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BRAIN RESEARCH

Research Report

GABA acts as a ligand chaperone in the early secretory pathway to promote cell surface expression of $GABA_A$ receptors

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ABSTRACT

GABA (γ-aminobutyric acid) is the primary inhibitory neurotransmitter in brain. The fast inhibitory effect of GABA is mediated through the $GABA_A$ receptor, a postsynaptic ligand-gated chloride channel. We propose that GABA can act as a ligand chaperone in the early secretory pathway to facilitate $GABA_A$ receptor cell surface expression. Forty-two hours of GABA treatment increased the surface expression of recombinant receptors expressed in HEK 293 cells, an effect accompanied by an increase in GABA-gated chloride currents. In time-course experiments, a 1 h GABA exposure, followed by a 5 h incubation in GABA-free medium, was sufficient to increase receptor surface expression. A shorter GABA exposure could be used in HEK 293 cells stably transfected with the GABA transporter GAT-1. In rGAT-1HEK 293 cells, the GABA effect was blocked by the GAT-1 inhibitor NO-711, indicating that GABA was acting intracellularly. The effect of GABA was prevented by brefeldin A (BFA), an inhibitor of early secretory pathway trafficking. Coexpression of $GABA_A$ receptors with the GABA synthetic enzyme glutamic acid decarboxylase 67 (GAD67) also resulted in an increase in receptor surface levels. GABA treatment failed to promote the surface expression of GABA binding site mutant receptors, which themselves were poorly expressed at the surface. Consistent with an intracellular action of GABA, we show that GABA does not act by stabilizing surface receptors. Furthermore, GABA treatment rescued the surface expression of a receptor construct that was retained within the secretory pathway. Lastly, the lipophilic competitive antagonist (+)bicuculline promoted receptor surface expression, including the rescue of a secretory pathway-retained receptor. Our results indicate that a neurotransmitter can act as a ligand chaperone in the early secretory pathway to regulate the surface expression of its receptor. This effect appears to rely on binding site occupancy, rather than agonist-induced structural changes, since chaperoning is observed with both an agonist and a competitive antagonist.

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1. Introduction

The $GABA_A$ receptor is an inhibitory neurotransmitter receptor associated with a variety of neurological and psychiatric disorders and is the target of several classes of therapeutic agents (Whiting, 2003). It has been estimated that approximately 30% of synapses in the brain contain $GABA_A$ receptors (Nutt, 2006). The receptor mediates the fast inhibitory actions of the ubiquitous neurotransmitter γ-aminobutyric acid (GABA). Upon binding GABA, an integral chloride channel within the receptor is gated, allowing chloride influx and leading to membrane hyperpolarization.

$GABA_A$ receptors belong to the cys-loop ligand-gated ion channel family, whose other members include the nicotinic acetylcholine, glycine and 5-HT3 receptors (Lester et al., 2004). Members of the cys-loop family of ligand-gated ion channels are pentameric in structure, with each subunit possessing a large extracellular N-terminus, four membrane spanning domains (M1–M4), a large cytoplasmic loop between M3 and M4, and an extracellular C-terminus. At least 17 $GABA_A$ receptor subunits have been identified. Assembly of the receptor is defined by discrete oligomerization residues that selectively limit the type of pentameric assemblages (Bollan et al., 2003). Although many receptor subtypes exist, the predominant receptor subtype in brain is composed of α1β2γ2 subunits, with two copies each of α1 and β2 subunits (McKernan and Whiting, 1996). Two GABA binding sites are present on each pentamer, with the GABA binding pocket formed at the αβ subunit interface by the subunit N-terminal regions (Amin and Weiss, 1993).

Dynamic cell surface expression of neurotransmitter receptors, including the $GABA_A$ receptor, has been intensely studied in recent years. It is now recognized that the regulation of receptor trafficking is an important mechanism for controlling synaptic efficacy and plasticity (Collingridge et al., 2004; Jacob et al., 2008; Luscher and Keller, 2004; Newpher and Ehlers, 2008). While receptor endocytosis and recycling are established determinants of receptor cell surface expression (Leidenheimer, 2008), the roles that receptor biogenesis and secretory pathway trafficking play in maintaining receptor surface populations are largely unexplored. It remains to be determined whether these processes are specifically regulated or are simply default processes governed by subunit translation rates and the endoplasmic reticulum (ER) quality control machinery.

It has been reported that mutation of agonist binding residues on glutamate receptors results in their ER retention, leading to the speculation that agonist occupancy is required for glutamate receptor biogenesis (Coleman et al., 2009; Fleck, 2006; Gill et al., 2009; Mah et al., 2005; Priel et al., 2006; Valluru et al., 2005). The ER retention of receptor ligand binding site mutants, however, may reflect terminal misfolding of the receptor subunits due to the mutation, rather than due to the absence of ligand binding. Thus, the hypothesis that neurotransmitter receptor biogenesis may be facilitated by, or require, the binding of neurotransmitter remains to be tested. Here, using recombinant $GABA_A$ receptors, we provide the first demonstration, to our knowledge, that a neurotransmitter can act as a ligand chaperone in the endoplasmic reticulum to promote the cell surface expression of its receptor.

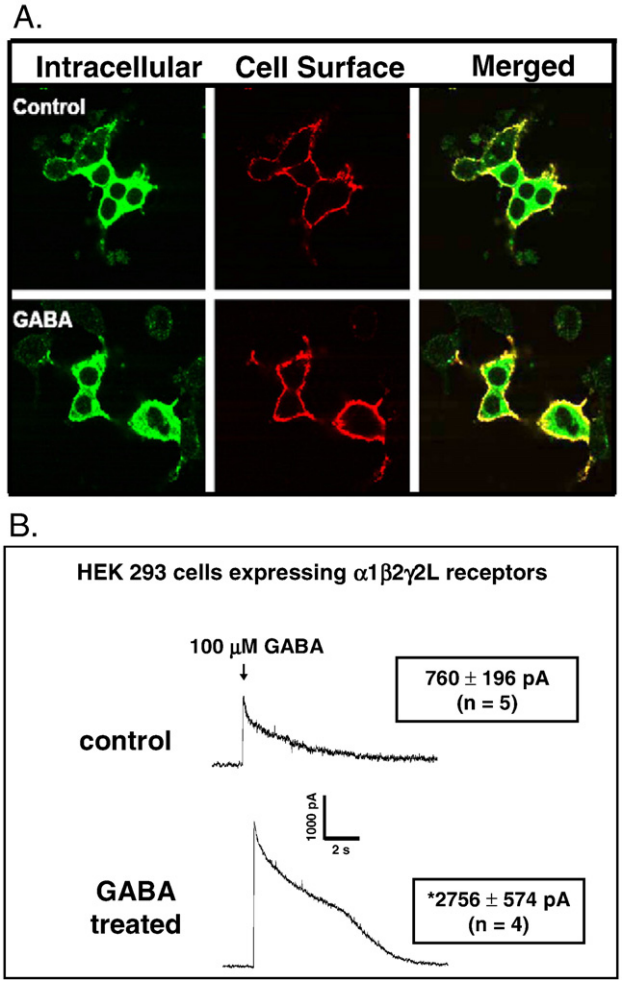

Fig. 1 – GABA treatment promotes surface expression of $GABA_A$ receptors composed of α1β2γ2L subunits. A) HEK 293 cells were transfected with α1, β2, and $γ2L^{V5}$ subunit cDNAs and incubated throughout the transfection and expression period in the absence or presence of 100 μM GABA. Forty-two hours post-transfection, cell surface and intracellular $GABA_A$ receptor populations were labeled by indirect immunofluorescence using an anti-V5 antibody and imaged by confocal microscopy (images representative of 20 independent experiments each performed in triplicate). B) The whole-cell patch-clamp technique was used to measure GABA-gated chloride currents. Following a 42 h GABA incubation period, GABA-containing medium was removed from culture dishes and replaced with GABA-free medium for at least 2 h prior to electrophysiological recording to avoid receptor desensitization from prolonged GABA treatment. GABA-gated chloride current peak amplitudes were measured in response to GABA (100 μM) applied with a solenoid-controlled superfusion system. Asterisk indicates that GABA-gated chloride current peak amplitudes were significantly different between cells incubated in GABA for 42 h. vs. control (*, $p \leq 0.01$, unpaired t-test).