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Clustering of GABA (A) Receptors

Ayla Arslan

International University of Sarajevo, Faculty of Engineering and Natural Sciences, Department of Genetics and Bioengineering, Hrasnicka Cesta 15, Ilidža 71210 Sarajevo, Bosnia and Herzegovina

Abstract

Targeting, clustering and immobilization of the neurotransmitter receptors is a complex process of molecular organization. At the postsynaptic level, anchoring protein model has been proposed for the mechanism of this organization. NMDA type of Glutamate receptors are anchored by the PSD 95 at the postsynaptic membrane. Gephyrin is essential for the anchoring of Glycine receptors and so Agrin for the Acetylcholine receptors at the post synaptic sites. In line with this, for GABA (A) receptors ($GABA_ARs$), the similar concept has been expected but the heterogeneity and complexity of $GABA_ARs$ make their analysis extremely difficult. This paper will briefly discuss the current perspectives on the clustering of selected $GABA_AR$ subtypes in terms of anchoring protein model.

Keywords: GABA (A) Receptors, neurotransmitter receptors, clustering

1. Introduction

Brain information processing depends on neural circuits, mediated by excitatory and inhibitory signals (Koch et al., 1983). These signals are generated by neurotransmitters like Glutamate, Acetylcholine, Glycine or GABA. When released from the pre-synaptic neuron, each neurotransmitter binds to its own receptor, leading to a change in its conformation and thus changing the receptors ionic permeability. It is this change in the ionic permeability that has the capability to alter the membrane potential of neurons which is approximately -70 mV at resting state. One of the factors important for this phenomenon is the clustering of neurotransmitter receptors targeted and immobilized in the special domains of the cell membrane. Targeting, clustering and immobilization of the neurotransmitter receptors is a complex process of molecular organization and at the postsynaptic level, anchoring protein model has been proposed for the clustering mechanism of neurotransmitter receptors like acetylcholine, glycine or NMDA receptors. For example NMDA type of Glutamate receptors are anchored by the PSD 95 at the postsynaptic membrane (Sheng and Pak, 1999; van Zundert, et al., 2004, Elias et al., 2008). Gephyrin is essential for the anchoring of Glycine receptors (Kirsch et al., 1993; Kirsch et al., 1995; Kneussel et al., 1999; Schrader et al., 2004; Feng, et al., 1998; Kimet al., 2006) and so Agrin for the Acetylcholine receptors (McMahan, et al., 1990; Bezakova and Ruegg, 2003; Kummer et al., 2006) at the post synaptic sites. Similarly for $GABA_ARs$ (Gamma-aminobutyric acid type A receptors), the same mechanism has been expected but the heterogeneity and complexity of these receptors make their analysis very difficult. In this paper, I will discuss the current perspectives on the clustering of $GABA_ARs$.

2. $GABA_ARs$: The chloride channels

$GABA_ARs$ are GABA gated heteropentameric chloride channels and major sites of inhibitory neurotransmission in the mammalian brain. The subunits are encoded by 19 genes ($\alpha1-\alpha6$, $\beta1-\beta3$, $\gamma1-\gamma3$, δ, ε, θ,π, $\rho1-\rho3$) and subunit composition determines the receptor's distribution both in cellular and sub-cellular level (Fritschy and Mohler, 1995; Sieghart and Sperk 2002; Sun et al., 2004).

Until last year, the structure of $GABA_ARs$ was based on the homology modeling (Sine and Engel, 2006) but the crystallized structure of homomeric $\beta3$ subunit containing $GABA_ARs$ ($GABA_AR$-$\beta3$cryst) at 3Å resolution have finally revealed the receptor structure for the first time (Miller and Aricescu, 2014). The receptor, composed of five subunits and arranged around a central pore, has a cylindrical shape with a height of 110Å and with a diameter of 60 to 80Å. Each subunit has a long N- terminus and a short C terminus both located extracellularly, four transmembrane domains*(*TM1–TM4), a large

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intracellular loop between the third and fourth transmembrane domains. Each extracellular domain has an amino-terminal α-helix (α1) followed by ten β–strands.

The most abundant receptor subtype of $GABA_AR$s is the γ2αβ subunit combination (γ2–$GABA_AR$s) with a stoichiometry of 2 α, 2 β and 1 γ2 subunit (Benke et al., 1991; Tretter et al., 1997; Sieghart and Sperk, 2002). Clustered at the postysynaptic sites (Fujiyama, et al., 2000, 2002), besides to nonsynaptic regions, γ2–$GABA_AR$s are in the close proximity of GABA release from presynaptic neuron and quickly respond to GABA by allowing chloride influx to the cell and thus causing a strong hyperpolarization and mediating fast phasic inhibition (Farrant & Nusser, 2005). On the other hand, some $GABA_AR$ subtypes are not located in the synapses. Electron microscopy studies clearly show that these receptors are non-synaptic (Nusser et al., 1998; Wei et al., 2003). So called extra-synaptic receptors, these receptors specifically contain δ subunit (Sommer et al., 1990) together with 2 α and 2 β subunits (Shivers et al., 1989; Jones et al., 1997, Patel et al., 2014), have higher affinity for GABA and mediate slow, tonic inhibition (Hausser and Clark, 1997; Farrant & Nusser, 2005). Another subset of GABA receptors containing α5, β and γ2 subunits are mostly extrasynaptic also (Kneussel, 2005; Loebrich et al., 2006). Therefore, different $GABA_AR$ subtypes have different subcellular distribution, i.e., synaptic and/or extrasynaptic, or perisynaptic. However the factors underlying the mechanisms involved in the differential distribution of receptor subtypes are not well understood. In this study we will focus on the clustering of γ2 and δ subunit containing receptor subtypes since these subunits involved in different physiological functions (phasic and tonic), never co-assemble, and have different subcellular distribution.

3. Clustering of $GABA_AR$subtypes: γ2–$GABA_AR$s and δ–$GABA_AR$s

$GABA_AR$s are assembled in the endoplasmic reticulum and microtubule-based transport mediates their mobility. Upon their arrival to the plasma membrane, the receptors diffuse laterally in the cell's surface but their interaction with specific adaptor proteins bound to scaffolding proteins with elements of cytoskeleton leads to the stabilization of neurotransmitter receptors at specific domains of plasma membrane (Tretter and Moss, 2008).Still, we cannot propose a mechanism for the membrane clustering for $GABA_AR$s in general but perhaps mechanisms may be proposed for different receptor subtypes.

In this context, γ2–$GABA_AR$s correspond to one receptor subtype. γ2 subunit is specifically essential for the process of synaptic clustering (Essrich et al., 1998). Despite this, synaptic currents have been detected in neurons obtained fromγ2knock-out mice. It appears likeγ3subunit is a candidate for clustering $GABA_AR$s at synapses in the absence of theγ2subunit at least for certain neurons (Kerti-Szigeti et al., 2014). Another molecular factor that might be important in the clustering of γ2–$GABA_AR$s is the Gephyrin. Current literature suggests that the clustering of γ2–$GABA_AR$s in the postsynaptic membrane is facilitated by interaction of gephyrin with the cytoplasmic domain ofαsubunit (Tretter et al., 2008, 2012; Mukherjee et al., 2011).However gephyrin is required for clustering of γ2–$GABA_AR$s that contain theγ2 subunit together with theα2 or α3 subunit but not withα1 subunit (Essrich et al., 1998; Kneussel et al., 1999, 2001; Tretter et all., 2011). Yeast 2-hybrid studies and glutathione S-transferase pull down assays show that Gephyrin interacts with $GABA_AR$ associated protein (GABARAP) (Kneussel et al., 2000; Kneussel and Loebrich, 2007). However, these two proteins are never co-localized in neurons and GABARAP is not found in the inhibitory synapses (Kneussel et al., 2000; Kittler, et al., 2001). Moreover studies of GABARAP deficient mice show that the number of $GABA_AR$s is not affected by the loss of GABARAP and immunostaining revealed no differences in the clustering of the γ2 subunit and the gephyrin (O'Sullivan et al., 2005)**.**Conversely, GABARAP is not essential for the clustering γ2–$GABA_AR$s but gephyrin is the anchoring protein of at least certain combinations of γ2–$GABA_AR$s clustered synaptically. Other $GABA_AR$ related proteins involve but not limited to some gephyrin interacting proteins like collybistin (Kins et al, 2000).

Although Gephyrin appears as a central protein for the postsynaptic clustering of some γ2–$GABA_AR$s, it is essentially dispensable for the clustering of extra-synaptic receptors (cited in Arslan et al., 2014). Extrasynaptic receptors are likely have their own assembly of proteins for their special arrangement in the extrasynaptic sites. For example radixin has been identified as anchoring protein for α5 containing $GABA_AR$s subtypes (Loebrich et al., 2006) which are extrasynaptic (Brünig et al., 2002). Is that the same case for the extrasynaptic δ–$GABA_AR$s? δ-$GABA_AR$s, are typically

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composed of α6 and β subunits in the cerebellum (Jones et al, 1997); and composed of α4 and βsubunits in the forebrain (Peng et al., 2002; Jia et al., 2005; Chandra et al., 2006). Electron microscopy data show that they are located extra-synaptically or perisynaptically in these regions (Nusser et al., 1998; Wei et al., 2003). There is no any protein identified so far associated with the process of extrasynaptic clustering of δ-$GABA_A$Rs but α subunit and δ subunit might have an active role in this process. Studies show that when a gephyrin-binding site is introduced into the intracellular domain of α6 and δ subunits, δ-$GABA_A$Rs became closer to the synaptic sites (Wu, et al., 2012). Besides, targeting of δ-γ2 chimeric subunits to synaptic or extrasynaptic sites has been found to be dependent on the co-assembly with the α2 or α6 subunit. (Wu, et al., 2012) Therefore, the α subunit isoforms, together with the γ2 and δ subunits is likely play a significant role in synaptic or extrasynaptic targeting of $GABA_A$Rs, respectively. This view has been further supported for the δ subunit by the studies of recombinant δ-γ2 subunits expressed in primary cultures of neurons. By focusing on γ2-$GABA_A$Rs and δ- $GABA_A$Rs, Arslan et al. (2014) comparatively analyzed the differential clustering of synaptic and extra-synaptic $GABA_A$Rs in hippocampal neurons and suggested that extra-synaptic clustering of δ-$GABA_A$Rs is dependent on the cytoplasmic loop of δ subunit probably via an active process (Arslan et al., 2014). As a result, these studies make the δ subunit and especially its cytoplasmic domain as strong candidate influential in the process of extrasynaptic clustering of corresponding receptor subtypes. Thus, in order to identify any anchoring protein which may be involved in the extrasynaptic clustering δ–$GABA_A$Rs, δ subunit and its cytoplasmic domain should be further analyzed by proteomics and yeast 2-hybrid screens.

4. Conclusion

The heterogeneity and complexity of $GABA_A$Rs make the analysis of their differential clustering extremely difficult. Despite this, some progress has been achieved in understanding the process of synaptic and extrasynaptic $GABA_A$Rs clustering. In line with anchoring protein model, Gephyrin is essential for the anchoring of some subtypes γ2–$GABA_A$Rs that contain the γ2 subunit together with the α2 or α3 subunit (but not with α1). Regarding δ–$GABA_A$Rs, there is less information but the emerging data for the importance of δ subunit may better guide the design of new studies aiming to identify novel proteins involved in the process. Thus, more progress is expected in the field to understand the molecular machinery involved in the process of differential clustering of $GABA_A$R subtypes.

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