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Allosteric modulation of ligand-gated ion channels

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Abstract

Ligand-gated ion channels (LGICs) are cell surface proteins that play an important role in fast synaptic transmission and in the modulation of cellular activity. Due to their intrinsic properties, LGICs respond to neurotransmitters and other effectors (e.g. pH) and transduce the binding of a ligand into an electrical current on a microsecond timescale. Following activation, LGICs open allowing an ion flux across the cell membrane. Depending upon the charge and concentration of ions, the flux can cause a depolarization or hyperpolarization, thus modulating excitability of the cell. While our understanding of LGICs has significantly progressed during the past decade, many properties of these proteins are still poorly understood, in particular their modulation by allosteric effectors. LGICs are often thought as a simple on–off switches. However, a closer look at these receptors reveals a complex behavior and a wide repertoire of subtle modulation by intrinsic and extrinsic factors. From a physiological point of view, this modulation can be seen as an additional level of complexity in the cell signaling process.

Here we review the allosteric modulation of LGICs in light of the latest findings and discuss the suitability of this approach to the design of new therapeutic molecules.

Keywords: Ligand-gated ion channels; Allosteric modulation; P2X receptors; Glutamate receptors; Nicotinic acetylcholine receptors; nAChRs; Divalent cations

1. Ligand-gated ion channels

The capacity of the central nervous system (CNS) to process information depends upon the ability of neurons to communicate and thus on the intimate process of synaptic transmission. While numerous processes participate in synaptic transmission, the contribution of ligand-gated ion channels (LGICs) is certainly one of the determining steps. Although these integral membrane proteins have been the focus of attention of numerous studies, important facets regarding their function remain obscure.

In mammals, LGICs are divided into three main families according to the number of transmembrane segments present in the subunits that form the channels (Fig. 1A). As their name suggests, LGICs are proteins that span the cell membrane and form both the binding site for the natural ligand and the ion-conducting pore, which can be opened and closed by the binding of the ligand. These ion channels result from the assembly of subunits that form a water-filled, ion-selective pore. The subunit composition of these channels can be homomeric or heteromeric in nature and, as a result they display a great diversity of physiological and pharmacological properties.

The first LGIC family is the P2X (adenosine triphosphate, ATP) receptors; these are cationic channels and are thought to contain three subunits. Each subunit contains two transmembrane segments separated by a large extracellular loop [1–3], the N- and C-terminal regions are thought to be located intracellularly (Fig. 1A). To date, seven P2X subunits have been identified, each of which can form functional homomeric receptors, although some doubt exists on the ability of $P2X_6$ receptors to form functional homomers, see [4]. Functional heteromeric receptors containing the $P2X_{2/3}$, $P2X_{1/5}$, $P2X_{2/6}$ and $P2X_{4/6}$ subunits have been characterized in heterologous expression systems, several of these heteromeric receptors have biophysical and pharmacological properties similar to

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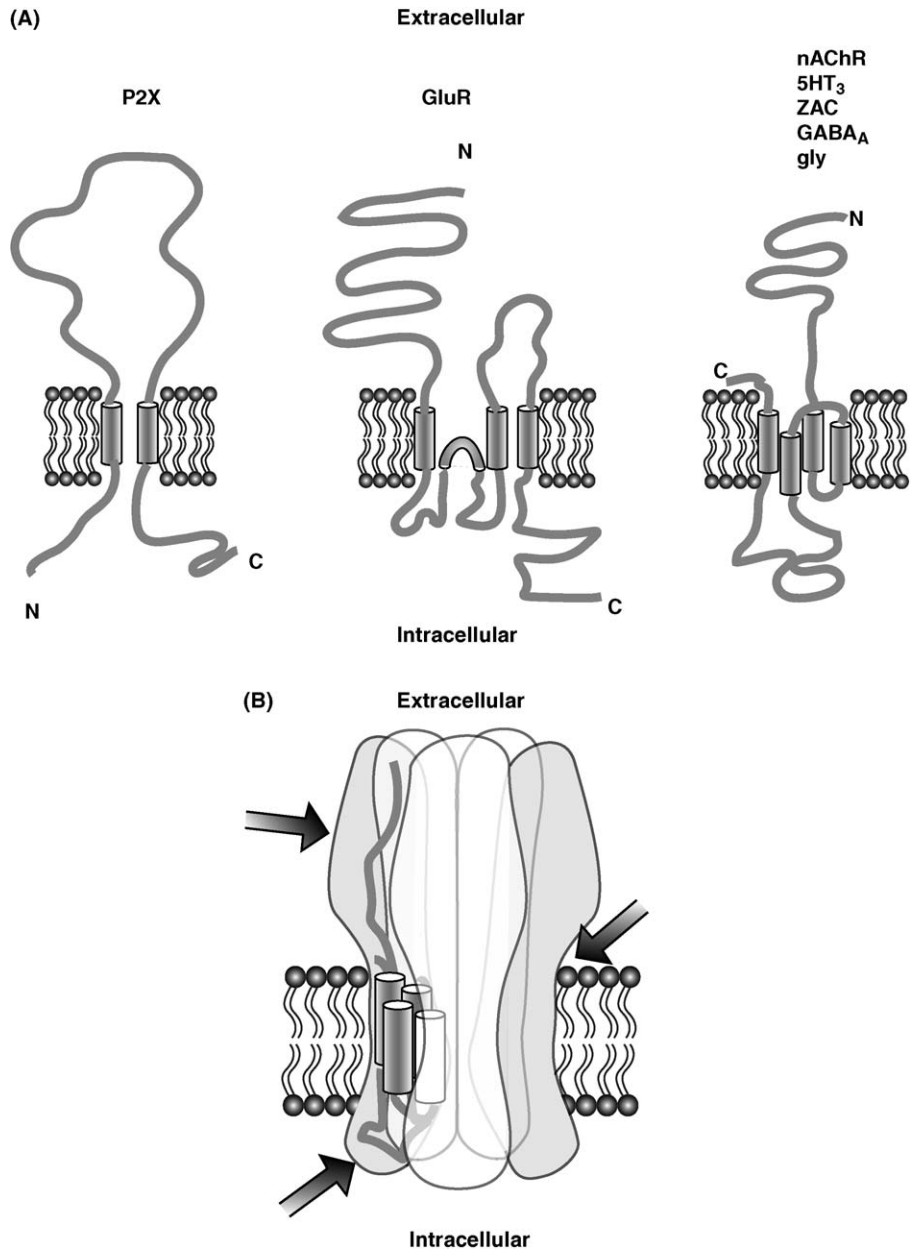

Fig. 1. Structure of the different LGICs. (A) Schematic representation of a single subunit from the three families of ligand-gated ion channels inserted in the cell membrane. Note the importance of both the extracellular and intracellular domain where different molecules can bind and interact. (B) Schematic representation of the typical structure of a four transmembrane LGIC such as the nAChR. Putative-binding sites for allosteric ligands are illustrated by the arrows.

native receptors suggesting that heteromeric P2X receptors may exist in native tissue. Immunoprecipitation experiments have shown that several other subunit combinations are also possible, and a number of alternatively spliced subunits have also been described [1–4].

The second family is the glutamate-activated cationic receptors, which include *N*-methyl-D-aspartate (NMDA) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid (AMPA) and kainate receptors. These are made up of four homologous subunits. Each subunit contains an extracellular amino-terminal domain which makes up half of the agonist-binding region, the first two transmembrane segments are separated by a ''P-loop'' and the second half of the agonist-binding region is formed by the extracellular loop between the second and third transmembrane segments (Fig. 1A). The C-terminal tail is variable in length and protrudes into the cytoplasm [5]. In addition, many subunits undergo alternative splicing or RNA editing which further increases their functional diversity. Functional NMDA receptors are formed by the co-assembly of an NR1 subunit and one of the four types of NR2 (A–D) subunits [6]. AMPA receptors are composed of the GluR 1–4 subunits and kainate receptors from multimeric assemblies of GluR 5–7 and KA-1/2 subunits [7].

Finally, the third and largest family is the ''Cys-loop receptor superfamily'' so called because of a conserved

cysteine loop in their extracellular domain. This family includes: nicotinic acetylcholine receptors (nAChRs), glycine receptors, 5-HT_3 (serotonin), zinc activated ZAC receptors and γ-aminobutyric-acid $GABA_A$ and $GABA_C$ also referred to as ρ receptors. The Cys-loop superfamily contains both anionic ($GABA_A$ and $GABA_C$, glycine), and cationic channels (nAChRs, 5-HT_3 and ZAC). These receptors are composed from five homologous subunits each made up of an extracellular amino-terminal domain and four transmembrane segments (TM1–TM4). The ligand-binding site is on the interface between adjacent subunits and the existence of numerous genes coding for distinct subunits that can combine to form a receptor contributes to the diversity of receptors within each family. The large loop located between transmembrane segments TM3 and TM4 makes up an intracellular domain, which is known to interact with intracellular proteins.

2. The concept of allosteric modulation

Binding of the endogenous ligand (neurotransmitter) to an LGIC causes opening of the channel. At least two models have been proposed to describe this. The induced fit model [8] predicts that the binding of an agonist molecule to the receptor protein induces a conformational change in the region of the ligand-binding site, which propagates to the pore region and causes opening of the ion-conducting pore. The second, allosteric model, predicts that the receptor protein constantly undergoes spontaneous changes between distinct conformational states. These transitions between states have different ''energy barriers''. Each of these conformational states has a different affinity for the ligand and the binding of a ligand to the LGIC preferentially stabilizes the receptor in a given state. Typically, the binding of an agonist molecule stabilizes the channel in the open state. This model was first proposed by Monod et al. to explain the observed behavior of proteins like haemoglobin [9] and was extended to LGICs by Karlin, who proposed that it could describe the functioning of nAChRs [10]. The high density of nAChRs found in the electric organ of the Electrophorus electricus (eel), and the discovery of the selective antagonist α-bungarotoxin, resulted in these receptors being extensively studied and becoming the basis for the first models to describe LGIC function. The binding site for the natural ligand, that activates the receptor, is known as the orthosteric-binding site. In addition the binding of ligands at other sites on the receptor surface can modify the functioning of the receptor. This concept of modulation of LGIC activity by the binding of a second ligand, or allosteric modulator, was introduced by Karlin [10] and termed, allosteric modulation. The binding site for an allosteric modulator is an allosteric-binding site, each receptor may have several allosteric-binding sites which are selective for different ligands. An elegant illustration of the conformational changes affecting a LGIC at rest, following agonist binding or in the presence of an allosteric modulator has been recently illustrated, using electron microscopy, for AMPA receptors [11].

Importantly the allosteric concept introduces another determining notion that is: binding of a molecule at any location on the protein complex can affect the stability of the complex and/or the energy barrier(s) for conformational changes. This is the principle of allosteric modulation. Typically, molecules that cause allosteric modulation are termed allosteric effectors and are divided in two classes. Positive allosteric effectors enhance the agonist-induced response whereas negative allosteric effectors reduce receptor function. In addition to modulating LGICs allosteric modulation can affect the function of a wide range of cellular proteins including enzymes and metabotropic membrane receptors [12,13].

Since allosteric effectors can bind to the receptor complex at many possible locations, as illustrated in Fig. 1B, their effects on receptor function are expected to vary as a result of the modification that they cause to the properties of the protein complex.

The simplest scheme that can adequately describe the activity of a LGIC is a two state scheme, where the receptor can be in either open or closed. According to this scheme the receptor can adopt two conformational states that are respectively: R the resting (closed) state, A the active (open) state (see Fig. 2A). In absence of an agonist ligand, the receptor is mainly in the resting R state and exposure to an agonist stabilizes the receptor in the A state. In this case a square shaped non-desensitizing current is observed, left of Fig. 2B. This two state model can be described by the allosteric equation described in the legend of Fig. 3. A modification of the energy barrier between different states, is characterized by a leftward shift of the concentration–response curve, a modification of the slope of the curve and a modification of the fraction of receptors that can be stabilized in the active state (proportional to the steady-state current when observing physiological responses) (see Fig. 3B). As the isomerization coefficient L decreases, an important phenotype emerges. Namely, the fraction of receptors in the A state in absence of ligand is inversely proportional to the L value. This implies that for L values in the range of 10, 10% of receptors should be in the active A state in absence of ligand. The arrow in Fig. 3B points to the intercept of the concentration–response curve and demonstrates that for low L values a fraction of receptors can be open in absence of ligand. Despite presenting a very low probability of opening, spontaneously openings have been observed at the neuromuscular junction receptors. It should be noted that the effects of an allosteric effector should affect not only the natural ligand, but also the action of any compound that interacts with the receptor. This implies that the pharmacology of the receptor can be significantly altered in the presence of

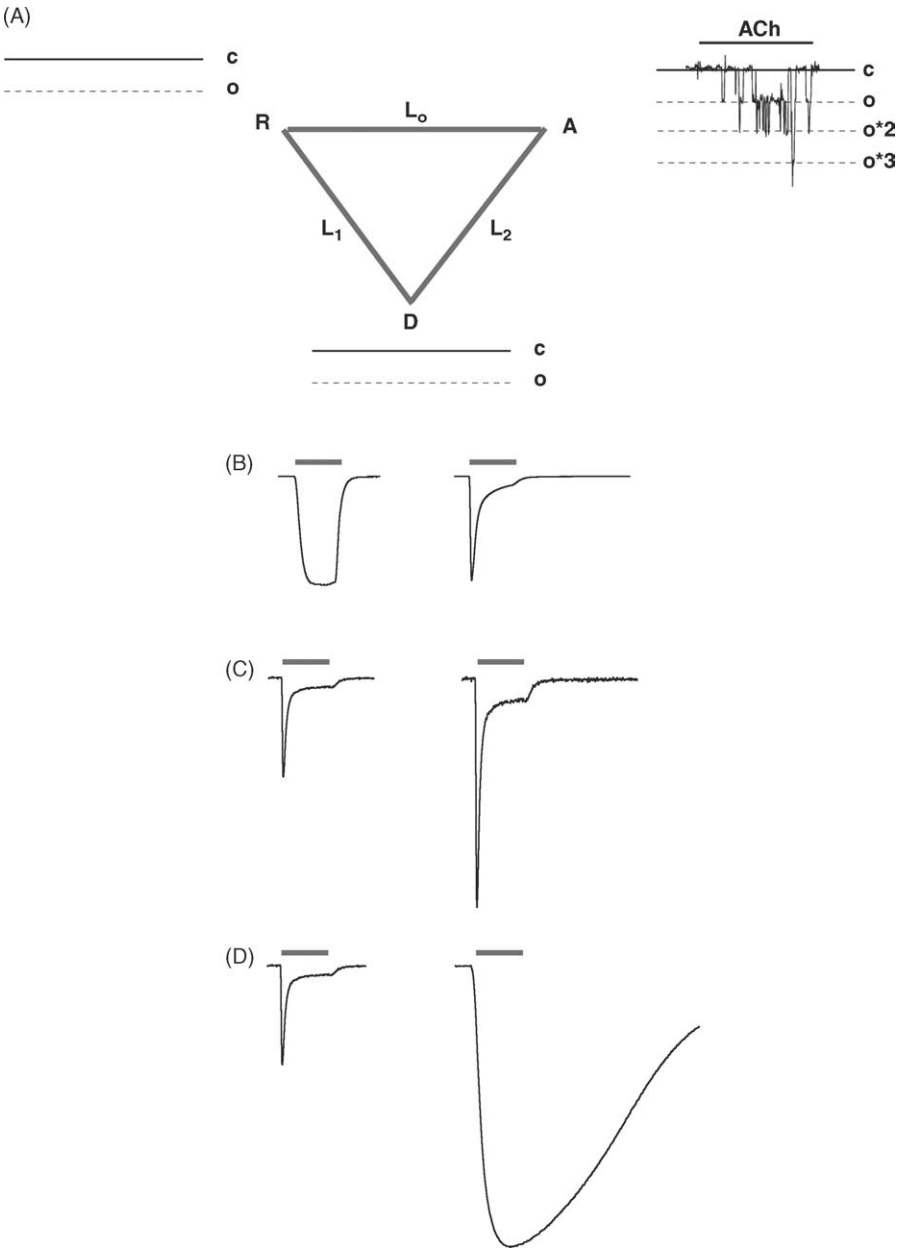

Fig. 2. Three state model and typical agonist evoked responses in the absence or presence of an allosteric effector. (A) The minimal three state model that can account for the properties of a LGIC. R is the resting closed state, A is the active open state and D is the desensitized closed state. L_0, L_1, L_2 are the isomerization coefficients that describe the energy barrier for the transition between the different states. Lines and dashed line indicate a closed and open state that would be recorded in single channel activity. A typical trace of a recording from a membrane patch containing at least three channels is shown for the active state. The bottom panel illustrates typical currents that would be recorded when a ligand-gated channel is activated. (B) Left trace illustrates the current that would be recorded if the channel does not desensitize. This typical current was recorded in an oocyte expressing the α7-L247T mutant. Currents displaying comparable characteristics can be recorded for slow desensitizing α4β2 nAChR, $GABA_A$ or NMDA receptors at low agonist concentrations. Right trace illustrates a typical current for a receptor that displays a marked desensitization (this current was recorded in an oocyte expressing the human α7 nAChR). Note the peak and plateau phase of the response observed during a constant agonist exposure (illustrated by the bar). (C) Effect of an allosteric modulator that changes the current amplitude but does not modify the response time course (e.g. 5-hydroxyindole at the α7 nAChR). (D) Effect of an allosteric modulator that changes both the amplitude and time course of the evoked current. This current is a typical example of the effect of an allosteric effector such as [1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea] on a cell expressing the human α7 nAChR. Note that comparable or even stronger effects would be observed for cyclothiazide effects at the AMPA receptor.

an allosteric effector. Similar observations were reported for the GPCRs and have been thoroughly analyzed in mathematical models [14].

However, many receptors show a decrease in the amplitude of the response in the continued presence of an agonist (Fig. 2B, right). This phenomenon is called ‘‘desensitization’’ and receptors in this state cannot be activated by subsequent agonist application. During prolonged agonist exposure the receptor progressively desensitizes, this can be accounted for by a transition into the desensitized (D)

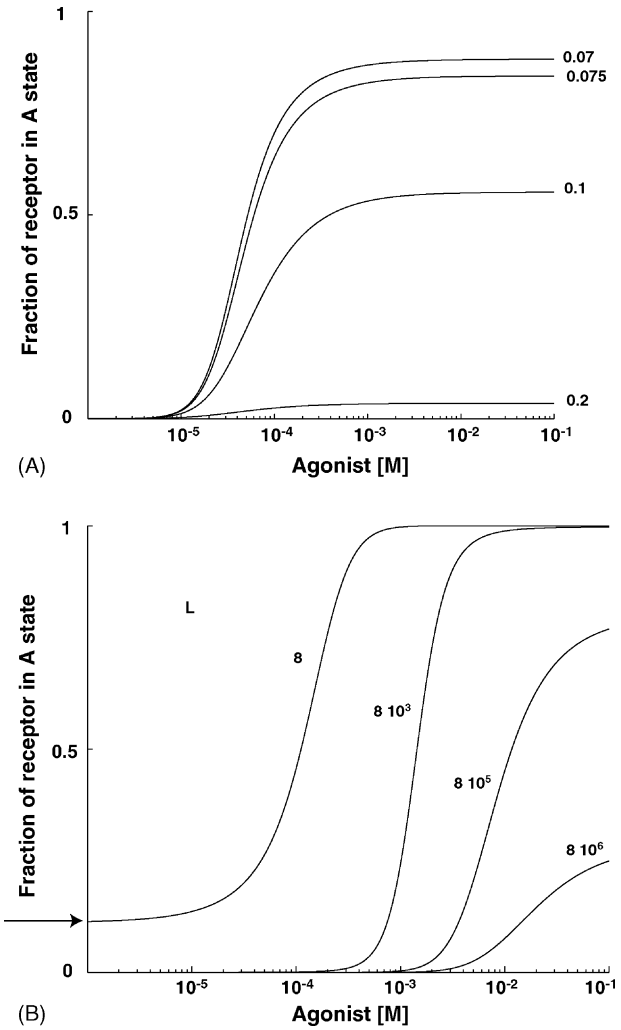

Fig. 3. Effects of allosteric effectors on the agonist concentration-response curve. (A) The K phenotype is characterized by a modification of the amplitude of the agonist-evoked dose-response with minimal modification of the apparent affinity and slope. (B) The L phenotype is characterized by a modification of the amplitude of evoked currents, a displacement of the half activation (EC_{50}) and a modification of the slope of the dose-response curve. Curves were computed using the allosteric equation for a two state model with $Y = 1/[1 + L(1 + C\alpha/(1 + \alpha))^n]$, where Y is the fraction of receptor in the A state, L the isomerization coefficient or equilibrium constant between the R and A state in absence of ligand $L = [R]/[A]$, C is the ratio of the dissociation constants (K_A/K_R), α is the concentration of ligand (M) and n is the number of binding sites. Computations were done using an $n = 5$. Curves plotted in (A) were obtained with an $L\,8 \times 10^4$ of, whereas curves plotted in (B) were obtained with a C value = 0.05. The arrow indicate that for low L coefficient a fraction of the receptors will be observed in the A state even in absence of ligand.

state (Fig. 2A). While more complex schemes can be proposed to simulate specific receptor properties, this basic three state model is well suited to discuss the effects of allosteric effectors.

Based on functional analysis of neuronal nicotinic acetylcholine receptors that contained mutations, Galzi et al. [15] proposed three possible mechanisms to describe the observed phenotypes. Structural changes can affect the affinity receptor for the ligand (K-phenotype), the transition energy barrier between states (L-phenotype) and the conductance (γ-phenotype). In a comparable manner, effects of allosteric effectors could be classified according to these definitions. Modeling of the function of LGIC with their multiple ligand-binding pockets and multiple configuration states is problematic. The corresponding transition scheme must be represented by a set of differential equations, containing a number of parameters proportional to the number of transitions (see for example [16–18]). Moreover, currently available techniques do not provide the ability to distinguish between multiple configurations. For example electrophysiological data do not permit to differentiate between multiple open or multiple closed states. To better understand the mechanisms of action of allosteric effectors there is a need for combined experimental techniques which would provide information on parameters such as, response time-course, ligand affinity, receptor configuration, etc.

Positive allosteric effectors can enhance the response amplitude with or without changes in the time-course of the response. Considering the three-state model, binding of an allosteric effector can affect either a single transition step, as described above, or simultaneously multiple transitions. Moreover, it can be predicted that modification of the A–D transition (Fig. 2A) should result in a modification of the response time course. One of the best examples of this is the change in the response kinetics of AMPA receptors caused by cyclothiazide, LY392098 or LY404187 [19,20].

3. Allosteric modulation of P2X receptors

The presence of allosteric sites on P2X receptors has been demonstrated, for instance, extracellular calcium ions selectively modulate $P2X_3$ receptors on rat dorsal root ganglion neurons, this was inhibited by magnesium [21]. Moreover, it was confirmed that modification of the intracellular calcium concentration had no effect, indicating that calcium must bind to the extracellular domain of the $P2X_3$ receptors to exert its modulation.

Modulation by protons (extracellular pH) or copper ions (Cu^{2+}) was observed both for the naïve $P2X_2$ receptors expressed in outer hair cells of the inner ear and recombinant receptors expressed in *Xenopus* oocytes [21]. Lowering the pH enhanced the ATP-evoked current up to 450%, while increasing the pH reduced the amplitude of the response. While copper also enhances responses of the $P2X_2$ receptors, albeit by a smaller degree than pH, these divalent ions have also been shown to inhibit $P2X_4$ receptors expressed in *Xenopus* oocytes [22]. The specificity of modulation of different receptor subtypes is further illustrated by the lack of effect of zinc at the $P2X_2$ receptors [23] whereas increasing the concentration of these divalent cations in the micromolar range enhances the function of $P2X_4$ receptors [22]. Cibacron blue, an antagonist of ATP at recombinant $P2X_1$ and $P2X_2$ receptors [24] has also been reported to be a positive allosteric modulator of

recombinant $P2X_3$ receptors [25]. Ivermectin is also a positive allosteric effector of the gating and kinetics of $P2X_4$ and $P2X_{4/6}$ channels [26]. Although none of these presently identified molecules are suitable therapeutics, they illustrate the ubiquitous nature of allosteric modulation of LGICs. Moreover, molecules such as ivermectin and cibacron blue may represent pharmacophores for structure-based design of novel allosteric ligands.

4. Allosteric modulation of glutamate receptors

Glutamate is the principal excitatory neurotransmitter in the CNS. Structure function studies and crystallography of the related bacterial glutamate-binding protein have revealed that the glutamate-binding site is formed by two regions of the extracellular domain joined by a flexible hinge region, which allows the structure to open and close in a manner akin to a clam shell. The agonist-binding site is located between these two regions and the agonist molecule stabilizes this binding site in a ''closed'' configuration, which corresponds to the conducting state of the receptor. Glutamate receptors are involved in long-term synaptic plasticity, which underlies learning and memory. NMDA receptors are cation permeable LGICs, expressed throughout the CNS and are critical for normal brain functioning. NMDA receptors have a complex activation profile. In the presence of high concentrations of glutamate, which is released from presynaptic nerve terminals, NMDA receptors have an extremely low probability of opening (approximately 10%). However, the amino acid glycine, which is present in cerebrospinal-fluid in the low micromolar range, acts as a positive allosteric modulator and, when the two molecules are bound on the receptor, the channel can open. In addition, the membrane must be depolarized to prevent the Mg^{2+} block.

The binding sites for these two molecules are located on different subunits and although the binding of the two molecules greatly increases the probability of channel opening, the presence of one agonist has a negative allosteric effect on the binding affinity of the other [27]. This is seen as a rapid activation of the receptor followed by an apparent receptor desensitization as one of the molecules dissociates from the complex [28,29]. NMDA receptors are also modulated by negative allosteric modulators, such as Zn^{2+} and ifenprodil, but important differences are seen in the sensitivity of different NMDA receptor subtypes to these modulators. The binding site for Zn^{2+} is located on the N-terminal domain of the NR2A subunit and the ifenprodil-binding site to the same domain of the NR2B subunit [30,31].

AMPA receptors display at least two distinct allosteric-binding sites, namely the cyclothiazide- and the 2,3-benzodiazepine-sites. The concept of positive and negative allosteric modulation of AMPA receptors by 2,3-benzodiazepine compounds is illustrated by the capability of LY303070 (GYKI 53784) to inhibit receptor function while exposure to LY404187 induces a strong potentiation of currents with a modification of the time course of recovery from desensitization [19,32].

5. Allosteric modulation of nicotinic acetylcholine receptors

nAChRs are a prototype of allosteric proteins (see for example [15,33]). Several studies have shown that the fast desensitizing homomeric α7 receptor typically illustrates the functioning of a LGIC and its sensitivity to different allosteric effectors. According to the allosteric model, these receptors have a high L_0 coefficient and therefore should be highly sensitive to positive allosteric effectors. In agreement with this prediction it has been shown that ACh-evoked currents at these receptors are potentiated by positive allosteric effectors such as divalent cations, steroid hormones, ivermectin, 5-hydroxyindole, cocaine methiodide, PNU-120596, etc. [34–38]. There is still a debate over the mechanism of action of many cholinergic drugs, a good example of this is the anticholinesterase galantamine. In addition to its anticholinesterase activity, the plant alkaloid galantamine is also a positive allosteric modulator of human α7 and α4β2 nAChRs [39,40]. Its successful use in the treatment of neurodegenerative disorders may lead the way for the development of new selective allosteric modulators [41,42].

6. Steroid modulation of nAChRs

A number of natural steroids, which are synthesized in the brain, are allosteric modulators of nAChRs, and probably represent an important physiological mechanism by which nAChR function is regulated in the brain. The effects of steroids at nAChRs can be either positive or negative. Many of these effects are specific for a particular receptor subtype for example, it was shown that progesterone acts as negative allosteric effector at α4β2 and α7 receptor subtypes [43] whereas 17β-estradiol acts as positive effector only at the α4β2 subtype [36,44]. The construction and expression of chimeric DNAs and mutation of residues demonstrated that the nature and position of the final four residues in the C-terminal was critical for modulation. Extending the terminal sequence by inserting residues also abolished potentiation [36]. The 17β-estradiol-binding site on the C-terminus is found to be distinct from the progesterone-binding site on the same receptor [36]. Hydrocortisone was shown to shorten the channel open time of the neonatal and adult neuromuscular junction nAChR [45,46], and dexamethasonne and corticosterone inhibit ACh-evoked $86Rb^+$ via α3β4 and muscle type receptors [47].

7. Modulation of nAChRs by protein kinases

Phosphorylation is an important mechanism in the regulation of many LGICs [48,49]. In addition to modulating receptor expression and subcellular localization, phosphorylation can also modulate channel properties such as desensitization and recovery from inactivation [50].

8. Endogenous modulators of nAChRs

Lynx-1 is an endogenous peptide isolated from the mammalian CNS, which has a "three-fingered" structure, analogous to the snake α-neurotoxins [51]. Lynx-1 forms stable complexes with α7 and α4β2 nAChRs potentiating ACh-evoked currents and increasing the rate of desensitization in vitro [51,52]. Recently, an endogenous secreted peptide, secreted mammalian ly6-uPAR related Protein 1 (SLURP-1), which shares a large degree of homology with Lynx-1, has been demonstrated to be a potent positive allosteric modulator of α7 nAChRs [53]. In humans, SLURP-1 is found in the blood and urine and is secreted from cervix, gums, stomach and esophagus with the highest concentrations found in keratinocytes of the epidermis [54].

The discovery of these peptides, with a high structural homology to snake α-neurotoxins, in humans raises the question of their role as endogenous allosteric modulators of LGIC function in vivo. In addition, non-peptidic modulation of nAChRs by serotonin has been reported [55].

9. Positive and negative modulation of $GABA_A$ receptors

$GABA_A$ receptors share a structural homology with the four transmembrane nAChRs, $5HT_3$, glycine and Zn^{2+} receptors. The degree of homology is such that modeling of the extracellular domain and ligand-binding site is now based on the crystal structure of the ACh-binding protein (AChBP) [56]. Several molecules that enhance the activity of these channels are in clinical use, the best known are the benzodiazepines [57–60]. Important progress, made in part with recombinant receptors and site directed mutagenesis experiments, have allowed the mapping of the benzodiazepine-binding site at the amino acid level [61]. These studies have elegantly shown that benzodiazepines bind at the interface between the α1 and γ2 subunits in the receptor complex and thereby explain, why only receptors containing the γ subunit are modulated by this class of allosteric effector. Binding of different ligands to this site can either potentiate the $GABA_A$ receptors or block the benzodiazepine effect (e.g. flumazenil = Ro 15-1788 is an antagonist of the benzodiazepine-binding site). A first study using cysteine-reactive compounds recently confirmed the contribution of certain amino acids to the benzodiazepine-binding site. This technique opens new possibilities for structure function studies of allosteric effectors based on the concept of cysteine scanning which allows to map-binding sites at any location on the receptor [62].

Many allosteric-binding sites have been identified on the $GABA_A$ receptor (see for example [63,64]) highlighting one of the important advantages of the search for allosteric effectors as compared to conventional agonists and antagonists. This multiplicity of potential-binding sites greatly increases the probability of finding a molecule that is selective for a particular receptor, however, the binding of an allosteric modulator to diverse sites on a receptor can be difficult to detect if using screening techniques which detect changes in the binding of labeled ligands. The increasing availability of high throughput screening on receptor function, such as FLIPR and automated electrophysiology systems is making the identification of these molecules easier.

These reports illustrate that specific protein residues must be present for each individual allosteric effector to bind and exert its action. For example, the differential sensitivity to neurosteroids of different $GABA_A$ receptor complexes highlights the role of the δ subunit. Use of microchimeras made between the $GABA_A$-ρ1 subunit and the related glycine-α1 subunit revealed the importance of the C-terminal end of this subunit for the receptor modulation by α-THDOC but not by allopregnanolone [65]. It is interesting to note the similar importance of the C-terminal end of the α4 nAChR subunit for the modulation by the steroid 17β-oestradiol [36,44]. In addition to its activity as a positive allosteric modulator at the α7 nAChR, ivermectin also has effects on GABA receptors, Williams and Risley [66] reported that ivermectin increases binding of benzodiazepines and benzodiazepine antagonists to rat cortical and cerebellar membrane preparations. These effects of ivermectin could be enhanced by bicuculline and inhibited by GABA suggesting the existence of an allosteric-binding site which is distinct from the GABA and benzodazepine sites. Ivermectin also potentiated GABA-activated currents in rat cortical neurons [67], mouse hippocampal neurons [68], and at recombinant human α1β1γ2, α1β2γ2, and α1β3γ2 $GABA_A$ receptors expressed in *Xenopus* oocytes.

10. Importance of allosteric modulators as therapeutics

While it would go beyond the scope of the current work to attempt to list all the possible therapeutic targets for allosteric effectors, a few examples of negative or positive allosteric effectors are discussed below. Searches of current literature and patents reveal that several pharmaceutical companies are developing positive and negative allosteric effectors for different LGIC families. In such

a quest, we shall keep in mind that screening strategies will determine, right from the beginning, the type of allosteric modulators that could be identified. Namely, protocols that use short-term agonist exposure will help to characterize non-competitive antagonists and/or positive allosteric modulators of resting and open states. While protocols based on long-term agonist exposure could lead to the identification of allosteric modulators of some desensitized states. Typical examples of allosteric effectors include negative allosteric effectors of the NMDA receptors such as ifenprodil or compounds which act similarly. The therapeutic target is to reduce the glutamate cytotoxicity observed following cerebrovascular injuries. By contrast with competitive antagonists that, at saturating concentrations, could totally inhibit LGIC functions, negative allosteric modulators are fine-tuning tools that could have a neutral behavior in normal physiological conditions but that could be very active in pathophysiological situations, without leading to complete receptor inhibition. This concept is illustrated by the NMDA and nicotinic open-channel blocker memantine. Inhibition of the receptors by memantine is much stronger when the receptors are over-activated.

But why should allosteric effectors be more suitable as drugs for blocking LGICs than competitive or non-competitive inhibitors? A first distinction between these classes of molecules is that a larger repertoire of allosteric effectors is expected than for the other inhibitors. The reason for this difference is that the binding of allosteric effectors is not restricted to the ligand-binding site or the ion-conducting pore, as in the case of competitive or open channel blockers. This advantage should allow the design of compounds that are more specifically targeted to particular receptor subtypes. In addition, it should be remembered that binding of a negative allosteric effector which affects the isomerization coefficient causes both a reduction of the receptor agonist sensitivity and activity. Positive outcomes of these advantages have already been observed and use of negative allosteric effectors of neuronal nicotinic receptors have been proposed for smoking cessation while negative effectors acting at the $GABA_A$ or $5HT_3$ receptors have been proposed for reduction of alcohol dependence. Wide spectrums of actions ranging from pain, to epilepsy to schizophrenia, etc., have been proposed for neurosteroids that increase or reduce $GABA_A$ receptor activity.

Positive allosteric effectors also show promise as therapeutical compounds. While benzodiazepines and their broad clinical use have already paved the way of positive allosteric effectors, newcomers include neuronal nicotinic acetylcholine modulators such as galantamine. The positive clinical outcomes reported in the treatment of neurodegenerative cholinergic disorders such as Alzheimer's is attributed, at least in part, to the allosteric effects of galantamine. Allosteric effectors showing subunit specificity for the neuronal nicotinic receptors such as (2-amino-5-keto)thiazole developed by Lilly or [1-(5-chloro-2,4-dimethoxy-phenyl)-3-(5-methyl-isoxazol-3-yl)-urea] developed by Pfizer are first examples of a wide range of molecules that will retain our attention in the future. Positive allosteric effectors of the AMPA receptors such as LY404187 have been proposed for the treatment of Parkinson's disease [69]. Alternatively, talampanel (a negative allosteric modulator of AMPA receptors) was developed to treat some epilepsies but is also assayed for protection of brain injuries, and therapeutic for Parkinsons disease. On the opposite positive allosteric effectors of the AMPA such as Org 24448 have been proposed to treat schizophrenia.

In view of the very broad range of allosteric effector applications an important future can be seen for these types of molecules that should provide additional benefits to the already know spectrum of compounds that are targeting LGICs.

Comparison of allosteric modulator effects versus agonists or antagonists reveals differences that are determinant for drug design (reviewed in [13,14]). Orthosteric agonists provoke sustained activation of the receptors even in the absence of a physiological activity of the corresponding neuronal network. For calcium permeable channels, such as NMDA or the nicotinic $\alpha 7$ receptors such activation can result in cytotoxic effects. In addition, sustained exposure can cause receptor desensitization that may result in the opposite of the desired effects. Thus, administration of orthosteric agonists needs very precise control of dosage and pharmacokinetic monitoring.

Use of orthosteric antagonists can lead, as a function of the drug concentration, to an insurmountable blockade of the receptors and therefore complete inhibition of the physiological response. Use of open channel blockers is associated with a use dependent effect that can also be insurmountable. Moreover, open channel blockers have a poor selectivity and are difficult to target to a precise receptor subtype.

In contrast, the effects of an allosteric effector are limited by the nature of the receptor modulation. Once all the effector-binding sites are saturated the receptor is maximally modulated and presence of a higher concentration of the modulator will not result in further effects. This ceiling effect has important advantages because it offers a much larger safety margin in drug administration and patient compliance. Overall, allosteric modulators offer several advantages over classic orthosteric compounds or open channel blockers and can be expected to have a bright future in drug discovery.

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