International Congress Series 1249 (2003) 235–244

Inverse agonists to explore the mechanisms of metabotropic glutamate receptor activity

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Received 16 April 2003; accepted 16 April 2003

Abstract

The most abundant excitatory neurotransmitter of the brain, glutamate, activates two sets of receptors: the ionotropic glutamate receptors responsible for the fast synaptic transmission and the metabotropic glutamate receptors (mGluRs) which regulate synaptic transmission. Thus, mGluRs are involved in fundamental physiological processes such as learning and memory, cardiac and movement control or pain. However, they also contribute to some neurological diseases. These receptors are then considered as good therapeutic targets. They share less than 10% sequence identity with the rhodopsin-related or the glucagon receptor-related receptors. Moreover, in addition to a heptahelical domain (HD) responsible for the coupling to G proteins, they possess a large extracellular domain containing the ligand binding site. Until recently, the functioning of these receptors was poorly understood. We have shown that several mGluRs displayed a constitutive activity, which was controlled by their C-terminal tail, and could be involved in long-term regulation of neuronal processes. Moreover, we showed that only noncompetitive allosteric compounds acting directly in the heptahelical domain were able to block this constitutive activity. These compounds allowed a better understanding of the mGluR activation process. Together with the recent determination of the crystal dimeric structure of the extracellular glutamate binding domain (BD) of mGluR1, our results led to a theoretical model for the functioning of this receptor.

Keywords: Metabotropic glutamate receptors; Inverse agonist; Constitutive activity; Activation mechanism, Allosteric regulation

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0531-5131/03 © 2003 Published by Elsevier B.V.
doi:10.1016/S0531-5131(03)00601-0

1. Introduction

1.1. mGluR subtypes and physiology

Glutamate is the main excitatory neurotransmitter of the central nervous system. It activates the ionotropic NMDA, AMPA and kainate receptors, which are responsible for the fast synaptic transmission. Glutamate activates also receptors coupled to G proteins called metabotropic glutamate receptors or mGluRs. Eight mGluRs (mGluR1-8) and a number of splice variants have been isolated [1,2]. They have been classified into three groups. mGluR1 and mGluR5 of group I are coupled to the phospholipase C pathway, while the receptors of group II (mGluR2 and mGluR3) and of group III (mGluR4, mGluR6, mGluR7 and mGluR8) are negatively coupled to the adenylyl cyclase pathway in heterologous systems [1,2]. The group I mGluRs are involved in long-term regulation of the synaptic transmission, supposed to be the basis for learning and memory processes [1]. They also control pain signals and, recently, important studies revealed the role of mGluR5 in cocaine addiction [3]. Many studies focussed on the role of the group II mGluRs in neurological disorders, such as anxiety or schizophrenia and drug addiction, and mGluR4 and mGluR7 could be involved in epilepsy [4,5]. The necessary development of the pharmacology for the understanding of the physiological roles of these receptors rendered the study of their structure and activation mechanism crucial.

1.2. Two domains for a receptor

mGluRs form the family 3 of G protein-coupled receptors (GPCRs), together with the calcium-sensing, $GABA_B$, and pheromone and taste receptors [2]. Like all GPCRs, the family 3 receptors have a heptahelical domain (HD; Fig. 1), but their most original feature is their large N-terminal extracellular region containing the binding domain (BD; Fig. 1), separated from the HD by a cystein-rich region (CRR), the function of which is unknown.

The BD has been shown to share sequence similarity with bacterial periplasmic proteins (BPP) involved in the transport of sugar, amino acids and ions in the periplasm of Gram-negative bacteria [6]. Residues of the BD involved in the binding of glutamate have been identified (for review, see Ref. [2]). A more definitive work was the recent publication of the crystal structure of the BD of mGluR1 [7]. The BD is formed by two lobes separated by a cleft in which glutamate binds (Fig. 1). The two lobes trap the agonists, as does a Venus flytrap to a fly [7]. The closed form is supposed to be the active form that turns on the HD. In contrast, antagonists stabilize the BD in an open inactive form [8,9] that does not turn on the HD.

Although the HD of mGluRs has only few residues in common with GPCRs of the other families, it couples to the same G proteins. Indeed, the intracellular second and third loops, together with the C-terminal tail, play a crucial role in the activation and in the selectivity of coupling to the G proteins [1,10,11]. The HD is also the target for many regulators [1], such as calmodulin, Pick1 or $\beta\gamma$ subunits of G proteins, that bind to the C-terminal tail or intracellular loops.

mGluRs are homodimers linked by a disulfide bond established between cystein residues of the BD (Fig. 2, and see Refs. [12,13]). The BD produced alone can form a

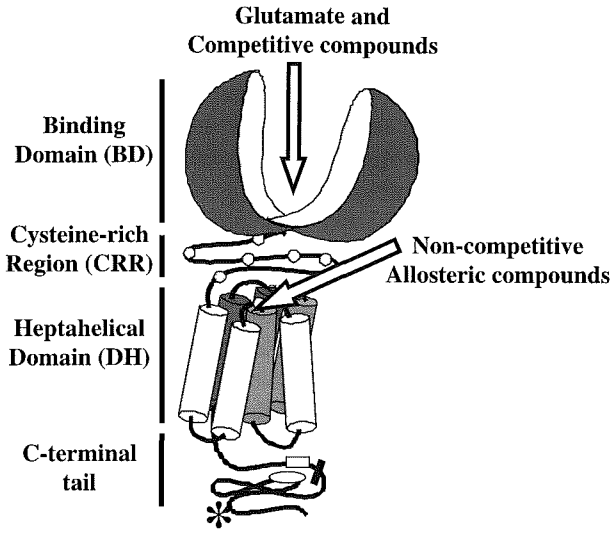

Fig. 1. Schematic structure of mGluR1a and 5. The two large domains of mGluRs are separated by the CRR. The site of binding of the competitive and noncompetitive compounds is shown. The length of the C-terminal tail is variable depending on the splice variant. The splicing site generating the short variants of mGluR1 is indicated by a black bar. The basic residue motif is shown by a white box, and the proline-rich region is circled in grey. The star represents the Homer binding site.

homodimer as revealed by the crystal structure of the dimer of mGluR1 BDs [7]. In the presence of glutamate, at least one of the two BD closes, and this is supposed to activate the HD. The model proposed that the closure of the BD brings the two links connecting the BD to the HD closer, modifying the position and may be the conformation of the activated HD [2,7,9]. However, the molecular events in the HD underlying the active state are not yet known. Then the discovery and study of constitutive activity of some mGluRs could

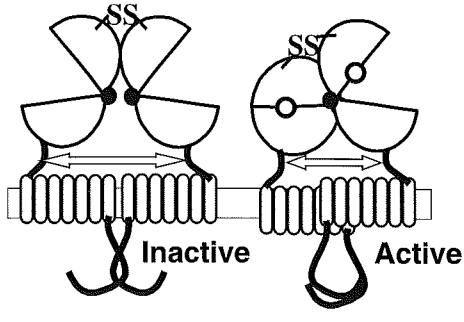

Fig. 2. Mechanism of activation of dimeric mGluRs. The dimer of mGluRs is stabilized by a disulfide bond between the first lobes of the two BD. Upon binding of glutamate, at least one BD closes, and this induces a rotation of the BD that brings the two HD closer. This is supposed to trigger the activation of the HD. The arrows indicate the distance between the two linkers connecting the BD to the CRR and the HD. Glutamate is represented by a white circle.

bring important information for understanding the mechanism underlying the activation of mGluRs.

2. Constitutive activity: from evidence to physiological roles

We have demonstrated that mGluR5 and the mGluR1a variant of mGluR1 displayed a constitutive activity when expressed in LLC-PK1 or HEK-293 cell lines [14–16]. The mGluR1a variant, such as mGluR5, possesses a long C-terminal tail of more than 350 residues in contrast to the other variants (Fig. 1; [14]). We showed that a motif of basic residues (RRKK in mGluR1 and KRR in mGluR5) in the C-terminal tail was involved in the generation of the constitutive activity [15]. Unexpectedly, this motif was located in a region common to all variants. The motif inhibited the constitutive activity in the short splice variants, and its inhibitory action was suppressed by a proline-rich domain present only in the long variant [15].

The control of the constitutive activity by splicing of the C-terminal tail was reinforced by our recent results obtained in collaboration with Fagni et al. In neurons, the constitutive activity of mGluR1a and mGluR5 was shut down by an interaction with the regulatory interacting proteins, called Homers, which bound to the long C-terminal tail of mGluR1a and mGluR5 ([17] and article by Fagni et al., this issue). An intense neuronal activity induced the breakage of the interaction, allowing mGluR1a and mGluR5 to display their constitutive activity. This process could play a role in synaptic plasticity [17]. These physiological studies required the use of powerful tools such as inverse agonists. However, only recently have these kinds of pharmacological tools been identified and made available, as will be shown below.

3. The quest for inverse agonists

To block the constitutive activity of mGluRs, inverse agonists are necessary. None of the known competitive antagonists (such as α-methyl-4-carboxy-phenylglycine, MCPG), the structure of which was derived from glutamate, was able to block the constitutive activity [14–16]. Fortunately, the systematic screening of chemical compounds by pharmaceutical companies led to the recent identification of new classes of selective and potent compounds acting on mGluRs and not derived from glutamate [2,18]. For example, 7-hydroxyiminocyclopropan[b]chromen-1a-carboxylic acid ethyl ester (CPCCOEt, Novartis) and (3a*S*,6a*S*)-6a-naphtalen-2-ylmethyl-5-methyliden-hexahydro-cyclopental[c]furan-1-on (BAY 36-7620, BAYER) specifically inhibited mGluR1, and 2-methyl-6-(phenylethynyl)pyridine (MPEP, Novartis) inhibited only mGluR5 [19–21].

Thus, in collaboration with teams from BAYER and Novartis, we demonstrated that BAY 36-7620 and MPEP were potent and efficient inverse agonists on mGuR1a and mGluR5, respectively [20,21]. CPCCOEt did not show any significant inverse agonist activity under normal conditions. Interestingly, the action of these compounds was noncompetitive, suggesting that these negative allosteric compounds did not bind to the BD of mGluRs as did the competitive antagonists such as MCPG (Fig. 1). Indeed, they

bound and acted directly in the HD. Residues of TM7 of mGluR1 were crucial for the selective action of CPCCOEt on mGluR1 [19]. Residues of TM7 and TM3 (such as P655 and S658) are necessary for the selective effect of MPEP on mGluR5 [20]. We also showed that the region comprising TM5, TM6 and TM7 of mGluR1 plays an important role for the selective action of BAY 36-7620 [21]. All these data suggested that there is an overlapping binding pocket in which these compounds bind. This has been reinforced by the study of the binding site of the recently identified positive allosteric compounds acting in the HD of mGluR1 [22].

4. Theoretical model of the constitutive activation of mGluRs

As for rhodopsine-related receptors, it was thought that mGluRs oscillate between an inactive and an active state [23]. However, because mGluRs are constituted of two domains, a BD and an HD, their constitutive activity could result of two different mechanisms. First, it could be due to the spontaneous closure, without glutamate, of the BD, which would turn on the HD (Fig. 3, 1 in circle). Indeed, mGluR1 BD could be closed without glutamate [7]. Moreover, the BD bound to the competitive antagonist MCPG is in an open form and could not be closed [8,9]. Thus, if the constitutive activity is due to the closure of the BD, then competitive antagonists, by preventing the closure, should be inverse agonists. However, as mentioned above, none of the competitive antagonists blocked the constitutive activity, indicating that the constitutive activity did not result from the spontaneous closure of the BD. The second possibility (Fig. 3, 2 in circle) was that the HD itself turned on spontaneously and activated the G proteins independently of the state (open or closed) of the BD. In this case,

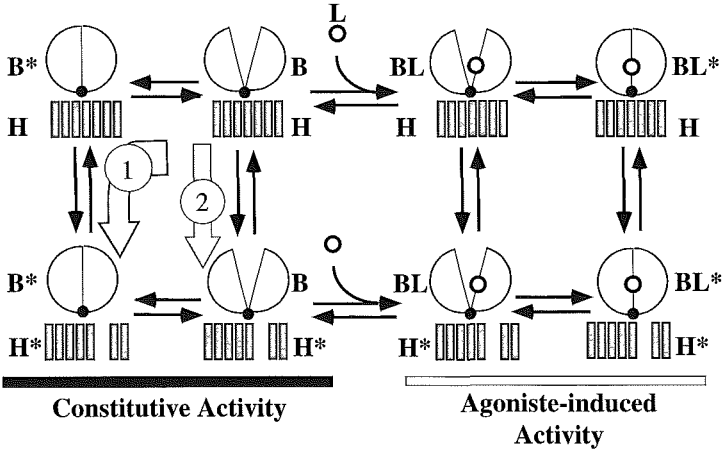

Fig. 3. Theoretical model of the constitutive activity. This model explains the possible ways (noted 1 and 2 in circles) to generate constitutive activity in mGluRs. When the seven TMs are split into two parts schematically, its means that they are in an active state. B: The binding domain (BD); H: the heptahelical domain (HD); *: activated state. The ligand, or glutamate, is represented by a white circle and named L

the competitive antagonists should be ineffective, and only compounds that bind and block directly the HD activation should display inverse agonist activity (Fig. 2). That is exactly what we observed (see above). Then the HD of some mGluRs can switch spontaneously to an active state as described for rhodopsine-related receptor. Thus, if we want to supress the constitutive activity of mGluRs, competitive antagonists should not be used.

A complete theoretical model has been established to explain the functioning of mGluRs [23]. This model predicted that there is a functional coupling, or an allosteric interaction, between the states (active or inactive) of BD and those of HD. As our results suggested that the spontaneous activation of the HD was not influenced (even partially) by the state (open/inactive or close/active) of the BD, one could consider that the functional coupling is loose between BD and HD in mGluRs. As discussed recently, pharmacological data obtained with mGluRs and $GABA_B$ receptors confirmed our model [23] and indicated that in $GABA_B$ receptors, the functional coupling was tighter than in mGluRs. Thus, depending on the allosteric interaction between BD and HD, the effect of regulators on the functioning of the receptors could be different and this could be of physiological importance [23]. The loose interaction observed in some mGluRs could be due to the presence of the CRR that is not present in $GABA_B$ receptors [23].

5. Conclusion

We showed that some mGluRs display constitutive activity that is blocked by allosteric compounds inhibiting the activation of the HD. We established a theoretical model to explain our results. However, because mGluRs are dimeric receptors, some intriguing questions have to be addressed to get a more precise view of the functioning of the receptor complex. As mentioned above, the agonist-induced closure of the BD would bring the HD closer in association and trigger their activation. Then do the allosteric antagonists block this association? And consequently, is a spontaneous association of the HD responsible for the constitutive activity of the receptor? Or is the constitutive activity the result of a spontaneous switch (blocked by allosteric antagonists) of the G protein coupling region of one of the HD?

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Discussion 19

T. Costa

Do you know what happens if you only transfect the rhodopsin-like cassette without the ligand-binding domain on top? Do you get any constitutive activity there?

L. Prezeau

That's something I want to do. I'm sure other people tried. I would be very curious to know if it has constitutive activity.

T. Schwartz

The real activity resides in the 7TM domain, but the ligand-binding domain is then going back and forth between two different conformations which we really can see. It's a little surprising that there is no effect from the 7TM domain back up on the other one. Are you really saying this is not happening?

L. Prezeau

We and others did not see a strong effect on agonist activity of the 7TM on the extracellular domain, when measuring the effect of GTP binding or other nucleotides to the G protein. We tried to explain this in our recently published review paper (Trends Pharmacol Sci 2002; 23:268-74). There's an allosteric regulation between these two domains, the extracellular domain and the heptahelical domain, and this coupling can be loose or tight. In the $GABA_B$ receptor this intramolecular coupling is pretty tight and in mGluRs it seems to be pretty loose. The difference between mGluRs and $GABA_B$ receptors is that you have a cysteine rich region (CRR) between the two domains in mGluR that is not present in the $GABA_B$ receptors, so maybe you have some flexibility here. We can also ask what is the advantage of having a loose coupling constant between these two domains. Maybe it's because you have regulation by intracellular proteins. That means if we are really seeing that mGluRs have a capability of being activated by an extracellular or intracellular signal, you may not want to have a real modification of the affinity of the drug if homer is binding and revealing the constitutive activity. If the receptor is constitutively active in cells because it has been stimulated, you don't want the affinity of the drugs to be increased, because you have glutamate everywhere. And then if you have higher affinity for glutamate, then you will stimulate again the receptor. A loose allosteric constant would just protect against this phenomenon.

H. Giles

Forgive me if you've already explained this, but how certain are you that the drug -your inverse agonist compound- is acting totally extracellularly, on the extracellular portion of the receptor? How certain are you that they are acting on the extracellular site?

L. Prezeau

We are sure that the inverse agonists act on the 7TM part of the receptor. We can say that substitution of several residues in the TMs modify the selectivity between the two receptors, mGlu1 and mGlu5. You can switch the selectivity just by changing these residues. Then the drug is acting on mGlu1 and not on mGlu5, or vice versa. So we think the selectivity for binding could be there, but selectivity also means access. So maybe it's just an access that you block or you reveal and then it's binding deeper in the protein. That could be that, but we think it's on the third intramembranar part or top of the transmembrane domains.

H. Giles

I was thinking about definitions, and I was wondering if we were going to get into a position where we could identify ligands which switch off constitutive activity, but actually don't behave as antagonists at all. I know you showed that this compound was behaving in a non-competitive way but was antagonizing the agonist. But one could envisage that you could actually have things that would switch off the receptor at an allosteric site, perhaps by interfering with G proteins which were completely separate, and I was just wondering what we'd call those.

L. Prezeau

Right, we can regulate the receptor by acting on different places. In our case, people did some homology modelling of the 7TMs of mGluRs, according to rhodopsin structure, and they saw that there is a kind of pocket which could correspond to the retinal pocket. Our compounds are probably acting in this kind of pockets. We can put them in a right conformation and have the position of the selective residues in the right way. At the last meeting on mGluRs, we discovered all these positive allosteric regulators, and they are acting on the same binding pocket. So we are pretty sure that there is a kind of "antique" pocket that is probably related to the pocket you have in the family 1 receptors, that is still there, and you can act on it and modify the 7TM just like family 1 receptors. But maybe will we discover new place to regulate the receptor, like interfering with the G protein coupling.

M. Brann

About the $GABA_B$ receptors, I was just wondering if you'd elaborate a bit more on the analogy of the family 3 receptors, and to what degree you think the concepts you're talking about really will be in common for $GABA_B$. We spoke about the calcium receptor, one of my particular interests, have you looked at it? How much does this compare with what you've seen in those other systems?

L. Prezeau

For Ca^{2+} receptors we don't know. We don't have the tools, so we can't use them. Another thing that is important is that the calcium receptor has a CRR. As the allosteric constant is loose or tight, depending on the receptors, may be will the calcium receptor behave like mGluRs. When you modify the G protein state by an oligonucleotide or things like that, sometimes you see a shift in affinity for drugs. You can see it with GABA, so there's a really nice coupling between G protein, heptahelical domain and the extracellular domain. But for mGluR1 and mGluR5, there is no modification of affinity of the agonist on the extracellular domain. So there is really a loose allosteric coupling between these two domains compared to $GABA_B$ receptors. They are not behaving in the same way.

T. Schwartz

Both those systems really are dimers. We also know a lot about the ligand binding, with only one of them binding the ligand is enough to give the activity in the system. Could you say a few more words about that?

L. Prezeau

For $GABA_B$ receptors we are sure that they are heterodimers. You have two different proteins, GB_1 and GB_2, and GB_1 is binding the ligand, the GABA molecule, and the coupling is done by GB_2. You can switch the heptahelical domain and it will still function the same. So there may be not a real talking between extracellular domain and heptahelical

domain, but just, as I showed you here, a rotation of the extracellular domains that brings together the heptahelical domains. So it's probably the same thing as in mGluRs. One is closed, then you have a rotation of the two extracellular domains, and you activate the intracellular heptahelical part. May be, like $GABA_B$ receptors, only one of them is activated.