Molecular and Cellular Neuroscience 56 (2013) 201–211

Contents lists available at ScienceDirect

Molecular and Cellular Neuroscience

journal homepage: www.elsevier.com/locate/ymcne

$GABA_A$ receptor membrane insertion rates are specified by their subunit composition

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ARTICLE INFO

Article history:
Received 16 January 2013
Revised 3 April 2013
Accepted 19 May 2013
Available online 25 May 2013

Keywords:
GABA-A receptor
Rate of insertion
Hippocampal neurons
Subunit composition
HEK293

ABSTRACT

γ Amino-butyric acid type-A receptors (GABARs) containing γ2 or δ subunits form separate pools of receptors in vivo, with distinct localization and function. We determined the rate of surface membrane insertion of native and recombinant γ2 and δ subunit-containing GABARs (γ2-GABARs and δ-GABARs). Insertion of the α-bungarotoxin binding site (BBS) tagged γ2 subunit (t-γ2)-containing GABARs in the surface membrane of HEK293 cells occurred within minutes and reached a peak by 30 min. In contrast, insertion of the BBS-tagged δ subunit (t-δ)-containing receptors required longer incubation and peaked in 120 min. Insertion of the t-γ2 subunit-containing receptors was not influenced by assembling α1 or α4 subunits. In contrast, insertion of the α4β3t-δ subunit-containing receptors was faster than those containing α1β3t-δ subunits. The rate of insertion of native GABARs in the surface membrane of cultured hippocampal neurons, determined by an antibody saturation assay, was similar to that of the recombinant receptors expressed in HEK293 cells. Insertion of the γ2-GABARs was rapid and new γ2-GABARs were detected on the surface membrane of cell soma and dendrites within minutes. In contrast, insertion of the δ-GABARs was slow and newly inserted receptors were initially present only in the surface membrane of cell soma and later also appeared over the dendrites. Thus the rate of insertion of GABARs was dependent on their subunit composition.

Introduction

The γ-amino butyric acid type-A receptors (GABARs) mediate inhibitory neurotransmission in the forebrain and spinal cord. Sixteen subunits, α1–6, β1–3, γ1–3, δ, ε, π and θ, co-assemble to form pentameric receptors (Whiting, 2003). The majority of native GABARs in the hippocampus are composed of 2α, 2β and either a γ2 or δ subunit (Sperk et al., 1997; Sun et al., 2004). Subunit composition determines the surface membrane localization, kinetics, and pharmacological properties of GABARs (Olsen and Sieghart, 2009). The γ2 subunit-containing receptors (γ2-GABARs) are expressed at the synaptic and extrasynaptic membranes, whereas the δ subunit-containing receptors (δ-GABARs) are exclusively extrasynaptic (Nusser et al., 1998; Wei et al., 2003). γ2-GABARs mediate synaptic and tonic inhibitions, whereas δ-GABARs mediate only tonic inhibition (Brickley et al., 1996; Farrant and Nusser, 2005). The γ2 and δ subunits also assemble with distinct α subunits. The majority of γ2-GABARs expressed in the hippocampus contain α1 subunits, while smaller fractions contain α2, α3, α4 or α5 subunits. On the other hand, δ-GABARs expressed on hippocampal dentate granule cells contain α4 subunits, whereas those expressed on interneurons contain α1 subunits (Glykys et al., 2007; Pirker et al., 2000).

The γ2 and δ subunits do not co-assemble in vivo (Araujo et al., 1998; Jechlinger et al., 1998; Quirk et al., 1995). However, it is not known whether the characteristics of secretion of γ2- and δ-GABARs are similar or distinct. Aspects of the exocytosis of GABARs are partially understood (Chen and Olsen, 2007; Twelvetrees et al., 2010; Vithlani et al., 2011). A prior study demonstrated the rapid insertion of tagged β3 subunit-containing GABARs at the surface membrane of cultured hippocampal neurons, and all receptors were inserted at extrasynaptic sites (Bogdanov et al., 2006). The γ2-GABARs, which can diffuse between synaptic and extrasynaptic domains (Triller and Choquet, 2005), become trapped in the synapses through interaction with gephyrin (Essrich et al., 1998). These observations raise the possibility that γ2- and δ-GABARs could be inserted via the same vesicles and that δ-GABARs remain extrasynaptic, while γ-GABARs move laterally and become incorporated at the synapses. Alternately, insertion of γ2- and δ-GABARs could be distinct.

We compared the rate of surface membrane appearance of γ2- and δ-GABARs in cultured hippocampal neurons and determined the influence of co-assembled α1 or α4 subunits on their exocytosis using γ2 and δ subunits tagged with the α-bungarotoxin binding site.

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http://dx.doi.org/10.1016/j.mcn.2013.05.003

Results

Insertion of t-γ2 and t-δ subunit-containing GABARs was distinct

Insertion of the receptors containing t-γ2 or t-δ subunits assembled with α1β3 subunits was determined in HEK293 cells. Cells expressing these receptors were incubated with unlabeled α-BT to block the binding site (BBS) on existing surface receptors and then incubated at 37 °C for 30 or 60 min. Newly inserted receptors were detected by incubation with labeled α-BT. Surface α-BT fluorescence (red) was evident in cells expressing α1β3t-γ2 subunit-containing receptors following incubation at 37 °C for 30 as well as 60 min (Fig. 1). In contrast, surface α-BT fluorescence was minimal in the cells expressing α1β3t-δ subunit-containing receptors following incubation at 37 °C for 30 min, but became visible after 60 min (Fig. 1). These observations raised the possibility that the time course of insertion of γ2- and δ-GABARs at the surface membrane could be distinct. In the subsequent studies the time course of insertion of γ2- and δ-GABARs was characterized.

Insertion of t-γ2 and t-δ subunit-containing receptors in HEK293 cells

Newly inserted α1β3t-γ2 subunit-containing receptors appeared within minutes (Fig. 2A). Surface fluorescence increased with time, until it reached a plateau at 30 min (Fig. 2A, Table 1); 42 ± 6% and 43 ± 6% of all surface-expressed receptors were newly inserted within 30 and 60 min, respectively. The normalized surface fluorescent area was plotted as a function of time (Fig. 2B). Previous studies have reported that the interaction between BBS and α-BT is stable over a period of 3 h (Bogdanov et al., 2006), and we also found a stable interaction (data not shown). Thus the increase in surface fluorescence was due to the appearance of new receptors at the surface membrane, and accordingly, the relationship was best described by a single-phase exponential association equation $Y = Y_0 + (Y_{max} - Y_0) * (1 - e^{(-k * time)})$, where Y_{max} was the IR area at the peak, Y_0 was the IR area at time 0, and k was the time constant in min^{-1}. The best fit of the data suggested an insertion time constant of 0.043 ± 0.007 min^{-1}, half-time was 12.5 ± 1.1 min (Fig. 2B).

We confirmed that the increase in surface fluorescence over time was due to the insertion of new receptors. The synthesis and insertion of receptors are mediated by temperature sensitive enzymes. Therefore incubation at room temperature is likely to slow insertion. Incubation at room temperature slowed the insertion of α1β3t-γ2 subunit-containing receptors (Fig. 2A, C). The surface α-BT fluorescence in the cells incubated at RT for 60 min was 0.036 ± 0.018 (n = 20 cells). Thus, only 11 ± 6% of all surface expressed receptors were newly inserted after 60 min of incubation at RT compared to 42 ± 6% at 37 °C ($p < 0.05$).

Additionally, blockade of ER to Golgi protein transport is also expected to inhibit surface insertion of newly synthesized receptors. Fungal toxin brefeldin-A blocks protein traffic from the ER to Golgi bodies by inhibiting ADP-ribosylation factor (ARF) (Helms and Rothman, 1992). Incubation at 37 °C for 60 min in the presence of brefeldin-A (5 μg/ml) also slowed the insertion (Fig. 2A, C), the surface α-BT fluorescence in these cells was 0.049 ± 0.007 (n = 15 cells), which corresponded to 22 ± 10% of all surface expressed receptors ($p < 0.05$).

The insertion of α1β3t-δ subunit-containing receptors was also studied. Initial appearance of α-BT surface fluorescence required 30 min for α1β3t-δ subunit-containing receptors, the surface fluorescence peaked at 120 min and remained stable thereafter (Fig. 3A,

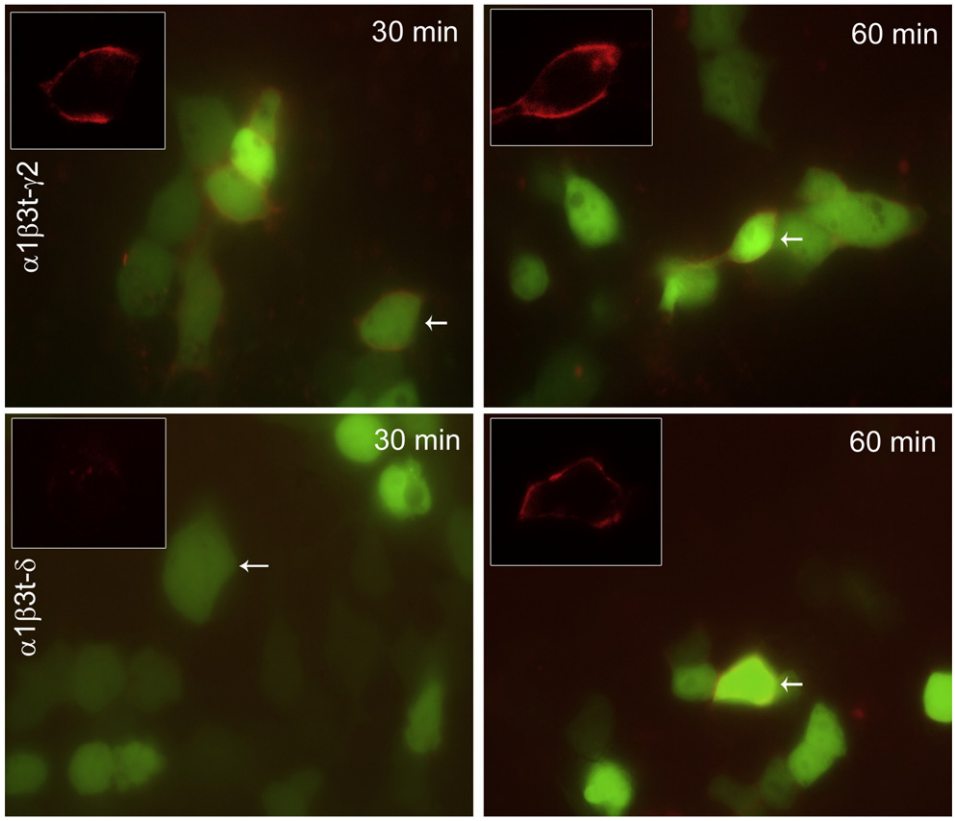

Fig. 1. Rapid appearance of γ2-GABARs on surface membrane of HEK293 cells. Surface α-BT fluorescence in the HEK293 cells expressing α1β3t-γ2 or α1β3t-δ subunit-containing receptors. Following blockade of existing surface receptors with unlabeled α-BT, the cells were incubated at 37 °C for 30 or 60 min, and newly inserted receptors were detected by incubation with labeled α-BT (red). GFP fluorescence was used to detect transfected cells. Insets in each panel show red fluorescence of surface α-BT in single cells from each panel to highlight the differences.