β -meATP induced currents /40, 130/. In the case of Csk-induced inhibition, intracellular Tyr³⁹³ was identified as the target of this kinase /40/. For Cdk5-modulation an increase of P2X3R serine phosphorylation was found, although is not clear if this effect is due a direct or indirect interaction with Cdk-5 /130/.

Calmodulin (CaM) is a calcium-dependent protein that activates downstream signaling events through different target proteins. It has been proposed that the P2X7R directly binds CaM, resulting in current facilitation and membrane blebbing in a calcium dependent manner; the proposed CaM-binding site corresponds to the Ile⁵⁴¹-Ser⁵⁵² sequence located in the C-terminal tail of P2X7 /152/.

Visinin-like protein 1 (VILIP1). A recent report described a novel interaction of the P2X2R with the neuronal calcium sensor VILIP1 /22/. The authors found that VILIP1 constitutively binds to P2X2Rs altering channel properties, like ATP-sensitivity and peak currents, opening the field to study if such interactions occur in other P2X subtypes.

LIPOPOLYSACCHARIDES

Lipopolysaccharides (LPS), also known as lipoglycans, are large molecules consisting of a lipid and a polysaccharide joined by a covalent bond. LPS are found in the outer membrane of Gram-negative bacteria, act as endotoxins and elicit strong immune responses in animals. A fascinating link between the P2X7R and inflammation emerged within the past few years suggesting that LPS modulate the receptor channel activity and therefore may participate as a relevant mediator in the inflammatory response. While it is known that the P2X7R is linked to the production of interleukin 1- β , a cutting edge publication showed that a sequence of the carboxy-end of the P2X7R contains a conserved LPS-binding domain (Figure 1). Denlinger et al. /46/ demonstrated that peptides derived from this P2X7R sequence bind LPS *in vitro*. Moreover, these peptides neutralize the ability of LPS to activate the extracellular signal-regulated kinases (ERK1, ERK2) and to promote the degradation of the inhibitor of kappaB-alpha isoform (L-kappaB-alpha) in tissue cultured macrophages. Collectively, these data suggest that the carboxy-terminal domain of P2X7R may directly coordinate several signal transduction events related to macrophage function and LPS action. In view that the LPS-binding domain is related to carboxy-end of the protein, and considering that the P2X7R is characterized by an extended C-terminal, it is unlikely that other P2XRs have a similar site. In addition, functional studies link the P2X7R, but not other P2XRs to the inflammatory response.

As to whether the interaction of LPS with the P2X7R is allosteric in nature remains to be detailed. However, it has not escaped our attention that endogenous lipids and particularly glycolipids may act as putative endogenous modulators at this site on the P2X7R. For example, lysophosphatidylcholine potentiates Ca^{2+} influx, apoptosis, and ERK activation in mouse microglial cells, effects which may be linked to P2X7R stimulation. Likewise, PIP_2 evoked a positive modulation of ATP-evoked currents /198/; BSA, 11-(5-dimethylaminonaphthalene-1-sulphonyl)amino)undecanoic acid or arachidonic acid markedly increased the potency of BzATP, to stimulate ethidium accumulation /119/ further supports this contention. It should be recalled that BzATP is a rather selective P2X7R agonist. Polymyxin B, and specifically its hydrophobic tail, positively modulate P2X7R-mediated ethidium uptake /61/. Recently, in support the role of lipids in P2X7R activity, Michel and Fonfria /118/. showed that a diverse range of lipids increased agonist potency at the P2X7R in functional and binding studies.

ALCOHOLS AS MODULATORS OF P2XRS

The sedative hypnotic properties of ethanol and longer chain alcohols have attracted humans since immemorial times. It was accepted for a long time that alcohols exert their psychotropic actions in a non-specific manner through interactions with membrane lipids, following a cut-off rule that relates alcohol potency to their hydrophobic nature. Independent of the lipid solubility of these drugs, it is now widely accepted that alcohols can directly modify protein function, in most cases, due to the interaction with TM domains or hydrophobic pockets within the protein structure. The most important targets of ethanol related to its intoxicating actions are neuronal voltage-gated and ligand-gated ionic channels. In the case of the latter, there are reports of alcohol effects on GABA_A , GABA_C , glycine, nicotinic, 5-HT₃, NMDA and AMPA/kainite receptors /37, 76, 123, 162/.

P2XRs are also modulated by alcohols; P2X2R and P2X4R are inhibited /42/ and P2X3R is potentiated by ethanol /41/. Several residues have been identified to be important in ethanol modulation of P2XRs. In the P2X4R, the extracellular His²⁴¹ has been proposed to be related with the mechanism of ethanol inhibition and recently TM-2 domain has been identified as key for ethanol inhibition /143, 188/. For the P2X2R, it has been demonstrated that the interface regions between TM domain and ectodomain are important for ethanol inhibition /7/.

PROPOFOL, KETAMINE AND TOLUENE

Propofol is an endovenous anesthetic that is widely used in clinic and has been reported to modulate the activity of neuronal GABA_A receptors /138/. It also potentiates P2X4R activity expressed in HEK293 cells and has no effect at P2X2R and P2X2/3R /168/. Propofol also potentiates the P2X7R-mediated currents in microglia, an effect that was mimicked by two other clinically relevant endovenous anesthetics, thiopental and ketamine /131/. In other study, neither ketamine nor propofol affected the P2X2Rs /68/.

Due to its intoxicating effects, toluene is an abused organic solvent; several studies report that it modulates several voltage and ligand-gated ion channels /65/. Among P2XRs, toluene potentiates the ATP-evoked currents in cells expressing homomeric P2X2R and P2X4R as well in cells expressing heteromeric P2X4/6R and P2X2/3R. In contrast in cells expressing homomeric the P2X3R, toluene inhibits ATP-evoked currents /184/.

CONCLUDING REMARKS

Physiological implications of allostereism. Allostereism adds a fundamental regulatory mechanism to ligand operated receptor channels, a mechanism common with enzymes, nuclear receptors and likely transporters. We now know that the activity of these proteins not only depends on ligand concentration or substrate availability at the receptor interface or milieu, but also on the accessibility of modulators both in the extracellular or intracellular setting. At the light of this concept, the synaptic role of these modulators has finally acquired full physiological significance; allostereism has widespread physiological implications for biosignaling. The P2XR family is no exception to this type of regulatory mechanisms, much like the pentameric Cys-loop receptors or the glutamate tetrameric receptors, highlighting the variety of regulatory mechanisms and the intricacies of these processes independent of the evolutionary origin of these proteins. Whilst some of the endogenous allosteric modulators act in the extracellular P2XR domains, others (such as ROS or kinase-dependent phosphorylations) occur at intracellular sites. Moreover, we also have discussed the action of IVM, a potent and efficacious P2XR modulator whose precise site and putative endogenous ligand remains to be established. In addition, exogenous compounds (like the alcohols and several anesthetic agents) that are not as potent modulators, are in the list of compounds that lack at present an endogenous ligand. The search for their corresponding endogenous ligands, if any, is of a great and urgent physiological relevance.

The finding that other endogenous compounds, like protons, ROS or neurosteroids, modulate P2XR activity also open promising opportunities to study the allosteric modulation in a more physiological context. For example, changes in cellular pH (as they have been observed during ischemia) could affect P23XR activity. In the case of oxidative stress or following abundant ROS production, either by pathophysiological conditions or drugs, we recently identified an intracellular redox sensor for the P2X2R that modulates its channel activity /30/. In our view, this is a novel and challenging concept adding that the physiological activity of this particular P2XR depends also on the metabolic state of the cell, a regulatory modality that adjusts cell communication depending on its minute-to-minute

metabolism. Likewise, the recent discovery that P2XRs are modulated by neurosteroids /34, 45/, adds new possibilities for altering P2XR signaling, and brain excitability, during specific periods of life such as the menstrual cycle days or during long-lasting stressful situations, or the burst of adolescence, pregnancy or menopause, among other pathophysiological conditions when the concentration of brain steroids or the pregnane-derived steroids change dramatically. Similarly, the influence of phosphoinositides on P2XR functioning, through interactions with positively charged amino acids located in the interface between the C-termini and the TM domain of the P2XRs; endogenous proteins like calmodulin, protein kinases and calcium-sensing protein also modulate the activity of P2XRs.

Pharmacological and plausible therapeutic applications and implications. In view of the interest in the P2X7R as a target for novel analgesics and anti-inflammatory agents, a substantial number of novel compounds with a potential clinical profile have been developed by major pharmaceutical companies. An abridged compendium showing some of these promising P2XRs agonists or competitive antagonists was collected by Gunosewoyo and Kassiou /71/. The main problem with most of these drugs is their poor selectivity among P2XRs subtypes. In this context, where allosteric modulators constitute a valuable alternative for future drug design, the identification and characterization of P2XR allosteric sites is a relevant issue. For example, the design of a drug that mimics the action of IVM could result in a specific P2X4R ligand; the same may apply to other P2XR modulators that might interact at allosteric receptor modulator sites.

Although at present the pharmacological abundance of the P2X armament is far from that developed for the GABA_A or nicotinic receptors, the emerging development of novel P2X7R antagonists seem to align along this trend. In two recent reports, Michel et al. /116, 117/ reported on novel selective P2X7R antagonists displaying a complex noncompetitive pharmacodynamic profile at this receptor. Upon further studies, some of these compounds better qualified as allosteric modulators rather than interacting directly at the ligand binding site. For example, GW791343 does not appear to interact at the ATP binding of the human P2X7R, but rather acts as a negative allosteric modulator. Interestingly, the predominant effect of GW791343 at the rat P2X7R was as a positive allosteric modulator; indicating in addition subtle differences in receptor species. Therefore novel mechanisms are at hand with species differences revealing that carefully controlled studies will be necessary before moving to human studies. These compounds and the insight of analyzing drugs as potential allosteric modulators is a powerful strategy to develop new tools for the purinergic field. Incidentally, in view of the potential of P2X7R antagonists as anti-inflammatory drugs, the opportunities of new mechanisms for the ease of chronic pain should be seriously evaluated /49/.

Evolutionary considerations. It is possible that all P2XR subtypes originated from an ancestral gene, which in turn likely evolved from another protein related to either a primitive ATP binding protein or a subunit of a primordial ionic channel. One of the main aspects of evolution of these proteins is conservation of trace metals coordination sites, as well illustrated by the presence of allosteric sites in today's P2XRs from microorganisms (*Schistosoma mansoni* – IVM and *Dictyostelium discoideum* – copper) /3, 63/ to mammals /32, 87, 160, 166/. It is challenging that although the amino acids involved in P2XR trace metal coordination do not appear to be aligned among the several P2XRs subtypes most subunits evolved a trace metal allosteric site. Even more challenging is the notion that while the ATP-gated currents elicited by P2X2R homomers is positively modulated by either zinc or copper that evoked by P2X7R homomers is inhibited by these metals. The most paradigmatic case within P2XRs is the P2X4R homomer, which has a distinct and separate site for the zinc as a positive modulator and for copper as a negative modulator. The

characterization of two sites, based on the identification of the amino acids involved in the differential coordination of these two trace metals was dissected by Coddou et al. /29, 32/. The tridimensional structure of the P2XRs based on the crystallized P2X4R from the zebra fish shows that the trace metal coordination site(s) for the P2X4R or the P2X2R are spatially close to the ATP binding site /96/. allowing us to propose that metals modify essentially the affinity of the P2XR for ATP as was determined experimentally /2, 29, 32/.

Similar considerations are valid and may be predicted for other allosteric regulators of P2XRs. It is interesting that most ligand-gated ionic channels are sensitive to modulation by IVM, but the mechanism(s) and even the precise site(s) remain as yet unclear for almost all ligand-gated channels. By far, the most exciting property of IVM as a P2XR allosteric modulator refers to its P2X4R specificity, posing a picture much different from that observed with trace metals. As to why the evolution of the IVM modulation differed so markedly from trace metals remain to be further studied and established. Steroids, as trace metals, are either positive or negative allosteric P2XR modulators. The identification of modulator steroid site(s) revealed that some sites are specific for the gonadal steroids, other sites show specificity for the neurosteroids, allowing us to propose that strict structural requirements may be interpreted in terms of receptors multiplicity, a hypothesis that should be examined. As to whether the IVM and the steroid site(s) share commonalities is an issue that awaits further experimentation but in view of the differences in P2XR subtype selectivity, it appears more teleological that these molecules evolved separate modulator sites.

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References

1. Acuna-Castillo C, Coddou C, Bull P, Brito J, Huidobro-Toro JP. Differential role of extracellular histidines in copper, zinc, magnesium and proton modulation of the P2X7 purinergic receptor. *J Neurochem.* 2007; 101:17–26. [PubMed: 17394459]
2. Acuna-Castillo C, Morales B, Huidobro-Toro JP. Zinc and copper modulate differentially the P2X4 receptor. *J Neurochem.* 2000; 74:1529–1537. [PubMed: 10737610]
3. Agboh KC, Webb TE, Evans RJ, Ennion SJ. Functional characterization of a P2X receptor from *Schistosoma mansoni*. *J Biol Chem.* 2004; 279:41650–41657. [PubMed: 15292267]
4. Ahrens J, Leuwer M, Demir R, Krampfl K, Foadi N, Haeseler G. The anaesthetic steroid alphaxalone positively modulates alpha-glycine receptor function. *Pharmacology.* 2008; 82:228–232. [PubMed: 18810247]
5. Alqallaf SM, Evans BA, Kidd EJ. Atypical P2X receptor pharmacology in two human osteoblast-like cell lines. *Br J Pharmacol.* 2009; 156:1124–1135. [PubMed: 19226284]
6. Aracena-Parks P, Goonasekera SA, Gilman CP, Dirksen RT, Hidalgo C, Hamilton SL. Identification of cysteines involved in S-nitrosylation, S-glutathionylation, and oxidation to disulfides in ryanodine receptor type 1. *J Biol Chem.* 2006; 281:40354–40368. [PubMed: 17071618]
7. Asatryan L, Popova M, Woodward JJ, King BF, Alkana RL, Davies DL. Roles of ectodomain and transmembrane regions in ethanol and agonist action in purinergic P2X2 and P2X3 receptors. *Neuropharmacology.* 2008; 55:835–843. [PubMed: 18639563]
8. Assaf SY, Chung SH. Release of endogenous Zn²⁺ from brain tissue during activity. *Nature.* 1984; 308:734–736. [PubMed: 6717566]
9. Becker D, Woltersdorf R, Boldt W, Schmitz S, Braam U, Schmalzing G, Markwardt F. The P2X7 carboxyl tail is a regulatory module of P2X7 receptor channel activity. *J Biol Chem.* 2008; 283:25725–25734. [PubMed: 18617511]

10. Bernier LP, Ase AR, Chevallier S, Blais D, Zhao Q, Boue-Grabot E, Logothetis D, Seguela P. Phosphoinositides regulate P2X₄ ATP-gated channels through direct interactions. *J Neurosci*. 2008; 28:12938–12945. [PubMed: 19036987]
11. Bernier LP, Ase AR, Tong X, Hamel E, Blais D, Zhao Q, Logothetis DE, Seguela P. Direct modulation of P2X₁ receptor-channels by the lipid phosphatidylinositol 4,5-bisphosphate. *Mol Pharmacol*. 2008; 74:785–792. [PubMed: 18523136]
12. Boue-Grabot E, Archambault V, Seguela P. A protein kinase C site highly conserved in P2X subunits controls the desensitization kinetics of P2X₂(2) ATP-gated channels. *J Biol Chem*. 2000; 275:10190–10195. [PubMed: 10744703]
13. Brake AJ, Wagenbach MJ, Julius D. New structural motif for ligand-gated ion channels defined by an ionotropic ATP receptor. *Nature*. 1994; 371:519–523. [PubMed: 7523952]
14. Brandle U, Spielmanns P, Osteroth R, Sim J, Surprenant A, Buell G, Ruppersberg JP, Plinkert PK, Zenner HP, Glowatzki E. Desensitization of the P2X₂(2) receptor controlled by alternative splicing. *FEBS Lett*. 1997; 404:294–298. [PubMed: 9119082]
15. Brown DA, Yule DI. Protein kinase A regulation of P2X₄ receptors: Requirement for a specific motif in the C-terminus. *Biochim Biophys Acta*. 2010; 1803:275–287. [PubMed: 20026202]
16. Burnstock G. Purinergic nerves. *Pharmacol Rev*. 1972; 24:509–581. [PubMed: 4404211]
17. Carratu MR, Renna G, Giustino A, De Salvia MA, Cuomo V. Changes in peripheral nervous system activity produced in rats by prenatal exposure to carbon monoxide. *Arch Toxicol*. 1993; 67:297–301. [PubMed: 8368938]
18. Casas-Pruneda G, Reyes JP, Perez-Flores G, Perez-Cornejo P, Arreola J. Functional interactions between P2X₄ and P2X₇ receptors from mouse salivary epithelia. *J Physiol*. 2009; 587:2887–2901. [PubMed: 19403602]
19. Changeux J, Leuzinger W, Huchet M. Specific binding of acetylcholine to acetylcholinesterase in the presence of eserine. *FEBS Lett*. 1968; 2:77–80. [PubMed: 11946273]
20. Changeux JP, Podleski T, Meunier JC. On Some Structural Analogies between Acetylcholinesterase and the Macromolecular Receptor of Acetylcholine. *J Gen Physiol*. 1969; 54:225–244. [PubMed: 19873643]
21. Changeux JP, Ryter A, Leuzinger W, Barrand P, Podleski T. On the association of tyrocidine with acetylcholinesterase. *Proc Natl Acad Sci USA*. 1969; 62:986–993. [PubMed: 5257018]
22. Chaumont S, Compan V, Toulme E, Richler E, Housley GD, Rassendren F, Khakh BS. Regulation of P2X₂ receptors by the neuronal calcium sensor VILIP1. *Sci Signal*. 2008; 1(41):ra8. [PubMed: 18922787]
23. Chiamvimonvat N, O'Rourke B, Kamp TJ, Kallen RG, Hofmann F, Flockerzi V, Marban E. Functional consequences of sulfhydryl modification in the pore-forming subunits of cardiovascular Ca²⁺ and Na⁺ channels. *Circ Res*. 1995; 76:325–334. [PubMed: 7859379]
24. Chow YW, Wang HL. Functional modulation of P2X₂ receptors by cyclic AMP-dependent protein kinase. *J Neurochem*. 1998; 70:2606–2612. [PubMed: 9603227]
25. Christopoulos A, May LT, Avlani VA, Sexton PM. G-protein-coupled receptor allostery: the promise and the problem(s). *Biochem Soc Trans*. 2004; 32:873–877. [PubMed: 15494038]
26. Clarke CE, Benham CD, Bridges A, George AR, Meadows HJ. Mutation of histidine 286 of the human P2X₄ purinoceptor removes extracellular pH sensitivity. *J Physiol*. 2000; 523(Pt 3):697–703. [PubMed: 10718748]
27. Clyne JD, LaPointe LD, Hume RI. The role of histidine residues in modulation of the rat P2X₂(2) purinoceptor by zinc and pH. *J Physiol*. 2002; 539:347–359. [PubMed: 11882669]
28. Clyne JD, Wang LF, Hume RI. Mutational analysis of the conserved cysteines of the rat P2X₂ purinoceptor. *J Neurosci*. 2002; 22:3873–3880. [PubMed: 12019306]
29. Coddou C, Acuna-Castillo C, Bull P, Huidobro-Toro JP. Dissecting the facilitator and inhibitor allosteric metal sites of the P2X₄ receptor channel: critical roles of CYS132 for zinc potentiation and ASP138 for copper inhibition. *J Biol Chem*. 2007; 282:36879–36886. [PubMed: 17962187]
30. Coddou C, Codocedo JF, Li S, Lillo JG, Acuna-Castillo C, Bull P, Stojilkovic SS, Huidobro-Toro JP. Reactive oxygen species potentiate the P2X₂ receptor activity through intracellular Cys430. *J Neurosci*. 2009; 29:12284–12291. [PubMed: 19793987]

31. Coddou C, Lorca RA, Acuna-Castillo C, Grauso M, Rassendren F, Huidobro-Toro JP. Heavy metals modulate the activity of the purinergic P2X4 receptor. *Toxicol Appl Pharmacol.* 2005; 202:121–131. [PubMed: 15629187]
32. Coddou C, Morales B, Gonzalez J, Grauso M, Gordillo F, Bull P, Rassendren F, Huidobro-Toro JP. Histidine 140 plays a key role in the inhibitory modulation of the P2X4 nucleotide receptor by copper but not zinc. *J Biol Chem.* 2003; 278:36777–36785. [PubMed: 12819199]
33. Coddou C, Morales B, Huidobro-Toro JP. Neuromodulator role of zinc and copper during prolonged ATP applications to P2X4 purinoceptors. *Eur J Pharmacol.* 2003; 472:49–56. [PubMed: 12860472]
34. Codocedo JF, Rodriguez FE, Huidobro-Toro JP. Neurosteroids differentially modulate P2X ATP-gated channels through non-genomic interactions. *J Neurochem.* 2009; 110:734–744. [PubMed: 19457083]
35. Compagnone NA, Mellon SH. Neurosteroids: biosynthesis and function of these novel neuromodulators. *Frontiers Neuroendocrinol.* 2000; 21:1–56.
36. Costa E. The Di Mascio Lecture. The allosteric modulation of GABAA receptors Seventeen years of research. *Neuropsychopharmacology.* 1991; 4:225–235. [PubMed: 1714281]
37. Crews FT, Morrow AL, Criswell H, Breese G. Effects of ethanol on ion channels. *Int Rev Neurobiol.* 1996; 39:283–367. [PubMed: 8894851]
38. Cully DF, Vassilatis DK, Liu KK, Parese PS, Van der Ploeg LH, Schaeffer JM, Arena JP. Cloning of an avermectin-sensitive glutamate-gated chloride channel from *Caenorhabditis elegans*. *Nature.* 1994; 371:707–711. [PubMed: 7935817]
39. Czirjak G, Petheo GL, Spat A, Enyedi P. Inhibition of TASK-1 potassium channel by phospholipase C. *Am J Physiol Cell Physiol.* 2001; 281:C700–C708. [PubMed: 11443069]
40. D'Arco M, Giniatullin R, Leone V, Carloni P, Birsa N, Nair A, Nistri A, Fabbretti E. The C-terminal Src inhibitory kinase (Csk)-mediated tyrosine phosphorylation is a novel molecular mechanism to limit P2X3 receptor function in mouse sensory neurons. *J Biol Chem.* 2009; 284:21393–21401. [PubMed: 19509283]
41. Davies DL, Kochegarov AA, Kuo ST, Kulkarni AA, Woodward JJ, King BF, Alkana RL. Ethanol differentially affects ATP-gated P2X(3) and P2X(4) receptor subtypes expressed in *Xenopus* oocytes. *Neuropharmacology.* 2005; 49:243–253. [PubMed: 15993446]
42. Davies DL, Machu TK, Guo Y, Alkana RL. Ethanol sensitivity in ATP-gated P2X receptors is subunit dependent. *Alcohol Clin Exp Res.* 2002; 26:773–778. [PubMed: 12068244]
43. Davis MJ, Wu X, Nurkiewicz TR, Kawasaki J, Gui P, Hill MA, Wilson E. Regulation of ion channels by protein tyrosine phosphorylation. *Am J Physiol Heart Circ Physiol.* 2001; 281:H1835–H1862. [PubMed: 11668044]
44. De Roo M, Boue-Grabot E, Schlichter R. Selective potentiation of homomeric P2X2 ionotropic ATP receptors by a fast non-genomic action of progesterone. *Neuropharmacology.* 2010; 58:569–577. [PubMed: 20004677]
45. De Roo M, Rodeau JL, Schlichter R. Dehydroepiandrosterone potentiates native ionotropic ATP receptors containing the P2X2 subunit in rat sensory neurones. *J Physiol.* 2003; 552:59–71. [PubMed: 12844512]
46. Denlinger LC, Fisette PL, Sommer JA, Watters JJ, Prabhu U, Dubyak GR, Proctor RA, Bertics PJ. Cutting edge: the nucleotide receptor P2X7 contains multiple protein- and lipid-interaction motifs including a potential binding site for bacterial lipopolysaccharide. *J Immunol.* 2001; 167:1871–1876. [PubMed: 11489964]
47. Dent JA, Davis MW, Avery L. *avr-15* encodes a chloride channel subunit that mediates inhibitory glutamatergic neurotransmission and ivermectin sensitivity in *Caenorhabditis elegans*. *EMBO J.* 1997; 16:5867–5879. [PubMed: 9312045]
48. DiChiara TJ, Reinhart PH. Redox modulation of hslo Ca^{2+} -activated K^{+} channels. *J Neurosci.* 1997; 17:4942–4955. [PubMed: 9185532]
49. Donnelly-Roberts DL, Jarvis MF. Discovery of P2X7 receptor-selective antagonists offers new insights into P2X7 receptor function and indicates a role in chronic pain states. *Br J Pharmacol.* 2007; 151:571–579. [PubMed: 17471177]

50. Donoso, MV.; Delpiano, AM.; Huidobro-Toro, JP. Modulator role of neuropeptide Y in human vascular sympathetic neuroeffector junctions. In: Zukowska, Z.; Feuerstein, GZ., editors. NPY family of peptides in Neurobiology, Cardiovascular and Metabolic Disorders: from Genes to Therapeutics. Switzerland Exs: Birkhauser, Verlag; 2006. p. 65-76.
51. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev.* 2002; 82:47–95. [PubMed: 11773609]
52. Drury AN, Szent-Gyorgyi A. The physiological activity of adenine compounds with especial reference to their action upon the mammalian heart. *J Physiol.* 1929; 68:213–237. [PubMed: 16994064]
53. Duckwitz W, Hausmann R, Aschrafi A, Schmalzing G. P2X5 subunit assembly requires scaffolding by the second transmembrane domain and a conserved aspartate. *J Biol Chem.* 2006; 281:39561–39572. [PubMed: 17001079]
54. Egan TM, Haines WR, Voigt MM. A domain contributing to the ion channel of ATP-gated P2X2 receptors identified by the substituted cysteine accessibility method. *J Neurosci.* 1998; 18:2350–2359. [PubMed: 9502796]
55. Egan TM, Khakh BS. Contribution of calcium ions to P2X channel responses. *J Neurosci.* 2004; 24:3413–3420. [PubMed: 15056721]
56. Emmett DS, Feranchak A, Kilic G, Puljak L, Miller B, Dolovcak S, McWilliams R, Doctor RB, Fitz JG. Characterization of ionotropic purinergic receptors in hepatocytes. *Hepatology.* 2008; 47:698–705. [PubMed: 18027885]
57. Ennion S, Hagan S, Evans RJ. The role of positively charged amino acids in ATP recognition by human P2X(1) receptors. *J Biol Chem.* 2000; 275:29361–29367. [PubMed: 10827197]
58. Ennion SJ, Evans RJ. 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.* 2002; 61:303–311. [PubMed: 11809854]
59. Evans JR, Bielefeldt K. Regulation of sodium currents through oxidation and reduction of thiol residues. *Neuroscience.* 2000; 101:229–236. [PubMed: 11068151]
60. Evans RJ. Orthosteric and allosteric binding sites of P2X receptors. *Eur Biophys J.* 2009; 38:319–27. [PubMed: 18247022]
61. Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M, Panther E, Di Virgilio F. The P2X7 receptor: a key player in IL-1 processing and release. *J Immunol.* 2006; 176:3877–3883. [PubMed: 16547218]
62. Foresti R, Hammad J, Clark JE, Johnson TR, Mann BE, Friebe A, Green CJ, Motterlini R. Vasoactive properties of CORM-3, a novel water-soluble carbon monoxide-releasing molecule. *Br J Pharmacol.* 2004; 142:453–460. [PubMed: 15148243]
63. Fountain SJ, Parkinson K, Young MT, Cao L, Thompson CR, North RA. An intracellular P2X receptor required for osmoregulation in *Dictyostelium discoideum*. *Nature.* 2007; 448:200–203. [PubMed: 17625565]
64. Franklin C, Braam U, Eisele T, Schmalzing G, Hausmann R. Lack of evidence for direct phosphorylation of recombinantly expressed P2X(2) and P2X (3) receptors by protein kinase C. *Purinergic Signal.* 2007; 3:377–388. [PubMed: 18404451]
65. Franks NP, Lieb WR. Inhibitory synapses Anaesthetics set their sites on ion channels. *Nature.* 1997; 389:334–335. [PubMed: 9311772]
66. Friday SC, Hume RI. Contribution of extracellular negatively charged residues to ATP action and zinc modulation of rat P2X2 receptors. *J Neurochem.* 2008; 105:1264–1275. [PubMed: 18194442]
67. Fujiwara Y, Kubo Y. Regulation of the desensitization and ion selectivity of ATP-gated P2X2 channels by phosphoinositides. *J Physiol.* 2006; 576:135–149. [PubMed: 16857707]
68. Furuya R, Oka K, Watanabe I, Kamiya Y, Itoh H, Andoh T. The effects of ketamine and propofol on neuronal nicotinic acetylcholine receptors and P2x purinoceptors in PC12 cells. *Anesth Analg.* 1999; 88:174–180. [PubMed: 9895088]
69. Galzi JL, Revah F, Bessis A, Changeux JP. Functional architecture of the nicotinic acetylcholine receptor: from electric organ to brain. *Annu Rev Pharmacol Toxicol.* 31:1991. 37–72.

70. Gerevich Z, Zadori ZS, Koles L, Kopp L, Milius D, Wirkner K, Gyires K, Illes P. Dual effect of acid pH on purinergic P2X₃ receptors depends on the histidine 206 residue. *J Biol Chem*. 2007; 282:33949–33957. [PubMed: 17890225]
71. Gunosewoyo H, Kassiou M. P2X purinergic receptor ligands: recently patented compounds. *Expert Opin Ther Pat*. 2010 Mar 8. in press.
72. Haefely WE. Pharmacology of the benzodiazepine receptor. *Eur Arch Psychiatry Neurol Sci*. 1989; 238:294–301. [PubMed: 2569974]
73. Haines WR, Migita K, Cox JA, Egan TM, Voigt MM. The first transmembrane domain of the P2X receptor subunit participates in the agonist-induced gating of the channel. *J Biol Chem*. 2001; 276:32793–32798. [PubMed: 11438537]
74. Haines WR, Voigt MM, Migita K, Torres GE, Egan TM. On the contribution of the first transmembrane domain to whole-cell current through an ATP-gated ionotropic P2X receptor. *J Neurosci*. 2001; 21:5885–5892. [PubMed: 11487611]
75. Hardie RC, Raghu P, Moore S, Juusola M, Baines RA, Sweeney ST. Calcium influx via TRP channels is required to maintain PIP₂ levels in Drosophila photoreceptors. *Neuron*. 2001; 30:149–159. [PubMed: 11343651]
76. Harris BD, Moody EJ, Gu ZQ, Skolnick P. Contribution of "diazepam-insensitive" GABA_A receptors to the alcohol antagonist properties of Ro 15-4513 and related imidazobenzodiazepines. *Pharmacol Biochem Behav*. 1995; 52:113–118. [PubMed: 7501652]
77. Harrison NL, Majewska MD, Harrington JW, Barker JL. Structure-activity relationships for steroid interaction with the gamma-aminobutyric acid A receptor complex. *J Pharmacol Exp Ther*. 1987; 241:346–353. [PubMed: 3033209]
78. Harrison NL, Simmonds MA. Modulation of the GABA receptor complex by a steroid anaesthetic. *Brain Res*. 1984; 323:287–292. [PubMed: 6098342]
79. Hirzel K, Muller U, Latal AT, Hulsman S, Grudzinska J, Seeliger MW, Betz H, Laube B. Hyperekplexia phenotype of glycine receptor alpha1 subunit mutant mice identifies Zn(2+) as an essential endogenous modulator of glycinergic neurotransmission. *Neuron*. 2006; 52:679–690. [PubMed: 17114051]
80. Hosie AM, Wilkins ME, Smart TG. Neurosteroid binding sites on GABA(A) receptors. *Pharmacol Ther*. 2007; 116:7–19.
81. Hu AQ, Wang ZM, Lan DM, Fu YM, Zhu YH, Dong Y, Zheng P. Inhibition of evoked glutamate release by neurosteroid allopregnanolone via inhibition of L-type calcium channels in rat medial prefrontal cortex. *Neuropsychopharmacology*. 2007; 32:1477–1489. [PubMed: 17151597]
82. Hu X, Gu J, Chen L, Huang Pu Y. [Studies on the crystal structure of ivermectin (H2Bla)]. *Yao Xue Xue Bao*. 1998; 33:449–452. [PubMed: 12016915]
83. Huang CL, Feng S, Hilgemann DW. Direct activation of inward rectifier potassium channels by PIP₂ and its stabilization by Gbetagamma. *Nature*. 1998; 391:803–806. [PubMed: 9486652]
84. Huidobro-Toro JP, Lorca RA, Coddou C. Trace metals in the brain: allosteric modulators of ligand-gated receptor channels, the case of ATP-gated P2X receptors. *Eur Biophys J*. 2008; 37:301–314. [PubMed: 17972073]
85. Ikeda T. Pharmacological effects of ivermectin, an antiparasitic agent for intestinal strongyloidiasis: its mode of action and clinical efficacy. *Nippon Yakurigaku Zasshi*. 2003; 122:527–538. [PubMed: 14639007]
86. Jelinkova I, Vavra V, Jindrichova M, Obsil T, Zemkova HW, Zemkova H, Stojilkovic SS. Identification of P2X₄ receptor transmembrane residues contributing to channel gating and interaction with ivermectin. *Pflugers Arch*. 2008; 456:939–950. [PubMed: 18427835]
87. Jelinkova I, Yan Z, Liang Z, Moonat S, Teisinger J, Stojilkovic SS, Zemkova H. Identification of P2X₄ receptor-specific residues contributing to the ivermectin effects on channel deactivation. *Biochem Biophys Res Comm*. 2006; 349:619–625. [PubMed: 16949036]
88. Jensik P, Cox T. ATP-induced internalization of amphibian epithelial P2X receptors is linked to channel opening. *Pflugers Arch*. 2002; 444:795–800. [PubMed: 12355180]
89. Jiang LH, Rassendren F, Spelta V, Surprenant A, North RA. Amino acid residues involved in gating identified in the first membrane-spanning domain of the rat P2X₂ receptor. *J Biol Chem*. 2001; 276:14902–14908. [PubMed: 11278888]

90. Jiang LH, Rassendren F, Surprenant A, North RA. Identification of amino acid residues contributing to the ATP-binding site of a purinergic P2X receptor. *J Biol Chem.* 2000; 275:34190–34196. [PubMed: 10940304]
91. Jindrichova M, Vavra V, Obsil T, Stojilkovic SS, Zemkova H. Functional relevance of aromatic residues in the first transmembrane domain of P2X receptors. *J Neurochem.* 2009; 109:923–934. [PubMed: 19425179]
92. Johansson T, Frandberg PA, Nyberg F, Le Greves P. Molecular mechanisms for nanomolar concentrations of neurosteroids at NR1/NR2B receptors. *J Pharmacol Exp Ther.* 2008; 324:759–768. [PubMed: 18006693]
93. Jornvall H. The alcohol dehydrogenase system. *Exs.* 1994; 71:221–229. [PubMed: 8032153]
94. Kardos J, Kovacs I, Hajos F, Kalman M, Simonyi M. Nerve endings from rat brain tissue release copper upon depolarization. A possible role in regulating neuronal excitability. *Neurosci lett.* 1989; 103:139–144. [PubMed: 2549468]
95. Kaufman RD. Biophysical mechanisms of anesthetic action: historical perspective and review of current concepts. *Anesthesiology.* 1977; 46:49–62. [PubMed: 188356]
96. Kawate T, Michel JC, Birdsong WT, Gouaux E. Crystal structure of the ATP-gated P2X(4) ion channel in the closed state. *Nature.* 2009; 460:592–598. [PubMed: 19641588]
97. Khakh BS, Egan TM. Contribution of transmembrane regions to ATP-gated P2X2 channel permeability dynamics. *J Biol Chem.* 2005; 280:6118–6129. [PubMed: 15556949]
98. Khakh BS, Proctor WR, Dunwiddie TV, Labarca C, Lester HA. Allosteric control of gating and kinetics at P2X(4) receptor channels. *J Neurosci.* 1999; 19:7289–7299. [PubMed: 10460235]
99. Koshimizu T, Tomic M, Koshimizu M, Stojilkovic SS. Identification of amino acid residues contributing to desensitization of the P2X2 receptor channel. *J Biol Chem.* 1998; 273:12853–12857. [PubMed: 9582314]
100. Koshimizu T, Tomic M, Van Goor F, Stojilkovic SS. Functional role of alternative splicing in pituitary P2X2 receptor-channel activation and desensitization. *Mol Endocrinol.* 1998; 12:901–913. [PubMed: 9658396]
101. Koshimizu TA, Kretschmannova K, He ML, Ueno S, Tanoue A, Yanagihara N, Stojilkovic SS, Tsujimoto G. Carboxyl-terminal splicing enhances physical interactions between the cytoplasmic tails of purinergic P2X receptors. *Mol Pharmacol.* 2006; 69:1588–1598. [PubMed: 16467187]
102. Krause RM, Buisson B, Bertrand S, Corringer PJ, Galzi JL, Changeux JP, Bertrand D. Ivermectin: a positive allosteric effector of the $\alpha 7$ neuronal nicotinic acetylcholine receptor. *Mol Pharmacol.* 1998; 53:283–294. [PubMed: 9463487]
103. Krusek J, Zemkova H. Effect of ivermectin on gamma-aminobutyric acid-induced chloride currents in mouse hippocampal embryonic neurones. *Eur J Pharmacol.* 1994; 259:121–128. [PubMed: 7957605]
104. Kuo YM, Zhou B, Cosco D, Gitschier J. The copper transporter CTR1 provides an essential function in mammalian embryonic development. *Proc Natl Acad Sci USA.* 2001; 98:6836–6841. [PubMed: 11391004]
105. Le Foil F, Castel H, Louiset E, Vaudry H, Cazin L. Multiple modulatory effects of the neuroactive steroid pregnanolone on GABAA receptor in frog pituitary melanotrophs. *J Physiol.* 1997; 504(Pt 2):387–400. [PubMed: 9365913]
106. Leinders-Zufall T, Shepherd GM, Zufall F. Regulation of cyclic nucleotide-gated channels and membrane excitability in olfactory receptor cells by carbon monoxide. *J Neurophysiol.* 1995; 74:1498–1508. [PubMed: 8989388]
107. Li Z, Migita K, Samways DS, Voigt MM, Egan TM. Gain and loss of channel function by alanine substitutions in the transmembrane segments of the rat ATP-gated P2X2 receptor. *J Neurosci.* 2004; 24:7378–7386. [PubMed: 15317863]
108. Linden DJ, Narasimhan K, Gurfel D. Protoporphyrins modulate voltage-gated Ca current in AtT-20 pituitary cells. *J Neurophysiol.* 1993; 70:2673–2677. [PubMed: 7907135]
109. Liu GJ, Brockhausen J, Bennett MR. P2X1 receptor currents after disruption of the PKC site and its surroundings by dominant negative mutations in FIEK293 cells. *Auton Neurosci.* 2003; 108:12–16. [PubMed: 14614959]

110. Liu X, Ma W, Surprenant A, Jiang LH. Identification of the amino acid residues in the extracellular domain of rat P2X(7) receptor involved in functional inhibition by acidic pH. *Br J Pharmacol.* 2009; 156:135–142. [PubMed: 19068080]
111. Liu X, Surprenant A, Mao HJ, Roger S, Xia R, Bradley H, Jiang LH. Identification of key residues coordinating functional inhibition of P2X7 receptors by zinc and copper. *Mol Pharmacol.* 2008; 73:252–259. [PubMed: 17959713]
112. Lorca RA, Coddou C, Gazitua MC, Bull P, Arredondo C, Huidobro-Toro JP. Extracellular histidine residues identify common structural determinants in the copper/zinc P2X2 receptor modulation. *J Neurochem.* 2005; 95:499–512. [PubMed: 16190872]
113. Lustig KD, Shiau AK, Brake AJ, Julius D. Expression cloning of an ATP receptor from mouse neuroblastoma cells. *Proc Natl Acad Sci USA.* 1993; 90:5113–5117. [PubMed: 7685114]
114. Madsen E, Gitlin JD. Copper and iron disorders of the brain. *Ann Rev Neurosci.* 2007; 30:317–337. [PubMed: 17367269]
115. Mathie A, Sutton GL, Clarke CE, Veale EL. Zinc and copper: pharmacological probes and endogenous modulators of neuronal excitability. *Pharmacol Ther.* 2006; 111:567–583. [PubMed: 16410023]
116. Michel AD, Chambers LJ, Walter DS. Negative and positive allosteric modulators of the P2X(7) receptor. *Br J Pharmacol.* 2008; 153:737–750. [PubMed: 18071294]
117. Michel AD, Clay WC, Ng SW, Roman S, Thompson K, Condreay JP, Hall M, Holbrook J, Livermore D, Senger S. Identification of regions of the P2X(7) receptor that contribute to human and rat species differences in antagonist effects. *Br J Pharmacol.* 2008; 155:738–751. [PubMed: 18660826]
118. Michel AD, Fonfria E. Agonist potency at P2X7 receptors is modulated by structurally diverse lipids. *Br J Pharmacol.* 2007; 152:523–537. [PubMed: 17700717]
119. Michel AD, Xing M, Humphrey PP. Serum constituents can affect 2'-& 3'-O-(4-benzoylbenzoyl)-ATP potency at P2X(7) receptors. *Br J Pharmacol.* 2001; 132:1501–1508. [PubMed: 11264244]
120. Migita K, Haines WR, Voigt MM, Egan TM. Polar residues of the second transmembrane domain influence cation permeability of the ATP-gated P2X(2) receptor. *J Biol Chem* 2001. 276:30934–30941.
121. Miller J, McLachlan AD, Klug A. Repetitive zinc-binding domains in the protein transcription factor IIIA from *Xenopus* oocytes. *EMBO J.* 1985; 4:1609–1614. [PubMed: 4040853]
122. Mizota K, Yoshida A, Uchida H, Fujita R, Ueda H. Novel type of Gq/11 protein-coupled neurosteroid receptor sensitive to endocrine disrupting chemicals in mast cell line (RBL-2H3). *Br J Pharmacol.* 2005; 145:545–550. [PubMed: 15821754]
123. Monaghan DT, Bridges RJ, Cotman CW. The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. *Ann Rev Pharmacol Toxicol.* 1989; 29:365–402. [PubMed: 2543272]
124. Monod J, Wyman J, Changeux JP. On the Nature of Allosteric Transitions: a Plausible Model. *J Mol Biol.* 1965; 12:88–118. [PubMed: 14343300]
125. Moore SF, Mackenzie AB. Species and agonist dependent zinc modulation of endogenous and recombinant ATP-gated P2X7 receptors. *Biochem Pharmacol.* 2008; 76:1740–1747. [PubMed: 18848528]
126. Nagaoka M, Nara M, Tamada T, Kume H, Oguma T, Kikuchi T, Zaini J, Moriya T, Ichinose M, Tamura G, Hattori T. Regulation of adenosine 5'-triphosphate (ATP)-gated P2X(4) receptors on tracheal smooth muscle cells. *Respir Physiol Neurobiol.* 2009; 166:61–67. [PubMed: 19429520]
127. Nagatsu T. Tyrosine hydroxylase: human isoforms, structure and regulation in physiology and pathology. *Essays Biochem.* 1995; 30:15–35. [PubMed: 8822146]
128. Nagaya N, Tittle RK, Friday C, Hume RI. Long-lasting inhibition of human P2X2 receptors by copper. Society for Neuroscience 39th Annual Meeting. 2009
129. Nagaya N, Tittle RK, Saar N, Dellal SS, Hume RI. An intersubunit zinc binding site in rat P2X2 receptors. *J Biol Chem.* 2005; 280:25982–25993. [PubMed: 15899882]
130. Nair A, Simonetti M, Fabbretti E, Nistri A. The Cdk5 Kinase Downregulates ATP-Gated Ionotropic P2X(3) Receptor Function Via Serine Phosphorylation. *Cell Mol Neurobiol.* 2009 Dec 4. in press.

131. Nakanishi M, Mori T, Nishikawa K, Sawada M, Kuno M, Asada A. The effects of general anesthetics on P2X7 and P2Y receptors in a rat microglial cell line. *Anesth Analg*. 2007; 104:1136–1144. [PubMed: 17456664]
132. Nakazawa K, Ohno Y. Effects of neuroamines and divalent cations on cloned and mutated ATP-gated channels. *Eur J Pharmacol*. 1997; 325:101–108. [PubMed: 9151945]
133. Newbolt A, Stoop R, Virginio C, Surprenant A, North RA, Buell G, Rassendren F. Membrane topology of an ATP-gated ion channel (P2X receptor). *J Biol Chem*. 1998; 273:15177–15182. [PubMed: 9614131]
134. Nicke A, Baumert HG, Rettinger J, Eichele A, Lambrecht G, Mutschler E, Schmalzing G. P2X1 and P2X3 receptors form stable trimers: a novel structural motif of ligand-gated ion channels. *EMBO J*. 1998; 17:3016–3028. [PubMed: 9606184]
135. Palmiter RD, Huang L. Efflux and compartmentalization of zinc by members of the SLC30 family of solute carriers. *Pflugers Arch*. 2004; 447:744–751. [PubMed: 12748859]
136. Pannaccione A, Castaldo P, Ficker E, Annunziato L, Tagliatela M. Histidines 578 and 587 in the S5-S6 linker of the human Ether-a-gogo Related Gene-1 K⁺ channels confer sensitivity to reactive oxygen species. *J Biol Chem*. 2002; 277:8912–8919. [PubMed: 11756457]
137. Paradiso K, Zhang J, Steinbach JH. The C terminus of the human nicotinic alpha4beta2 receptor forms a binding site required for potentiation by an estrogenic steroid. *J Neurosci*. 2001; 21:6561–6568. [PubMed: 11517245]
138. Patten D, Foxon GR, Martin KF, Halliwell RF. An electrophysiological study of the effects of propofol on native neuronal ligand-gated ion channels. *Clin Exp Pharmacol Physiol*. 2001; 28:451–458. [PubMed: 11380521]
139. Paukert M, Osteroth R, Geisler HS, Brandle U, Glowatzki E, Ruppersberg JP, Grunder S. Inflammatory mediators potentiate ATP-gated channels through the P2X(3) subunit. *J Biol Chem*. 2001; 276:21077–21082. [PubMed: 11264291]
140. Peers C, Dallas ML, Scragg JL. Ion channels as effectors in carbon monoxide signaling. *Commun Integr Biol*. 2009; 2:241–242. [PubMed: 19641743]
141. Peters JA, Kirkness EF, Callachan H, Lambert JJ, Turner AJ. Modulation of the GABAA receptor by depressant barbiturates and pregnane steroids. *Br J Pharmacol*. 1988; 94:1257–1269. [PubMed: 2850060]
142. Pochynyuk O, Staruschenko A, Tong Q, Medina J, Stockand JD. Identification of a functional phosphatidylinositol 3,4,5-trisphosphate binding site in the epithelial Na⁺ channel. *J Biol Chem*. 2005; 280:37565–37571. [PubMed: 16154997]
143. Popova M, Asatryan L, Ostrovskaya O, Wyatt LR, Li K, Alkana RL, Davies DL. A point mutation in the ectodomain-transmembrane 2 interface eliminates the inhibitory effects of ethanol in P2X4 receptors. *J Neurochem*. 2010; 112:307–317. [PubMed: 19878433]
144. Prestwich GD. Phosphoinositide signaling; from affinity probes to pharmaceutical targets. *Chem Biol*. 2004; 11:619–637. [PubMed: 15157873]
145. Priel A, Silberberg SD. Mechanism of ivermectin facilitation of human P2X4 receptor channels. *J Gen Physiol*. 2004; 123:281–293. [PubMed: 14769846]
146. Prossnitz ER, Arterburn JB, Smith HO, Oprea TI, Sklar LA, Hathaway HJ. Estrogen signaling through the transmembrane G protein-coupled receptor GPR30. *Ann Rev Physiol*. 2008; 70:165–190. [PubMed: 18271749]
147. Ralevic V, Burnstock G. Receptors for purines and pyrimidines. *Pharmacol Rev*. 1998; 50:413–492. [PubMed: 9755289]
148. Rassendren F, Buell G, Newbolt A, North RA, Surprenant A. Identification of amino acid residues contributing to the pore of a P2X receptor. *EMBO J*. 1997; 16:3446–3454. [PubMed: 9218787]
149. Richardson J, Thomas KA, Rubin BH, Richardson DC. Crystal structure of bovine Cu,Zn superoxide dismutase at 3 Å resolution: chain tracing and metal ligands. *Proc Nat Acad Sci USA*. 1975; 72:1349–1353. [PubMed: 1055410]
150. Roberts JA, Evans RJ. Cysteine substitution mutants give structural insight and identify ATP binding and activation sites at P2X receptors. *J Neurosci*. 2007; 27:4072–4082. [PubMed: 17428985]

151. Roberts JA, Valente M, Allsopp RC, Watt D, Evans RJ. Contribution of the region Glu81 to Val200 of the extracellular loop of the human P2X1 receptor to agonist binding and gating revealed using cysteine scanning mutagenesis. *J Neurochem.* 2009; 109:1042–1052. [PubMed: 19519776]
152. Roger S, Pelegrin P, Surprenant A. Facilitation of P2X7 receptor currents and membrane blebbing via constitutive and dynamic calmodulin binding. *J Neurosci.* 2008; 28:6393–6401. [PubMed: 18562610]
153. Samways DS, Egan TM. Acidic amino acids impart enhanced Ca²⁺ permeability and flux in two members of the ATP-gated P2X receptor family. *J Gen Physiol.* 2007; 129:245–256. [PubMed: 17325195]
154. Schiess AR, Partridge LD. Pregnenolone sulfate acts through a G-protein-coupled signal-like receptor to enhance short term facilitation in adult hippocampal neurons. *Eur J Pharmacol.* 2005; 518:22–29. [PubMed: 15996654]
155. Sedlacek M, Korinek M, Petrovic M, Cais O, Adamusova E, Chodounska H, Vyklicky L Jr. Neurosteroid modulation of ionotropic glutamate receptors and excitatory synaptic transmission. *Physiol Res.* 2008; 57(Suppl 3):S49–57. [PubMed: 18481915]
156. Selye H. On the Hormonal Activity of a Steroid Compound. *Science.* 1941; 94:94. [PubMed: 17774030]
157. Shan Q, Hadrill JL, Lynch JW. Ivermectin, an unconventional agonist of the glycine receptor chloride channel. *J Biol Chem.* 2001; 276:12556–12564. [PubMed: 11278873]
158. Sigel E, Baur R. Effect of avermectin B1a on chick neuronal gamma-aminobutyrate receptor channels expressed in *Xenopus* oocytes. *Mol Pharmacol.* 1987; 32:749–752. [PubMed: 2447480]
159. Silberberg SD, Chang TH, Swartz KJ. Secondary structure and gating rearrangements of transmembrane segments in rat P2X4 receptor channels. *J Gen Physiol.* 2005; 125:347–359. [PubMed: 15795310]
160. Silberberg SD, Li M, Swartz KJ. Ivermectin Interaction with transmembrane helices reveals widespread rearrangements during opening of P2X receptor channels. *Neuron.* 2007; 54:263–274. [PubMed: 17442247]
161. Sim JA, Park CK, Oh SB, Evans RJ, North RA. P2X1 and P2X4 receptor currents in mouse macrophages. *Br J Pharmacol.* 2007; 152:1283–1290. [PubMed: 17934511]
162. Sommer B, Seeburg PH. Glutamate receptor channels: novel properties and new clones. *Trends Pharmacol Sci.* 1992; 13:291–296. [PubMed: 1380742]
163. Springer J, Arison BH, Hirshfield JM, Hoogsteen K. The absolute stereochemistry and conformation of avermectin B2a. *J Am Chem Soc.* 1981; 203:4221–4224.
164. Suh BC, Inoue T, Meyer T, Hille B. Rapid chemically induced changes of PtdIns(4,5)P₂ gate KCNQ ion channels. *Science.* 2006; 314:1454–1457. [PubMed: 16990515]
165. Swope SL, Moss SJ, Raymond LA, Huganir RL. Regulation of ligand-gated ion channels by protein phosphorylation. *Adv Second Messenger Phosphoprotein Res.* 1999; 33:49–78. [PubMed: 10218114]
166. Tittle RK, Hume RL. Opposite effects of zinc on human and rat P2X2 receptors. *J Neurosci.* 2008; 28:11131–11140. [PubMed: 18971456]
167. Tombaugh, GC.; Somjen, GG. pH Modulation of Voltage-Gated Ion Channels. In: Kaila, K.; Ramson, BR., editors. *pH and Brain Function* United States. Wiley-Liss; 1998. p. 395-416.
168. Tomioka A, Ueno S, Kohama K, Goto F, Inoue K. Propofol potentiates ATP-activated currents of recombinant P2X(4) receptor channels expressed in human embryonic kidney 293 cells. *Neurosci lett.* 2000; 284:167–170. [PubMed: 10773425]
169. Torres GE, Egan TM, Voigt MM. Identification of a domain involved in ATP-gated ionotropic receptor subunit assembly. *J Biol Chem.* 1999; 274:22359–22365. [PubMed: 10428806]
170. Torres GE, Haines WR, Egan TM, Voigt MM. Co-expression of P2X1 and P2X5 receptor subunits reveals a novel ATP-gated ion channel. *Mol Pharmacol.* 1998; 54:989–993. [PubMed: 9855626]
171. Toulme E, Soto F, Garret M, Boue-Grabot E. Functional properties of internalization-deficient P2X4 receptors reveal a novel mechanism of ligand-gated channel facilitation by ivermectin. *Mol Pharmacol.* 2006; 69:576–587. [PubMed: 16282518]

172. Traynelis, SF. pH Modulation of Ligand-Gated Ion Channels. In: Kaila, K.; Ransom, BR., editors. pH and Brain Function United States. Wiley-Liss; 1998. p. 417-446.
173. Tsutsui K, Sakamoto H, Ukena K. A novel aspect of the cerebellum: biosynthesis of neurosteroids in the Purkinje cell. *Cerebellum*. 2003; 2:215-222. [PubMed: 14509571]
174. Tucker SJ, Baukrowitz T. How highly charged anionic lipids bind and regulate ion channels. *J Physiol*. 2008; 131:431-438.
175. Valera S, Hussy N, Evans RJ, Adami N, North RA, Surprenant A, Buell G. A new class of ligand-gated ion channel defined by P2x receptor for extracellular ATP. *Nature*. 1994; 371:516-519. [PubMed: 7523951]
176. Varela-Nallar L, Toledo EM, Larrondo LF, Cabral AL, Martins VR, Inestrosa NC. Induction of cellular prion protein gene expression by copper in neurons. *Am J Physiol Cell Physiol*. 2006; 290:C271-281. [PubMed: 16148034]
177. Vial C, Tobin AB, Evans RJ. G-protein-coupled receptor regulation of P2X1 receptors does not involve direct channel phosphorylation. *Biochem J*. 2004; 382:101-110. [PubMed: 15144237]
178. Virginio C, Church D, North RA, Surprenant A. Effects of divalent cations, protons and calmidazolium at the rat P2X7 receptor. *Neuropharmacology*. 1997; 36:1285-1294. [PubMed: 9364483]
179. Wareham K, Vial C, Wykes RC, Bradding P, Seward EP. Functional evidence for the expression of P2X1, P2X4 and P2X7 receptors in human lung mast cells. *Br J Pharmacol*. 2009; 157:1215-1224. [PubMed: 19552691]
180. Webb TE, Simon J, Krishek BJ, Bateson AN, Smart TG, King BF, Burnstock G, Barnard EA. Cloning and functional expression of a brain G-protein-coupled ATP receptor. *FEBS lett*. 1993; 324:219-225. [PubMed: 8508924]
181. Wildman SS, Brown SG, Rahman M, Noel CA, Churchill L, Burnstock G, Unwin RJ, King BF. Sensitization by extracellular Ca(2+) of rat P2X(5) receptor and its pharmacological properties compared with rat P2X(1). *Mol Pharmacol*. 2002; 62:957-966. [PubMed: 12237343]
182. Wildman SS, King BF, Burnstock G. Modulatory activity of extracellular H+ and Zn2+ on ATP-responses at rP2X1 and rP2X3 receptors. *Br J Pharmacol*. 1999; 128:486-492. [PubMed: 10510462]
183. Wilkinson WJ, Gadeberg HC, Harrison AW, Allen ND, Riccardi D, Kemp PJ. Carbon monoxide is a rapid modulator of recombinant and native P2X(2) ligand-gated ion channels. *Br J Pharmacol*. 2009; 158:862-871. [PubMed: 19694727]
184. Woodward JJ, Nowak M, Davies DL. Effects of the abused solvent toluene on recombinant P2X receptors expressed in HEK293 cells. *Brain Res Mol Brain Res*. 2004; 125:86-95. [PubMed: 15193425]
185. Wu L, Bauer CS, Zhen XG, Xie C, Yang J. Dual regulation of voltage-gated calcium channels by PtdIns(4,5)P2. *Nature*. 2002; 419:947-952. [PubMed: 12410316]
186. Wu L, Cao K, Lu Y, Wang R. Different mechanisms underlying the stimulation of K(Ca) channels by nitric oxide and carbon monoxide. *J Clin Invest*. 2002; 110:691-700. [PubMed: 12208870]
187. Wu L, Wang R. Carbon monoxide: endogenous production, physiological functions, and pharmacological applications. *Pharmacol Rev*. 2005; 57:585-630. [PubMed: 16382109]
188. Xiong K, Stewart RR, Hu XQ, Werby E, Peoples RW, Weight FF, Li C. Role of extracellular histidines in agonist sensitivity of the rat P2X4 receptor. *Neurosci lett*. 2004; 365:195-199. [PubMed: 15246547]
189. Xue BG, Whittemore ER, Park CH, Woodward RM, Lan NC, Gee KW. Partial agonism by 3alpha,21-dihydroxy-5beta-pregnan-20-one at the gamma-aminobutyric acidA receptor neurosteroid site. *J Pharmacol Exp Ther*. 1997; 281:1095-1101. [PubMed: 9190841]
190. Yan Z, Li S, Liang Z, Tomic M, Stojilkovic SS. The P2X7 receptor channel pore dilates under physiological ion conditions. *J Gen Physiol*. 2008; 132:563-573. [PubMed: 18852304]
191. Yan Z, Liang Z, Obsil T, Stojilkovic SS. Participation of the Lys313-Ile333 sequence of the purinergic P2X4 receptor in agonist binding and transduction of signals to the channel gate. *J Biol Chem*. 2006; 281:32649-32659. [PubMed: 16954225]

192. Yan Z, Liang Z, Tomic M, Obsil T, Stojilkovic SS. Molecular determinants of the agonist binding domain of a P2X receptor channel. *Mol Pharmacol*. 2005; 67:1078–1088. [PubMed: 15632318]
193. Yi CL, Liu YW, Xiong KM, Stewart RR, Peoples RW, Tian X, Zhou L, Ai YX, Li ZW, Wang QW, Li CY. Conserved extracellular cysteines differentially regulate the inhibitory effect of ethanol in rat P2X4 receptors. *Biochem Biophys Res Comm*. 2009; 381:102–106. [PubMed: 19351603]
194. Zafari AM, Ushio-Fukai M, Akers M, Yin Q, Shah A, Harrison DG, Taylor WR, Griendling KK. Role of NADH/NADPH oxidase-derived H₂O₂ in angiotensin II-induced vascular hypertrophy. *Hypertension*. 1998; 32:488–495. [PubMed: 9740615]
195. Zemkova H, Kucka M, Li S, Gonzalez-Iglesias AE, Tomic M, Stojilkovic SS. Characterization of purinergic P2X4 receptor channels expressed in anterior pituitary cells. *Am J Physiol Endocrinol Metab*. 2010; 298:E644–651. [PubMed: 20009029]
196. Zemkova H, Yan Z, Liang Z, Jelinkova I, Tomic M, Stojilkovic SS. Role of aromatic and charged ectodomain residues in the P2X(4) receptor functions. *J Neurochem*. 2007; 102:1139–1150. [PubMed: 17663752]
197. Zhao Q, Logothetis DE, Seguela P. Regulation of ATP-gated P2X receptors by phosphoinositides. *Pflugers Arch*. 2007; 455:181–185. [PubMed: 17479282]
198. Zhao Q, Yang M, Ting AT, Logothetis DE. PIP(2) Regulates the Ionic Current of P2X Receptors and P2X(7) Receptor-Mediated Cell Death. *Channels (Austin)*. 2007; 1:46–55. [PubMed: 19151591]

Figure 1.

Amino acid residues identified in allosteric modulation of P2X receptors. IVM, ivermectin, LPS, lipopolysaccharides; CaM, calmodulin; ?, residues not identified.

Figure 2.

Zinc and IVM interaction studies in cells expressing the wild-type and mutant P2X4Rs. Representative recordings of macroscopic currents of oocytes injected with cDNAs encoding for the wild-type P2X4R (A), C132A-P2X4R (B) and P2X4/2 chimera (C). Oocytes were challenged with 1 μM ATP alone (1st column) or 1 μM ATP plus 10 μM zinc (2nd column), or 1 μM ATP plus 3 μM IVM (3rd column), or both zinc and IVM plus 1 μM ATP (4th column). Zinc and IVM were pre-applied for 2 minutes before the ATP pulse. The combined application of both agents elicited statistically larger effects than that evoked by each agent separately only in the wild-type P2X4R.

Table 1

Effects of allosteric modulators on homomeric P2X receptors

Modulators	P2X1R	P2X2R	P2X3R	P2X4R	P2X5R	P2X7R	References
Zinc (μM)	1: inhibition	30: potentiation ^{a,b}	10: potentiation ^a	5: potentiation ^a	10: potentiation ^a	80: inhibition	1, 2, 27, 29, 32, 111, 112, 178, 181, 182
Copper (μM)		3: potentiation ^a		10: inhibition		2: inhibition	1, 2, 29, 32, 111, 112, 178
Cadmium (μM)	inhibition	3: potentiation ^a	inhibition	20: potentiation		100: inhibition	2, 31, 112, 132
Mercury [*] (μM)		3: potentiation ^a		10: inhibition		10: inhibition	30, 31
Protons (pH)	6.3: inhibition	7.3: potentiation	6.0: inhibition	7.0: inhibition	inhibition	6.1: inhibition	1, 26, 28, 70, 110, 192
H ₂ O ₂ (mM)		0.3: potentiation		1: inhibition			30
Myxothiazol (mM)		0.3: potentiation					30
Rotenone (mM)		0.3: potentiation					30
Mercury ^{**} (μM)		3: potentiation ^a		10: inhibition			30
CO		potentiation	no effect	inhibition			183
IVM (μM)		no effect	no effect	3: potentiation		no effect	86, 87, 91, 98, 145, 160, 171
progesterone	no effect	potentiation	no effect	no effect			34, 44, 45
Alfaxalone (nM)				0.4: potentiation			34
Alloprenolone (nM)				0.3: potentiation			34
THDOC				0.1: potentiation			34
DHEA		potentiation	inhibition				45
phosphoinositides	potentiation	√ desensitization	potentiation	potentiation	potentiation	potentiation	10, 11, 67, 197
PKA		↑ desensitization		potentiation			15, 24
Cdk5			inhibition				130
Src			inhibition				40
PKC		√ desensitization					12
Ethanol		inhibition	potentiation	inhibition			7, 41, 42, 143, 188
Propofol		no effect	no effect	potentiation		potentiation	68, 131, 168
Ketamine		no effect				potentiation	68, 131
Toluene		potentiation		potentiation			184

^aBiphasic effects, suggesting the existence of more than one allosteric site;

^btt human P2X2R, zinc acts as an inhibitor;

* mercury as a heavy metal;

** mercury as a source for ROS;

↑ facilitates desensitization;

↓ slows desensitization. Numbers in front of the nature of effects indicate EC₅₀/IC₅₀ values in units shown in the first column.