International Congress Series 1249 (2003) 245–251

Control of constitutive activity of metabotropic glutamate receptors by Homer proteins

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Received 16 April 2003; accepted 16 April 2003

Abstract

The postsynaptic metabotropic glutamate receptor subtypes 1a and 5 (mGluR1a and mGluR5) control excitatory synaptic transmission in the mammalian brain. These receptors are coupled to inositol trisphosphate- and ryanodine-sensitive Ca^{2+} stores via G proteins. The scaffolding protein Homer1b, Homer1c, Homer2, or Homer3 physically links mGluR1a or mGluR5 to these intracellular Ca^{2+} stores. The short splice variant Homer1a represents an exception within the family of Homer proteins. This protein is the product of an immediate early gene induced after intense neuronal activity that disrupts the mGluR1a/5–Ca^{2+} store scaffold. In the absence of agonist, Homer3 stabilizes mGluR1a and mGluR5 in an inactivate state, whereas Homer1a competes with Homer3 on mGluR1a and mGluR5, and directly activates these receptors. This is the first evidence showing that an intracellular protein can induce a constitutive activity of a G protein-coupled receptor, in response to intrinsic neuronal activity.

Keywords: Neurons; Metabotropic glutamate receptors, Constitutive activity; Homer

The excitatory neurotransmitter glutamate activates both ionotropic and metabotropic receptors (mGluRs). The mGluRs are classified into three groups (groups I, II, and III) based on sequence homology (Fig. 1) [1]. Group I mGluRs (mGluR1 and mGluR5; Fig. 1) are preferentially located at postsynaptic sites [2] and activate a PLC pathway [1]. The so-

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0531-5131/03
doi:10.1016/S0531-5131(03)00600-9

Fig. 1. Interaction domain of mGluRs with Homer proteins. The figure represents the carboxyl-terminus of mGluRs The white box indicates the position of the Homer protein interaction domain. Note that only mGluR1a, mGluR5a, and mGlouR5b splice variants display such a domain

called Homer proteins form multimeric complexes (Fig. 2) and link the carboxyl-terminus of mGluR1a, mGluR5a, or mGluR5b splice variants to inositol trisphosphate or ryanodine receptors (IP3R and RyR) [3–6] (Fig. 3A). This applies to all Homer proteins except to the short splice variant Homer1a, which exists only as a monomer (Fig. 2A). Homer1a is the product of an immediate early gene induced by intense neuronal activity [3,7,8], whereas multimeric Homer proteins are constitutively expressed [5,6]. Once expressed, Homer1a competes in a dominant negative manner with the multimeric Homer proteins and disrupts the mGluR1a/5–IP3/RyR complex (Fig. 3B). The various physiological roles of Homer proteins have been recently reviewed [9,10]. Here we will focus on the effects of these proteins in the agonist-independent activity of mGluRs.

The long mGluR1a and mGluR5—but not the short mGluR1b, mGluR1c, and mGluR1d—splice variants (Fig. 1) display marked constitutive activity in heterologous expression systems [11]. Interestingly, when transfected in cultured cerebellar granule neurons, neither mGluR1a nor mGluR5 displays detectable constitutive activity [12]. The two specific mGluR1 and mGluR5 inverse agonists, BAY-36-7620 [13] and 2-methyl-6-(phenylethynyl)pyridine (MPEP) [14], respectively, have been used to show that the endogenous mGluR1a also does not display constitutive activity in these neurons. On the

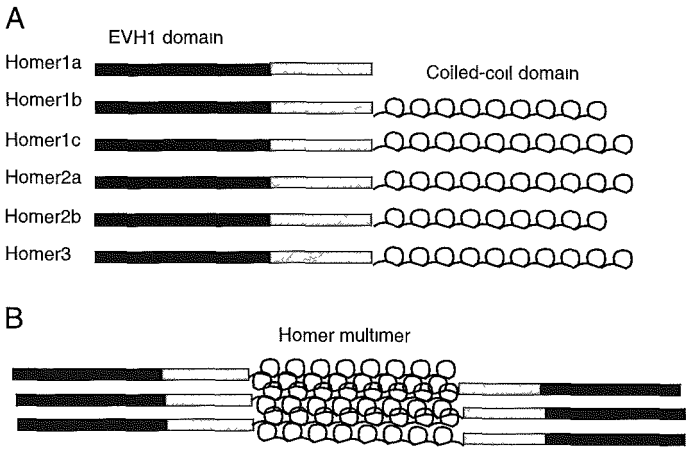

Fig. 2. The family of mouse Homer proteins. (A) Three genes (*Homer1*, *Homer2*, and *Homer3*) give rise to a family of Homer proteins and splice variants. All the Homer proteins display a homologous amino-terminal (EVH1-like) domain (black band) and a carboxyl-terminal coiled coil domain (curled band) separated by a divergent amino acid sequence (gray band), with the exception of the Homer1a splice variant that does not display the coiled coil domain. (B) Coiled coil domain interactions allow Homer proteins to form up to hexamers. Therefore, Homer1a is found only as a monomer.

other hand, blockade of Homer3 synthesis by a specific antisense oligonucleotide induces marked mGluR1a constitutive activity in cultured cerebellar granule cells. The antisense does not affect mGluR1a synthesis, indicating that the effect does not result from functional overexpression of the receptor [12]. One can therefore tentatively conclude that Homer3 stabilizes mGluR1a in an inactive conformation, in the absence of agonist (Fig. 3A).

Is the interaction between mGluR1a and Homer3 responsible for this effect? The F1128R mGluR5a mutant does not interact with Homer proteins and displays constitutive activity when transfected in cultured cerebellar granule cells [12]. Therefore, interaction between Homer3 and mGluR5 prevents constitutive activity of the receptor in neurons. Given the similarities between mGluR5 and mGluR1a, one can assume that the same conclusion also applies to mGluR1a. The following experiments confirm this hypothesis.

One can predict from these results that disruption of the interaction between mGluR1a and Homer3 by Homer1a should result in constitutive activity of the receptor. Indeed, transfection of recombinant Homer1a in cultured cerebellar granule cells induces constitutive activity of the receptor [12]. Furthermore, induction of endogenous Homer1a in these neurons, by a mixture of kainate-positive NMDA, triggers a marked constitutive activity of mGluR1, accompanied by the appearance of a significant amount of mGluR1a–Homer1a complex and reduced amount of mGluR1a–Homer3 complex [12]. Plasma membrane distribution of the receptor is not affected by the kainate-positive NMDA treatment. Therefore, activity-dependent expression of the immediate early gene, *Homer1a*, is sufficient to replace mGluR1a–Homer3 by mGluR1a–Homer1a functional complexes and to induce a constitutive activity of the new mGluR1a–Homer1a complexes in neurons (Fig. 3B).

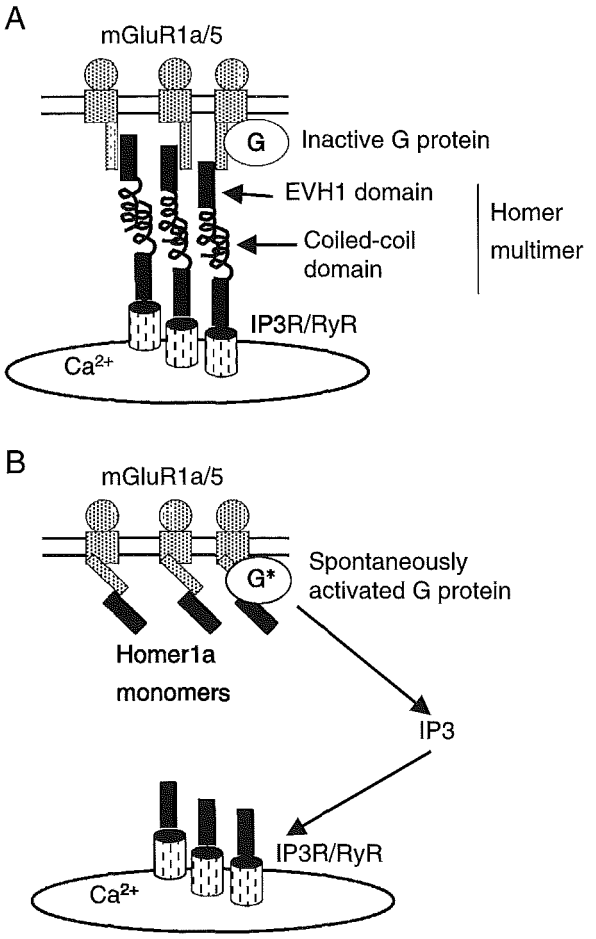

Fig. 3. Functional interactions between Homer proteins and receptors. (A) Homer proteins bind to mGluR1a or mGluR5 and IP3R or RyR. Thus, the coiled coil domain-containing Homer proteins create a physical link between these mGluRs and intracellular Ca^{2+} stores. We hypothesize that physical constraints caused by these multiprotein complex to the carboxyl-terminus of mGluR1a or mGluR5 prevent spontaneous activation of the G protein by these receptors. (B) Homer1a competes in a dominant negative manner with the coiled coil domain-containing Homer proteins on mGluR1a/5 and IP3R/RyR. Because Homer1a is a monomeric protein, it disrupts the mGluR1a/mGluR5–IP3R/RyR complex and creates only negligible constraints to the carboxyl-terminal domain of mGluRs. This causes spontaneous activation of the G protein (G*) by the receptor.

We tentatively suggest that the multimeric mGluR1a/5–Homer3–IP3R/RyR scaffold would create sufficient physical constraints to the mGluR1a/5 carboxyl-terminus to block spontaneous activation of the receptor associated G protein, in the absence of agonist (Fig. 3A). The interaction of monomeric Homer1a or the absence of Homer interaction (as it is the case in heterologous expression systems or with the tested mGluR5 mutant) with mGluRs would decrease these physical constraints and allow the receptors to spontaneously activate the G protein (Fig. 3B). These results provide the first evidence that an

intracellular protein can activate a G protein-coupled receptor independently of any extracellular receptor agonist binding.

Acknowledgements

We thank Dr. Rainer Khun (Novartis Pharma, Basel, Switzerland) and Dr. Thomas Müller (Bayer, Leverkussen, Germany) for kindly providing us with MPEP and BAY-36-7620, respectively.

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Discussion 20

R. Leurs

I'm a bit ignorant in this area, but you showed in the beginning that there are six different homer proteins. How about the role of these 1b, 1c, 2a, and 2b with respect to this specific property?

L. Fagni

So far with tests, it's only the effect of Homer1a on the Homer3 protein because this Homer3 protein was the endogenous one. We don't know whether this Homer1a protein could induce a similar effect in neurons that express also Homer2 proteins. Our results would predict that the effect would be the same. That there is no obvious difference between the multimer that is formed by Homer2 or Homer3 protein.

R. Leurs

So Homer2 can also constrain your glutamate receptors.

L. Fagni

We did not perform the experiment, but we can predict that it would be the same.

T. Schwartz

Concerning the mechanism, do you think it's very likely that the actual binding of Homer to this protein-rich domain, pretty far out in the C-terminal, will actually, by itself, conformationally restrain the receptor, or don't you think that it brings in other factors that may interact with the receptor?

L. Fagni

We said that the interaction between Homer1a and mGluR is responsible for this constitutive activity. And if we transfer the metabotropic glutamate receptors in HEK 293 cells, these receptors are constitutively active. But if we transfer the receptor with Homer3, we cannot block this constitutive activity. I think we need not only the Homer3 dimer, but also the SHANK protein and the IP3 receptor and all this network of proteins to add enough constraint to the C terminus of the receptor to block the constitutive activity.

T. Wurch

Is there a difference in the pharmacological properties of the constitutive activity in the neurons in which the Homer1a is induced and the HEK 293 cells? Did you compare the two conditions in the inverse agonism?

L. Prezeau

On HEK 293 cells, we can easily measure the affinity or potency of these drugs, but in neurons it wasn't that easy. But the concentration we used was really compatible with what we observed in HEK 293 cells.

T. Wurch

I was wondering if the presence of the Homer1a protein will modify something of the binding of the G protein.

L. Fagni

No, there was no modification in the binding.

G. Milligan

When I was looking at the slides on the maxi K channel assay, the level of constitutive activity was that uncovered by removing the Homer. Am I missing something here? Or did you just add a little bit of agonist?

L. Fagni

I don't have any clear explanation for that. These are supposed to be maximal concentrations, so I don't know. We didn't check higher concentrations than this.

R. Leurs

You have two different situations: you first have the Homer3 bound to the receptor, which might constrain it, at least to some extent, which you can overcome with an agonist, but apparently not completely.

L. Prezeau

If you look at the IP3 increase activity on the basal, the constitutive activity is rather low—it's 50% increase. When you compare to the agonist-induced activity, it's really smaller than the agonist-induced activity. But if you look at the trace for the BK channel, you see that the BK channel activity is really huge upon the constitutive activity of the receptor. I think that you have another cascade that is induced by a low concentration of IP3 released, and the subsequent calcium released. And maybe kinases or other molecules could modify the whole pathway and the channel activity. And indeed you need a little bit of time to go back to normal when you add the inverse agonist, and it's a more long-term effect than the agonist-induced effect. I think the scale of time is different.

L. Fagni

Another explanation could be that, by measuring the BK activity, we can reach a saturating activation of the channel, whereas by measuring IP_3 formation we don't reach this saturation effect.

P. Kitabgi

In some experiments, you transfected neurons with Homer1a. I guess those were primary cultured neurons. What is the efficiency of transfection in this system and how do you pick up, in your electrophysiological experiments, the neurons that you transfected?

L. Fagni

We get from 5% to 15%, the rate of transfection in this neuron by lipofection. We monitored the transfected receptor by cotransfecting GFP with Homer1a. We check that 90% of neurons expressing GFP also express the transfected protein.