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$GABA_A$ receptor subunit composition and competition at synapses are tuned by $GABA_B$ receptor activity

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ABSTRACT

$GABA_BRs$ have a well-established role in controlling neuronal excitability and presynaptic neurotransmitter release. We examined the role of $GABA_BR$ activity in modulating the number and lateral diffusion of $GABA_ARs$ at inhibitory synapses. Changes in diffusion of $GABA_ARs$ at synapses were observed when subunit heterogeneity was taken into account. While α1-$GABA_ARs$ were unaffected, α2- and α5-$GABA_ARs$ showed inverse changes in enrichment and diffusion. The intracellular TM3-4 loop of α2 was sufficient to observe the changes in diffusion by $GABA_BR$ activity, whereas the loop of α5 was not. The opposing effect on α2- and α5-$GABA_ARs$ was caused by a competition between $GABA_ARs$ for binding slots at synapses. Receptor immobilization by cross-linking revealed that α5-$GABA_AR$ trapping at synapses is regulated by modulation of α2-$GABA_AR$ mobility. Finally, PKC activity was determined to be part of the signaling pathway through which $GABA_BR$ activity modulates α2-$GABA_AR$ diffusion at synapses. These results outline a novel mechanism for tuning inhibitory transmission in a subunit-specific manner, and for the first time describe competition between $GABA_ARs$ with different subunit compositions for binding slots at synapses.

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Introduction

Inhibitory transmission is at the forefront of research into the etiology and treatment of neurological diseases (Lüscher et al., 2011). Lateral diffusion of receptors in and out of synapses in the membrane is an important mechanism for determining $GABA_AR$ number in the synapse (Gerrow and Triller, 2010). This lateral mobility and confinement of $GABA_ARs$ at synapses can be modulated by interaction with scaffolding molecules, such as gephyrin (Maric et al., 2011; Mukherjee et al., 2011; Saiepour et al., 2010; Tretter et al., 2011). Diverse aspects of $GABA_AR$ trafficking remain poorly understood due to their heterogeneity. In the hippocampus, most $GABA_ARs$ are made with two α subunits (α1, α2, or α5), two β subunits (β1 or β3), and one γ2 subunit (Kittler et al. 2004, Tretter and Moss, 2008). α1- and α2-subunits both contain binding domains for gephyrin, and are mostly synaptic (Mukherjee et al., 2011; Tretter et al., 2008), whereas the α5-subunit is mainly found extrasynaptically (Brünig et al., 2002; Caraiscos et al., 2004; Wu et al., 2012). Monitoring receptor trafficking while accounting for subunit heterogeneity is important since $GABA_ARs$ with different subunit compositions have different pharmacological and physiological properties.

$GABA_BRs$ are g-protein-coupled receptors (GPCRs), ubiquitous in the central nervous system (CNS), and implicated in numerous neurological and psychiatric diseases (Gassmann and Bettler, 2012). Presynaptic $GABA_BRs$ are present at inhibitory and excitatory terminals, where they inhibit neurotransmitter release. Postsynaptic $GABA_BRs$ open G protein-activated inwardly rectifying potassium channels (GIRKs; also known as inwardly rectifying K+ Kir3 channels), which inhibit neuronal activity by hyperpolarizing the membrane, and preventing the opening of voltage-gated calcium channels. At excitatory synapses, activation of $GABA_BRs$ modulates plasticity via presynaptic and postsynaptic mechanisms (Chalifoux and Carter, 2010; Davies and Collingridge, 1996; Davies et al., 1991). At inhibitory synapses, previous studies have predominantly shown a presynaptic effect on GABA release (Otis and Mody, 1992). Whether $GABA_BR$ activity affects receptor trafficking at inhibitory synapses has been less studied. We show that $GABA_BR$ activity affects $GABA_AR$ enrichment at inhibitory synapses, in association with a change in their rate of lateral diffusion at synapses via PKC signaling. Interestingly, this effect was dependent on α-subunit composition, and revealed competition between $GABA_ARs$ with different subunit compositions for binding slots at synapses.

Results

$GABA_BR$ activity changes synaptic enrichment and diffusion of $GABA_ARs$

To characterize whether $GABA_BR$ activity influences $GABA_AR$ trafficking at inhibitory synapses, we used hippocampal neurons cultured from mRFP–gephyrin knock-in mice, >21 days in vitro, where synapses were identified by mRFP–gephyrin clusters, and using antibodies

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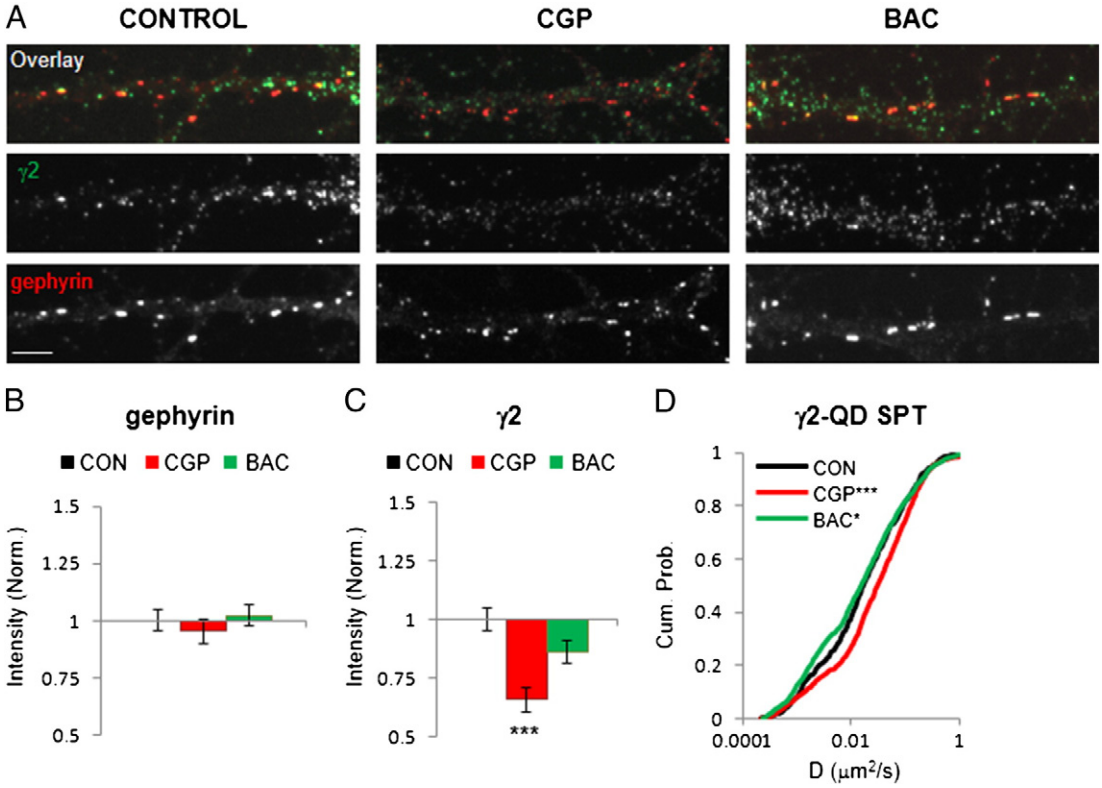

Fig. 1. $GABA_BR$ activity affects $GABA_AR$ enrichment and diffusion at synapses. (A) Representative images of γ2 subunit of $GABA_ARs$ (green) in cultured hippocampal neurons from mRFP–gephyrin (red) knock-in mice under control conditions, and after 1-hour incubation with the $GABA_BR$ antagonist CGP55845 (CGP, 1 μM) or baclofen (BAC, 10 μM). Scale bar = 5 μm. (B) Normalized gephyrin cluster intensity (mean ± s.e.m.) with CGP (red, 95.4 ± 5.4%, n = 40 neurons), and baclofen (green, 102 ± 4.8%, n = 52 neurons). (C) Normalized γ2 cluster intensity at gephyrin. CGP = 65.8.±2.6%, BAC = 86.0 ± 0.5. T-test, ***p < 0.001. (D) Cumulative probability of γ2-QD diffusion coefficient (log scale; Median; CON = 0.017 $\mu m^2/s$, n = 467 QDs; CGP = 0.031 $\mu m^2/s$, n = 832 QDs; BAC = 0.015 $\mu m^2/s$, n = 1070 QDs). KS test, *p < 0.05, ***p < 0.001.

against the γ2-subunit (Fig. 1A). Neurons were incubated for 1 h with the $GABA_BR$ antagonist CGP55845 (CGP, 1 μM), or agonist baclofen (BAC, 10 μM). No changes in gephyrin fluorescence were recorded compared with normalized controls (Fig. 1B). CGP decreased fluorescence intensity of γ2 at gephyrin compared with normalized controls (Fig. 1C, $p = 8 \cdot 10^{-9}$). BAC modestly decreased γ2 fluorescence intensity at gephyrin ($p = 0.047$). This postsynaptic change, where $GABA_BR$ activity alters the amount of γ2 independently of gephyrin, is a novel finding and suggests modulation of $GABA_AR$ trafficking by $GABA_BR$ activity.

$GABA_ARs$ diffuse laterally on the neuronal membrane surface, and trapping of diffusing receptors by scaffolding molecules determines receptor number at synapses (Gerrow & Triller, 2010). This trapping may be measured by changes in diffusion coefficient via quantum dot (QD)-based single particle tracking (SPT). Using this technique, we determined whether altering $GABA_BR$ activity effects $GABA_AR$ lateral mobility at synapses. In control neurons, γ2-QDs had diffusive behaviors at synapses as previously reported (Bannai et al., 2009). When $GABA_BR$ activity was decreased by CGP, cumulative analysis of the rate of diffusion revealed that γ2-QDs had a significantly higher diffusion coefficient, evidenced by a significant shift of the cumulative distribution curve to the right of the control curve (Fig. 1D; $p = 5 \cdot 10^{-6}$). When $GABA_BR$ activity was increased by BAC, a small decrease in the median diffusion coefficient was recorded, resulting in a shift of the cumulative distribution curve to the left of the control curve, particularly in the slowest quartile (Fig. 1D; $p = 0.02$). This suggests that $GABA_BR$ activity alters $GABA_AR$ accumulation at synapses by changing their lateral diffusion. However, the effects with BAC present an interesting paradox. Diffusion data suggest trapping of $GABA_ARs$ at synapses, whereas immunocytochemistry (ICC) suggests a loss of $GABA_ARs$. A possibility to be examined next, is that $GABA_BR$ activity affects a subset of $GABA_ARs$ as evidenced by the shift in diffusion in the slowest quartile.

Subunit-specific regulation of $GABA_AR$ diffusion by $GABA_BR$ activity

To determine how different $GABA_AR$ isoforms are modulated by $GABA_BR$ activity, we performed ICC and QD-SPT using antibodies against α1, α2, and α5. In the hippocampus, most synaptic $GABA_ARs$ contain α1 or α2, while α5-$GABA_ARs$ are generally extrasynaptic (Brünig et al., 2002; Caraiscos et al., 2004; Mukherjee et al., 2011; Tretter et al., 2008). Given the effect on γ2, a predominantly synaptic subunit, we hypothesized that $GABA_BR$ activity would affect α1 and/or α2 subunits, and have no effect on α5 at synapses.

All three α-subunits in our mRFP–gephyrin knock-in mice cultures showed a distribution in agreement with previous data and in vitro (Brünig et al., 2002; Tretter et al., 2008), and in situ (Pirker et al., 2000). Expression of α1 was highest in interneurons, and was clustered at gephyrin (Fig. 2A). α2 clusters also co-localized with gephyrin (Fig. 2B), whereas α5 expression was predominantly extrasynaptic (Fig. 2C). Correspondingly, each α-subunit had different diffusion properties at synapses. α1- and α2-QDs had slower median rates of diffusion, whereas α5-QD diffusion was consistent with molecules not particularly enriched at synapses, and had the fastest median rate of diffusion.

α1 containing $GABA_ARs$ showed no significant changes in receptor/gephyrin fluorescence intensity ratio (AU) with CGP or BAC compared with normalized controls (Fig. 2D). Diffusion properties, such as diffusion coefficient, were also unchanged (Fig. 2E). For α2 containing $GABA_ARs$, receptor/gephyrin fluorescence intensity ratio was decreased with CGP and increased with BAC (Fig. 2F, $p = 0.003$ and $5 \cdot 10^{-5}$). The diffusion coefficient of α2-QDs was increased with CGP (Fig. 2G, $p = 2 \cdot 10^{-8}$), and decreased with BAC ($p = 3 \cdot 10^{-7}$). These changes in enrichment and diffusion were unaffected by blockade of clathrin-mediated internalization (Fig. S1).

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