Allosteric Receptor Modulation in Drug Targeting

edited by
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Preface

Allosteric modulation of receptors for endogenous ligands has, in recent years, attracted considerable interest from the drug research community. There may be a number of reasons for this but not least of these must surely be the success of the benzodiazepine class of anxiolytic/sedative drugs. These positive receptor modulators were first introduced at the end of the 1960s in response to the need for safer sedatives that would not produce the respiratory depression associated with the action of the existing barbiturate class of drugs. The benzodiazepines allosterically modulate the ionotropic GABA\textsubscript{A} receptor to enhance the action of the inhibitory transmitter GABA. In the absence of GABA the benzodiazepines are inert, which is a feature of allosteric drug action. The allosteric agent requires the presence of the receptor agonist in order to exert its effect. This characteristic can be of considerable benefit over directly acting receptor agonists that act at orthosteric sites. Excessive activation of the receptor system cannot occur because the maximal stimulation that can arise is dependent upon the concentration of the natural receptor ligand. This, in turn, is less likely to produce receptor desensitisation or tolerance as can occur with full receptor agonists.

So what does the term “allosteric modulator” mean? Neubig and colleagues (2003, Pharmacol Rev, 55, 597–606) have defined this term, under the auspices of the International Union of Pharmacology, as “a ligand that increases or decreases the action of an agonist or antagonist by combining with a distinct site on the receptor macromolecule.” Thus, in the example of the benzodiazepines there exist distinct receptors on the GABA\textsubscript{A} receptor macromolecule that on activation increase the maximal response to the agonist, GABA, as well as increasing the apparent sensitivity to it.

The concept of allostery in drug action was introduced more than 40 years ago (Monod et al. 1963;1965, J Mol Biol 6, 306–329, 12, 88–118) at a time when the structures of receptors were beginning to emerge. The original
focus of interest in allosterism was associated with ionotropic cholinergic receptors. This association then expanded to encompass metabotropic as well as other ionotropic receptors, such that there are now many examples to provide the potential for application to therapeutics. The obvious application is in agonist enhancement but negative modulation is also a possibility though examples of this are relatively uncommon.

This volume provides some examples of the receptors where allosteric activity has been defined including ionotropic \( \text{GABA}_A \), 5-HT3, glutamatergic and nicotinic receptors, as well as metabotropic G-protein–coupled receptors such as mGluR, muscarinic, \( \text{GABA}_B \), and \( \alpha \)-adrenoceptors. However, before considering these examples, it is important that the underlying features, principles, and characteristics of allosteric mechanisms are described. As a consequence, the first section comprises three contributions that provide these details as well as modelling allosteric action. Stephen Daniels, Terry Kenakin, and David Hall, all of whom are recognized experts in the principles of drug action, have prepared these chapters.

Among the many examples of allosteric modulation included in this volume are the benzodiazepines and the coverage given by Hanns Möhler in section 2 provides up-to-date information on their characteristics. Subsequent chapters in this section are focused on the other ionotropic receptors within the same structural superfamily. Section 3 is devoted to G-protein coupled receptors (GPCRs) and commences with an introductory chapter by Ad Ijzerman and colleagues on the general concept of allosterism at GPCRs. This is followed by comparative information on receptor examples. While the information is not exhaustive it is hoped that sufficient is provided to enable the reader to gain a clear understanding of their comparative features. There is no doubt that receptor allosterism is and will continue to become more important in the quest to find drug targets for a variety of diseases. Perhaps the material included in this volume will provide the background data required to facilitate this process.

Norman G. Bowery
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Cell surface receptors have long been a target for drug development with the intent to modulate receptor-mediated signalling to correct a pathophysiological state or provide symptomatic relief. However, traditional agonist, antagonist, or channel-blocking drugs are frequently associated with a high incidence of side effects and the development of tolerance and dependence. The binding site for the endogenous agonist is likely to be a highly conserved structural region, for a given class of receptor [e.g., the γ-aminobutyric acid type A receptors (GABA\textsubscript{A}-Rs)] and therefore unlikely to allow great selectivity, in agonist or antagonist activity, between different subtypes of a receptor class. This is probably one of the reasons for the difficulty in establishing a clinical role for glutamate receptor antagonists (e.g., CPPene) for the treatment of various neurological disorders (e.g., epilepsy, stroke), where despite pharmacological efficacy, the side effect profile precludes their use (1). Similarly, the ion channel associated with the ionotropic receptors is also likely to be highly conserved between different subtypes of a particular receptor class. This again offers little opportunity for selectivity and the likelihood of an unacceptable side effect profile.
THE ADVANTAGES OF ALLOSTERIC COMPARED TO ORTHOSTERIC MODULATION

As an alternative, drugs that act at a site spatially distinct from that at which the endogenous agonist acts, an allosteric as opposed to an orthosteric site, may overcome these problems. The benzodiazepines, which enhance the activity of GABA<sub>A</sub> receptors, are a classic example of such allosteric modulators (2). Allosteric modulators offer a number of advantages over conventional agonist or antagonist drugs.

First, they frequently exhibit little or no intrinsic activity since their mode of action is to enhance or inhibit the action of the endogenous agonist. In this respect, they only elicit an effect in the tissue(s) stimulated by the endogenous agonist; an effect which is also in synchrony with the frequency of the physiological stimulation. In principle, this should reduce the likelihood of the target receptor desensitizing, even in the continued presence of the allosteric modulator, thus removing one of the mechanisms for acquired tolerance.

Second, the action of an allosteric modulator is saturable; i.e., once the allosteric sites are fully occupied, no further allosteric effect is observed (3). Allosteric drugs should, therefore, be safer under conditions of overdose than conventional orthosteric drugs.

The third significant attraction of allosteric drugs is the possibility that an effective allosteric site could be found which is specific to one subtype of a receptor class because they can be targeted at nonconserved sites. Thus, benzodiazepines act as positive (diazepam, flunitrazepam) or negative (flumazenil) allosteric modulators at the α<sub>1,2,3,5βγ2</sub> but are without effect at α<sub>4,6βγ2</sub> subtypes of the GABA<sub>A</sub> receptor (2). In addition to allosteric site definition, it is possible that drugs may bind to an allosteric site but fail to express any cooperative effect (either positive or negative). This is exemplified by the action of N-chloromethyl brucine at muscarinic acetylcholine receptors (mACh-Rs); it is a positive allosteric modulator at M<sub>3</sub>, a negative allosteric modulator at M<sub>2</sub>, and is without effect at M<sub>1</sub> or M<sub>4</sub> subtypes of the mACh receptor, despite demonstrating equivalent binding affinity (4). N-chloromethyl brucine has been termed a “neutral” allosteric modulator at the M<sub>1,4</sub> subtypes of the mACh receptor.

Finally, allosteric modulators may be effective at various peptide and hormone G-protein coupled receptors in which the topographical arrangement of the orthosteric site makes it difficult for a small molecule to mimic the endogenous ligand (5).

THE DETECTION OF ALLOSTERIC FUNCTION

Until recently the recognition of allosteric modulators in drug discovery programs has been hampered by the almost universal use of equilibrium
radioligand binding assays in high-throughput screening systems. Although an allosteric modulator may alter binding at the orthosteric site (6,7), the use of an inappropriately high concentration of the orthosteric ligand may mask the allosteric effect or, in the case of negative allosteric modulators, may cause the allosteric interaction to resemble antagonism (3). As an alternative to equilibrium binding assays it is possible to use radiolabeled techniques to measure the rate of association/dissociation of the orthosteric ligand. Such measurements are frequently more successful at detecting allosteric interactions than equilibrium binding methods (8).

However, functional assays are far more likely to reliably detect allosteric interactions and, with the advent of a variety of different techniques (reporter systems, yeast and melanophore systems, fluorescence-based intracellular calcium measurements), are becoming adapted for high-throughput screening. Such methods are clearly capable of demonstrating allosteric interactions when nonequilibrium radiolabeled binding methods fail (9). Nevertheless, there can be difficulties, even with functional assays, in defining the receptor selectivity of an allosteric effect. The endogenous fatty acid, oleamide, can activate 5-hydroxytryptamine receptors type 7 (5-HT\textsubscript{7}), expressed in HeLa cells, in the absence of the orthosteric agonist 5-HT via an allosteric mechanism (10,11). However, the 5-HT\textsubscript{7} selective antagonist, clozapine, failed to inhibit the oleamide effect, although the oleamide-induced signalling was not seen in cells not transfected with 5-HT\textsubscript{7} receptors.

It would seem therefore that to maximize the likelihood of detecting allosteric effects, including inverse agonism, a functional assay is necessary. There are disadvantages, including difficulties in ascribing effects to a specific receptor or the activation of nonreceptor-mediated signalling, but these may be offset by the judicious use of radiolabeled binding methods as secondary screens (3).

**ALLOSTERIC MODULATION OF IONOTROPIC RECEPTORS**

**Anxiolytics**

The GABA\textsubscript{A} receptor has binding sites for many neuroactive substances including barbiturates, benzodiazepines, convulsants, general anesthetics, and neurosteroids. Of these, the benzodiazepines have been recognized as classic allosteric modulators and they have been in widespread clinical use since the early 1960s as anxiolytics and to treat insomnia. There is a wide spectrum of benzodiazepines including full agonists with varying pharmacodynamic and pharmacokinetic properties (e.g., diazepam, flunitrazepam, lorazepam), partial agonists (e.g., bretazenil, imidazenil), inverse agonists (e.g., methyl-6,7-dimethoxy-4-ethyl-\beta-carboline-3-carboxylate) and partial inverse agonists (e.g., N-methyl-\beta-carboline-3-carboxamide) that are anxiogenic and either convulsant or proconvulsant and full antagonists.
(e.g., flumazenil) that are without effect (12–14). It is now known that the benzodiazepines bind to the GABA<sub>A</sub> receptor and increase the number of channel openings when GABA is bound (full/partial agonists), which causes an increased chloride flux into the cell that results in a hyperpolarization of the resting membrane potential and hence a reduced likelihood of triggering an action potential (15,16). Conversely, the inverse agonists decrease the number of channel openings when GABA is bound, depolarizing the cell and increasing excitability. The antagonists have no effect on channel opening and thus do not affect the resting membrane potential. Despite the widespread clinical use of the benzodiazepines it became clear that they suffer a number of drawbacks. Acutely, they induce sedation and cognitive dysfunction and chronically they produce tolerance and both physical and psychological dependence with patients suffering severe withdrawal effects (14). In consequence their use has declined in recent years and there is a general guidance that their use should be restricted to the short-term (less than 4 weeks).

It was hoped that the partial agonists would provide the anxiolytic effect without the sedation and dependence associated with chronic use of the full agonists. This, however, is not the case. There has therefore been much recent interest in establishing exactly where the different benzodiazepines bind and whether it would be possible to develop subtype selective agents that would have anxiolytic properties but without the sedation and dependence. Recent experiments using molecular genetic techniques have begun to establish the benzodiazepine binding sites and to dissect the GABA<sub>A</sub> receptor subunits involved in sedation, anxiety, amnesia, and convulsive activity (2,13,17). The classic benzodiazepines (diazepam, flunitrazepam) bind to GABA<sub>A</sub> receptors that comprise α<sub>1</sub>, α<sub>2</sub>, α<sub>3</sub>, or α<sub>5</sub> subunits in combination with any β and γ<sub>2</sub> subunits. This receptor population accounts for approximately three-quarters of the total GABA<sub>A</sub> receptor population (13). GABA<sub>A</sub> receptors containing α<sub>4</sub> or α<sub>6</sub> subunits are insensitive to benzodiazepines.

Mice having GABA<sub>A</sub> receptors containing α<sub>1</sub> subunits that have been rendered insensitive to diazepam (by site-directed mutagenesis of a histidine residue for an argentine in the α subunit) show little sedation, and this was shown to be specific to ligands binding at the benzodiazepine site because barbiturates and neurosteroids were still as effective as in wild type mice (2). In similar experiments, the α<sub>1</sub> subunit was shown to be associated with anterograde amnesia, a significant side effect of benzodiazepines. The anticonvulsant activity of diazepam was tested against pentylenetetrazole-induced seizures in mice expressing benzodiazepine insensitive α<sub>1</sub> subunits, and it was shown that the anticonvulsant properties of diazepam are only partially expressed through the α<sub>1</sub> containing GABA<sub>A</sub> receptors. In contrast, the anticonvulsant properties of the α<sub>1</sub>-selective imidazopyridine, zolpidem, are wholly mediated through its α<sub>1</sub>/γ<sub>2</sub> binding (2).

The α<sub>5</sub> subunit appears to regulate cognitive processes, (13). There are data that suggest that the α<sub>2</sub> subunit regulates anxiety (2); however, more
recently, Atack et al. (2) have provided evidence that the α3 subunit also mediates anxiety. At present there are no satisfactory α2 and α3 selective ligands which will restrict further progress towards achieving the target of a benzodiazepine which does not cause sedation, impair cognitive processes, and induce dependence. It should be noted that approximately 25% of GABA_A receptors contain mixed α subunit types (e.g., α1α6βγ2, α1α3βγ2) and these receptors will display pharmacology which reflects the binding characteristics of each α subunit. This may make the search for the ideal benzodiazepine ligand more difficult.

**General Anesthetics**

The search for a molecular mechanism that would explain how general anesthetics induce a reversible loss of consciousness has lasted over 100 years. Despite considerable progress in recent years, in line with the general increase in understanding the regulation of cellular signalling through the combined application of functional studies with molecular biological and genetic techniques, we are still far from being able to define the essential pharmacology required to induce anesthesia. Thus, even the “best” modern anesthetics in clinical use (sevoflurane, desflurane, propofol) coupled with “best practice” in modern anesthesia still have the capacity to induce a fatal overdose with doses only three to four times greater than that necessary for anesthesia.

General anesthetics are capable of acting as allosteric modulators of all members of the cys-loop group of ionotrophic receptors nicotinic acetylcholine (nACh), 5-HT_3, GABA_A, glycine). However, their actions are not always consistent nor necessarily linked to their potency as general anesthetics.

Thus, neuronal α7 and muscle αβγδ nACh receptors, which are inhibited by thiopentone, do not differentiate in potency between the optical isomers of thiopentone, and the neuronal α4β2 receptor shows the opposite potency ratio between the optical isomers compared to that observed for anesthetic potency (18). Volatile anesthetics potentiate heteromeric neuronal nACh receptors (e.g., α3βδ) at concentrations much less than those which produce general anesthesia (18,19), while neuromuscular junction (αβγδ) and neuronal α7 receptors are relatively unaffected by volatile anesthetics. Although the exact mechanism by which volatile anesthetics modulate heteromeric neuronal nicotinic receptors, the extracellular loop linking M2 and M3 transmembrane segments appears vital (20). Heteromeric neuronal nACh receptors may, therefore, play a part in regulating via an allosteric mechanism the action of volatile, but not intravenous, anesthetics.

Volatile anesthetics potentiate currents recorded from recombinant 3-HT_3 receptors whereas many intravenous anesthetics inhibit 5-HT-stimulated [C14]guanidinium influx into NIE/115 neuroblastoma cells (21). This latter effect may arise from a noncompetitive antagonism rather than via an allosteric inhibition.
Perhaps of more relevance to general anesthesia are the actions of general anesthetics at $\text{GABA}_A$ and glycine receptors. The great majority of the $\text{GABA}_A$ receptor subtypes are potentiated by volatile anesthetics, and this activity correlates well with their anesthetic potency (22). The intravenous anesthetics (propofol, barbiturates, etomidate, and the anesthetic steroid 5α-pregnan-3α-ol-20-one) all potentiate $\text{GABA}_A$ receptors (23). Glycine receptors are also potentiated by barbiturates and propofol but not etomidate or 5α-pregnan-3α-ol-20-one (23). Glycine receptors are also potentiated by volatile anesthetics (24,25). The gaseous anesthetics xenon and nitrous oxide have no appreciable effect on $\text{GABA}_A$ receptors, but do potentiate glycine receptors (25–27).

The identification of a subset of $\text{GABA}_A$ receptors as being implicated in a mechanism linked to sedation led to an elegant set of experiments in which the action of etomidate, which is selective for receptors containing $\beta_2$ and $\beta_3$ subunits, was tested in genetically modified mice having etomidate-insensitive $\beta_2$ subunits (28). It was concluded that while the $\beta_3$ subunit was associated with etomidate’s anesthetic potency, the hypnotic and sedative properties were mediated via different mechanisms. The authors proposed that the “hypothesis of anesthesia producing a general global depression should be revised.” This certainly appears to be the case, and while the cys-loop ionotropic receptors are important targets for general anesthetics, allosteric interactions at these receptors are far from the complete story.

The ionotropic glutamate receptors N-methyl-D-aspartate (NMDA), $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainic acid (KA), named after their selective agonists, are in general insensitive to general anesthetics. Those that do have effect, such as ketamine and xenon, appear to do so through noncompetitive mechanisms rather than allosteric mechanisms (29,30).

It would seem that: simple gaseous anesthetics (xenon, nitrous oxide) act by allosteric potentiation of glycine receptors, and as noncompetitive antagonists of glutamate receptors; volatile anesthetics act as positive allosteric modulators of many subtypes of $\text{GABA}_A$ receptors, glycine receptors, 5-HT$_3$ receptors, and heteromeric neuronal nACh receptors; and intravenous anesthetics (propofol, barbiturates, steroids) act primarily as positive allosteric modulators at $\text{GABA}_A$ receptors. It is probable that these actions do not wholly define the anesthetic properties of these substances and that a more complex pattern of neuronal modification is involved. This awaits further experimental dissection but the necessary tools now appear to be available.

**Neurosteroids**

Neuroactive steroids are synthesized in the brain and act to regulate neuronal excitability via modulation of cell-surface receptors (31), in contrast to the classical genomic activity of steroids to regulate gene expression. The
3α-reduced metabolites of progesterone and deoxycorticosterone (e.g., 3α, 5α-tetrahydroprogesterone; 3α,5α-tetrahydrodeoxycorticosterone), pregnenolone sulfate, and dehydroepiandrosterone sulfate are positive allosteric modulators of specific neurotransmitter receptors, especially the GABA_A receptor. More recently, evidence has accumulated of negative modulation of a variety of other receptor types (Table 1). The concentration required for the negative modulation of many of these receptors is quite high, low micromolar concentrations rather than the nanomolar concentrations required for the positive modulation of GABA_A receptors. This raises questions as to the likelihood that these interactions have physiological significance.

In addition to any putative role as pharmaceuticals, where their similarity of action to the benzodiazepines suggests their side effect profile may be counterindicative, the neurosteroids are important endogenous modulators of physiological function. The fatigue associated with pregnancy, postmenstrual syndrome, and postnatal depression have all been linked to fluctuations in neurosteroid concentrations (31). Given this, there will be considerable interest in applying functional molecular biological and genetic

**Table 1** Allosteric Modulation by a Variety of Neuroactive Steroids of Ionotropic and Metabotropic Receptors

<table>
<thead>
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<th>Negative</th>
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<tbody>
<tr>
<td>GABA_A</td>
<td>3α,5α-THP</td>
<td>PS</td>
</tr>
<tr>
<td></td>
<td>3α,5β-THP</td>
<td>DHEA-S</td>
</tr>
<tr>
<td></td>
<td>3α,5α-THDOC</td>
<td></td>
</tr>
<tr>
<td>nACh</td>
<td>Progesterone</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>Progesterone</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>PS</td>
<td></td>
</tr>
<tr>
<td>5HT_3</td>
<td>Estradiol (17α and β)</td>
<td>Progesterone</td>
</tr>
<tr>
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</tr>
<tr>
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<td>3α,5α-THP</td>
<td></td>
</tr>
<tr>
<td>NMDA</td>
<td>PS</td>
<td>17β-Estradiol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pregnanolone-S</td>
</tr>
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<td>Pregnanolone hemisuccinate</td>
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<td>AMPA</td>
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<td>PS</td>
</tr>
<tr>
<td>KA</td>
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<td></td>
</tr>
<tr>
<td>Oxytocin</td>
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<td></td>
</tr>
<tr>
<td>Sigma type 1 (σ1)</td>
<td>DHEA-S</td>
<td>PS</td>
</tr>
</tbody>
</table>

*Abbreviations:* THP, tetrahydroprogesterone; PS, pregnenolone sulfate; DHEA-S, dehydroepiandrosterone sulfate; THDOC, tetrahydrodeoxycorticosterone.

*Source:* From Ref. 31.
techniques to carefully dissect the complex pathways neurosteroids affect and to analyze the details of their interaction with the various receptor targets so that more selective pharmaceuticals can be designed to take advantage of these therapeutic routes.

**Neurodegeneration**

Galantamine acts as a positive allosteric modulator of neuronal nACh receptors. It increases the probability of channel openings, enhancing the apparent potency, but not the efficacy, of nicotinic agonists, in activating various nACh receptor subtypes (32,33). Allosteric modulation of nACh receptors is not common for all cholinesterase inhibitors, for example, it is shown by neither donepezil nor rivastigmine (33). Although details of the allosteric mechanism await clarification, all possess a cationic nitrogen (at physiological pH) that is located at a fixed distance from a phenolic group (32,34). By positively modulating presynaptic nACh receptors, galantamine facilitates GABAergic and glutamatergic neurotransmission. It is only recently that the allosteric action of galantamine at nACh receptors has been associated with its therapeutic effectiveness in the treatment of Alzheimer’s disease (35,36). Alzheimer’s disease is a multifactorial disorder but it has been suggested that cognitive impairments in Alzheimer’s disease are the result of modification of cholinergic modulation of glutamatergic and GABAergic neurotransmission, especially in the hippocampus and frontal cortex (37,38). Developments in this field may lead to improved therapies for other degenerative neurological pathologies, such as Parkinson’s and Huntington’s diseases.

**Endogenous Allosteric Modulation**

Zinc (Zn\(^{2+}\)) is now well established as an endogenous allosteric modulator at postsynaptic receptors, including all the glutamate receptors (NMDA, AMPA, KA), the adenosine triphosphate-activated purinoceptors P2X family of receptors, the GABA\(_A\) receptors (39), and the glycine receptor (40). Zinc modulation is highly dependent on subunit composition (41), and whether the modulation is positive or negative may depend on the zinc concentration (40).

GABA\(_A\) receptor subtypes \(\alpha\beta\gamma\) (the overwhelming majority) are relatively insensitive to Zn\(^{2+}\) whereas the \(\alpha\beta, \alpha\beta\delta,\) and \(\alpha\beta\epsilon\) subtypes are highly sensitive to inhibition by Zn\(^{2+}\) at a concentration in the low micromolar range (41). This has led to the suggestion that the physiological role of zinc is not to modulate the postsynaptic GABA\(_A\) receptors responsible for neuronal inhibition, but to regulate the tonic inhibition provided by the extrasynaptic GABA\(_A\) receptors (41). One exception to this may be in individuals suffering absence epilepsy. Studies have revealed that these individuals express a mutated form of the \(\gamma_2\) subunit of the GABA\(_A\) receptor, which
renders it insensitive to benzodiazepines (42–45). It has been shown further that this mutation not only renders the receptor insensitive to benzodiazepines but makes it far more sensitive to inhibition by Zn$^{2+}$ (46). It was suggested that the altered inhibition of the receptor by zinc may lead to the abnormal spike-and-wave discharges characteristic of absence epilepsy. If this turns out to be correct, then a novel therapeutic strategy for absence epilepsy may involve an antagonist at the zinc binding site to prevent these abnormal neuronal discharges. If the targeting were to involve the mutated γ2 subunit, then the action should be highly selective, thus minimizing side effects.

In addition to zinc and the neurosteroids (see preceding text), other endogenous allosteric modulators include sodium, calcium, l-amino acids, glycine, amidated lipids (e.g., oleamide), peptides (e.g., 5-hydroxytryptamine moduline), and arachadonic acid. It has even been shown that classic neurotransmitters, 5-HT and histamine, modulate the nACh and NMDA receptors (3). Thus, while synthetic pharmaceuticals that act at allosteric sites are attractive therapeutic modulators of ionotropic receptors, it is clear that many of these receptors are allosterically modulated under normal physiological conditions by endogenous substances. This undoubtedly adds to the complexity that needs to be unraveled before a truly comprehensive picture of synaptic transmission mediated by ionotropic receptors emerges. However, it does suggest that there may be alternative, more subtle means available to modulate a particular neurotransmission process which avoids the need to target the receptor directly.

**ALLOSTERIC MODULATION OF METABOTROPIC RECEPTORS**

Metabotropic receptors, otherwise known as G-protein coupled receptors, represent the largest group of therapeutic targets. Most ligands act at the orthosteric site as agonists, competitive antagonists, or inverse agonists (to inhibit constitutive activity) (5). Individual metabotropic receptors can demonstrate enormous variation in effect through a number of mechanisms, including post-transcriptional mechanisms (splice variation, exon skipping, intron retention), receptor dimerization, multiple signalling pathways (different receptors activating the same G-protein, one receptor activating more than one G-protein, and one G-protein activating different signalling pathways, through different second messenger enzymes and ion channels), and G-protein subunit exchange (47). To add to this complexity, it is now known that metabotropic receptors may be regulated by endogenous allosteric mechanisms (5).

**Endogenous Allosteric Modulation of Metabotropic Receptors**

Sodium ions interact with a highly conserved aspartate residue in the cytoplasmic region of transmembrane segment 2 (TM2) of a number of
metabotropic receptors, including adrenoceptor 2A (\(\alpha_2A\)), dopamine receptors (\(D_2,4\)), adenosine receptors (\(A_1, A_2a, A_3\)), and neurotensin receptors (NTS1) (48–52). It is suggested that Na\(^+\) binding to the negatively charged aspartate changes the receptor conformation so as to decrease agonist binding affinity. This, in turn, inhibits G-protein activation. The more recent studies indicate that additional residues in the cytoplasmic regions of TM1, TM3, and TM7 may also be binding sites for Na\(^+\) (52,53).

As with the ionotropic receptors (see preceding text), zinc (Zn\(^{2+}\)) allosterically modulates metabotropic receptors, including dopamine (\(D_1,2\)), tachykinin (NK\(_3\)), melanocortin (MC\(_1\)), and adrenoceptor (\(\beta_2\)) receptors (51,54–59). Unlike sodium, zinc is stored in synaptic vesicles and co-released, in a calcium-dependent manner, with neurotransmitters, reaching synaptic concentrations of 300 \(\mu\)M (60). Thus, while Na\(^+\) provides a tonic inhibition to a wide variety of metabotropic receptors, Zn\(^{2+}\) can produce a spectrum of physiologically relevant effects. Allosteric sites for Zn\(^{2+}\) have been identified on intracellular loops linking transmembrane domains (\(\beta_2\)) as well as at cytoplasmic regions of transmembrane domains (MC\(_1\), NK\(_3\)).

The calcium-sensing receptor is potentiated by \(L\)-amino acids through binding sites located in the extracellular N-terminal region (61,62). Calcium itself potentiates GABA\(_B\) heterodimeric receptors, through binding at a site proximal to the GABA\(_B1\) orthosteric site (63). Oleic acid and oleamide potentiate 5-HT\(_{2,7}\) (10,64) receptors and 5-HT-moduline inhibits 5-HT\(_{1B/1D}\) receptors (65,66). Oleamide has been associated with the induction of sleep, and therefore manipulation of the 5-HT\(_{2,7}\) receptors through the oleamide binding site may offer novel therapeutics to overcome insomnia that would be free of the side effects associated with benzodiazepines. The 5-HT\(_{1B/1D}\) receptors regulate the release of 5-HT, and thus inhibiting these receptors would lead to an increase in serotonergic signalling. The 5-HT moduline binding site may therefore represent an opportunity for novel therapeutic manipulation of serotonergic signalling in the treatment of anxiety and depression.

**Synthetic Allosteric Modulators of Metabotropic Receptors**

The metabotropic receptors do not share an overall amino acid sequence identity. There exists a number of distinct topologies with N- and C-terminal regions of varying size and complexity, but all possess seven \(a\)-helical transmembrane domains. All, when activated by ligand binding, activate an associated G-protein that in turn initiates various intracellular signalling cascades and/or activates or inhibits ion channel function (47). The metabotropic receptor family is normally divided into three “superfamilies,” A, B, and C, based on amino acid sequence homology (Table 2). The endogenous agonists of the family A (“rhodopsin-like”) metabotropic receptors bind to a crevice formed by transmembrane domains 3, 5, 6, and 7 (e.g., rhodopsin, adrenoceptors) or occasionally to extracellular
regions of the receptor (e.g., glycoprotein hormone receptors). Family B metabotropic receptors bind peptides to both extracellular and transmembrane regions. Family C metabotropic receptors (e.g., glutamate, GABA) bind the endogenous agonists exclusively in the large N-terminal domain (5).

Many synthetic ligands for both families A and C metabotropic receptors have been developed that act to allosterically modulate these receptors.

### Allosteric Modulators of Family A Metabotropic Receptors

Allosteric modulators have been identified for many family A metabotropic receptors, including \( \alpha_{1A} \) and \( \alpha_{2A} \) adrenoceptors, adenosine receptors, chemokine receptors, dopamine receptors, serotonin receptors, and muscarinic receptors (5). The first mACH allosteric modulators (e.g., gallamine) were shown to inhibit cardiac mACH receptors. A wide range of structurally diverse compounds have since been shown to act allosterically. Although the most sensitive mACH receptor subtype to allosteric modulation is M2, no clearly subtype-specific allosteric modulator has been identified, probably due to the high degree of amino acid sequence identity between the five mACH subtypes. Most of the allosteric modulators, including the negative modulator gallamine and the positive modulator alcuronium, bind through a “common allosteric site” on the extracellular side of the orthosteric site. The extracellular loops linking the transmembrane domains (especially the second and third) are crucial to this site. More recently, specific amino acid residues in mACH-M2 receptors that are critical, but not exclusive, for alkane-bisammonium and caracurine V allosteric ligand binding have been identified (Tyr177 in the second extracellular loop and Thr423 in TM7).
Some allosteric ligands (e.g., KT5720 and various WIN62,577 analogs) do not act through the common allosteric site demonstrating that there are multiple allosteric sites on the muscarinic receptors.

**Allosteric Modulators of Family C Metabotropic Receptors**

A large number of potent allosteric inhibitors and potentiators of metabotropic glutamate receptors have been identified which are highly selective for specific subtypes (Table 3) (5). Additionally, potentiators and inhibitors have been identified for the calcium-sensing receptor and three allosteric potentiators have been identified for the heterodimeric GABA$_{B1/2}$ receptor.

The binding sites of allosteric inhibitors for glutamate receptors (CPCCOEt, BAY36–7620, EM-TBPC, R 214127, and MPEP) and allosteric

<table>
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<tr>
<td>mGluR1</td>
<td>Ro 01–6128</td>
<td>CPCCOEt</td>
</tr>
<tr>
<td></td>
<td>Ro 67–7476</td>
<td>PHCCC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BAY 36–7620</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EM-TBPC</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R 214127</td>
</tr>
<tr>
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<td>GSK compounds</td>
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<td></td>
<td>NPS 2390</td>
</tr>
<tr>
<td>mGluR2</td>
<td>LY 487379</td>
<td>Ro 64–5229</td>
</tr>
<tr>
<td>mGluR4</td>
<td>MPEP</td>
<td>Ro 01–6128</td>
</tr>
<tr>
<td>mGluR5</td>
<td>DF3</td>
<td>Ro 67–7476</td>
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<tr>
<td>Calcium receptor</td>
<td>NPS R-467</td>
<td>PHCCC</td>
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<td>MPEP</td>
</tr>
<tr>
<td>GABA$_{B1/2}$</td>
<td>GS 39783</td>
<td>Calhex 231</td>
</tr>
<tr>
<td></td>
<td>CGP 7930</td>
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</tr>
<tr>
<td></td>
<td>CGP 13501</td>
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</tr>
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</table>

**Table 3** Allosteric Modulators of Family C Metabotropic Receptors

Abbreviations: CPCCOEt, 7-(hydroxyimino)cyclopropan[b]chromen-1a-carboxylic acid ethyl ester; PHCCC, N-phenyl-7-(hydroxyimino)cyclopropa[b]chromen-1a-carboxamide; BAY36–7620, (3aS,6aS)-6a-naphthalen-2-ylmethyl-5-methyldien; EM-TBPC, 1-ethyl-2-methyl-6-oxo-4(1,2,4,5-tetrahydro-benzof[d]azepin-3-yl)-1,6-dihydro-pyrimidine-5-carbonitrile; R 214127, [(3)H]-[3,4-dihydro-2H-pyran[2,3-b]quinolin-7-yl]-2-phenyl-1-ethanone; NPS 2390, 2-quinoxaline-carboxamidem-N$_1$-adamantan-1-yl; MPEP, 2-methyl-6-(phenylethynyl)-pyridine; Calhex 231, (1S,2S,1'R)-N1-(4-chlorobenzoyl)-N2-[1-(1-naphthyl)ethyl]-1,2-diaminocyclohexane; Ro 01–6128, diphenyl-acetyl-carbamic acid ethyl ester; Ro 67–7476, (S)-2-(4-fluoro-phenyl)-1-(toluene-4-sulfonyl)-pyrroolidine; LY 487379, N-(4-(2-methoxyphenoxypyphenyl)-N-(2,2,2-trifluoro-ethoxysulfonyl)pyrid-3-ylmethylamine; GS 39783, N,N'-dicyclopentyl-2-(methylthio)-5-nitro-4,6-pyrimidinediamine; CGP7930, 2,6-di-tert-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol; CGP 13501, aldehyde analog of CGP7930.

*Source:* From Ref. 5.
potentiators for glutamate receptors (Ro 01–6128, Ro 67–7476, LY 487379) and allosteric potentiators for calcium-sensing receptors (NPS R-467 and NPS R-568) reside exclusively within the 7TM domains, removed from the orthosteric site located in the N-terminal domain (5). The specific residues responsible for the subtype selectivity have been identified and are widely dispersed throughout the transmembrane regions. It has been concluded that all the allosteric modulators bind in a crevice defined by the four transmembrane domains TM3, TM5, TM6, and TM7 (5). A common allosteric site in the transmembrane domain of the family C GPCR, which mediates both allosteric potentiation and inhibition, mirrors the extracellular common allosteric site in the mACh receptors, and may underlie the structural differences between allosteric inhibitors and potentiators of the family C receptors. Allosteric inhibitors of mGluR1 or mGluR5 (PHCCC, SIB-1893, and MPEP) are weak allosteric potentiators of mGluR4 receptors. A series of benzaldazine analogs (e.g., DFB in Table 3) exhibit at mGluR5 receptors a range of activity from allosteric potentiation to allosteric inhibition to neutral cooperativity.

The number of allosteric ligands identified for mACh receptors and mGluRs compared to other GPCRs probably reflects an intensive search for allosteric modulators for these receptors, given their importance in a range of cardiovascular and neurological pathologies. For example, modulation of mGluR1 may well be a novel means of controlling hyperalgesia and also excitotoxicity, while mGluR5 inhibition may reduce anxiety and assist in the treatment of drug dependence and motor dysfunction in Parkinson’s disease (67).

CONCLUSIONS

Despite the obvious potential therapeutic benefits, in terms of efficacy and safety, of the prototypic allosteric modulators, the benzodiazepines, the search for compounds which would provide the clinical profile without the side effects was unsuccessful. Partly as a consequence, with the emerging knowledge of receptor structure, the development of combinatorial chemistry, and the need to introduce suitably high throughput screening methods, the wealth of new compounds to test tended to be of the agonist/antagonist type. The driving philosophy appears to have been a belief that, by using the improving knowledge of receptor structure, compounds more selectively targeted to specific receptor subtypes would be designed which, because of their selective binding, would provide efficacy and fewer side effects. It appears that this effort has not been rewarding in terms of clinically useful new therapeutics, but it has driven forward our understanding of the regulation of receptor function.

Our vastly improved knowledge of receptor structure and the relationship between structure and function has derived from successes in
defining ever more precisely receptor structure, using both imaging and molecular biological techniques, and in combining functional analysis with molecular biological and genetic techniques. These developments have led to a resurgence of interest in understanding how allosteric modulation of receptor function comes about and what a fundamental aspect of the normal physiological regulation of ionic signalling it is. Metabotropic receptor regulation, in particular, now seems to be almost bewilderingly complex, with genetic, structural, allosteric, and physiological mechanisms all operating simultaneously. However, with the techniques available it should be possible to develop strategies that utilize unique allosteric binding sites to subtly alter selective receptor function in ways that will provide therapeutic benefit without unwanted toxicology.

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Figure 6 Effects of various parameters on displacement curves to allosteric antagonists. The potency of the antagonist, as indicated by the abscissal location parameter of the displacement curve, is inversely proportional to the strength of the initial stimulus given the receptor (either initial agonist response or initial binding of radioligand tracer). The maximal displacement is a function of the magnitude [A]/[K A] dependent and [B]/[K B] dependent.
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