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BRAIN RESEARCH

Research Report

Investigating the role of protein folding and assembly in cell-type dependent expression of α7 nicotinic receptors using a green fluorescent protein chimera

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ARTICLE INFO

Article history:
Accepted 17 January 2009
Available online 3 February 2009

Keywords:
Multimeric protein assembly
Alpha7 nicotinic subunit
GFP
Alpha-bungarotoxin binding
Cell-dependent expression
Fluorescent protein

ABSTRACT

To test the hypothesis that cell-dependent expression of α7 receptors is due to differences in protein folding or assembly, we constructed a chimeric rat α7 subunit with green fluorescent protein (GFP) at the receptor C-terminal. Expression of α7-GFP in *Xenopus* oocytes resulted in currents that were indistinguishable from wild type receptors but were only 33% of control. ^{125}I-α-bungarotoxin (αBGT) binding at the oocyte surface was reduced to 23% of wild type. Transfection of α7-GFP into GH4C1 cells produced fluorescence that was less intense than GFP alone, but showed significant α-BGT binding compared to transfection with GFP. In contrast, α7-GFP transfection in SH-EP1, HEK293 and CHO-CAR cells produced fluorescence without αBGT binding. Flow cytometry of cells transfected with α7-GFP indicated fluorescence in both SH-EP1 and GH4C1 cells, but surface toxin binding sites and sites immunoprecipitated using anti-GFP antibodies were undetectable in SH-EP1 cells, suggesting a problem in folding/assembly rather than trafficking. Surprisingly, integrated fluorescence intensities in GH4C1 cells transfected with α7-GFP did not correlate with amounts of cell surface or immunoprecipitable αBGT binding. Therefore, GFP folding at the C-terminal of the α7-GFP chimera is cell-line independent, but toxin binding is highly cell-line dependent, suggesting that if altered protein folding is involved in the cell-type dependence of α7 receptor expression, the phenomenon is restricted to specific protein domains. Further, C-terminal GFP-labeled α7 receptors decreased the efficiency of folding/assembly not only of chimeric subunits, but also wild-type subunits, suggesting that the C-terminal is an important domain for α7 receptor assembly.

1. Introduction

Neuronal nicotinic acetylcholine receptors (nAChRs) exist as pentamers but show great pharmacological diversity due to various combinations of subunits. Twelve subunits (α2–α10 and β2–β4) have been identified and cloned (Arneric et al., 2007). α7 subunits (Couturier et al., 1990), α8 subunits cloned from chick (Schoepfer et al., 1990), and α9 subunits (Elgoyhen et al., 1994) all form functional nAChR in oocytes that are blocked by the snake toxin α-bungarotoxin (α-BGT). Of these,

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0006-8993/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.brainres.2009.01.046

α7-containing receptors are the most widely expressed in the nervous system and are involved in enhancing fast excitatory transmission through presynaptic mechanisms (McGehee et al., 1995), regulating peripheral inflammatory responses (de Jonge and Ulloa, 2007), motoneuronal death (Hory-Lee and Frank, 1995), and neural development (Liu et al., 2007).

Although α7 is widely expressed in the CNS (Dominguez del Toro et al., 1994), successful functional expression of this receptor subtype has been restricted to relatively few mammalian cell lines (Gopalakrishnan et al., 1995; Kassner and Berg, 1997; Puchacz et al., 1994; Quik et al., 1996). Cooper and Millar (1997) reported that FLAG-epitope labeled α7 receptors are expressed in a variety of cell lines after transfection with plasmids, although no α-BGT binding was detectable. In contrast, two cell lines that express endogenous α7 receptors, rat PC12 cells and human neuroblastoma cells (SH-SY5Y) successfully express FLAG-labeled α7 receptors that bind alpha-bungarotoxin. Sweileh et al. (2000) report similar results using adenovirus to insert the gene for rat α7 into cell lines. Of five cell lines tested, only two cell lines, GH4C1 from rat pituitary and SH-EP1 from human neuroblastoma, expressed immunoprecipitable α7 receptors that bound ^{125}I-αBGT. However, all five cell lines expressed significant quantities of α7 protein detected on Western blots. These and other studies (e.g. Aztiria et al., 2000) suggest that the capacity of cells to properly fold and/or process α7 receptor subunits may be limited to only a few cell lines. Further studies suggest that specific amino sequences in the α7 subunit confer this property (Dineley and Patrick, 2000), and that functional expression of α7 receptors requires the proper formation of disulfide bonds and/or palmitoylation within the subunits (Drisdel et al., 2004; Rakhilin et al., 1999). This phenomenon that α7 receptors are expressed as protein, but not processed into functional receptors may occur in vivo. For instance, Domingez del Toro et al. (1994) report that Purkinje cells in rat cerebellum are strongly immunopositive for α7 receptor, but little or no ^{125}I-αBGT binding is observed in rat cerebellum (Clarke et al., 1985).

Cooper and Millar (1997) reported that conformational-dependent antibodies failed to bind to α7 receptors expressed in non-permissive cell lines, suggesting that protein folding plays an important role in α7 receptor expression. This hypothesis is attractive since α7 receptor subunits are easily denatured, and often appear on Western blots as high molecular weight aggregates, even in cell lines that express functional α7 receptors (Cooper and Millar, 1998; Pugh et al., 1995). To further characterize α7 receptor folding, assembly and transport to the cell surface, we constructed a chimeric rat α7-subunit with green fluorescent protein (GFP) at the receptor C-terminal. GFP fluorescence is absolutely dependent on the proper folding of the active center (Prendergast, 1999) and has been used as an indicator of folding in chimeric proteins (Waldo et al., 1999). Preliminary accounts of these experiments have been reported (Loring et al., Soc. Neurosci. Abstr. 2001, 2008).

2. Results

2.1. *Expression of α7-GFP in oocytes*

Injection of α7-GFP mRNA into *Xenopus* oocytes gives rise to currents induced by application of varying concentrations of acetylcholine (Fig. 1A). The shape of the response is similar to that of wild-type α7, demonstrating rapid desensitization. However, the magnitude of the α7-GFP response is only

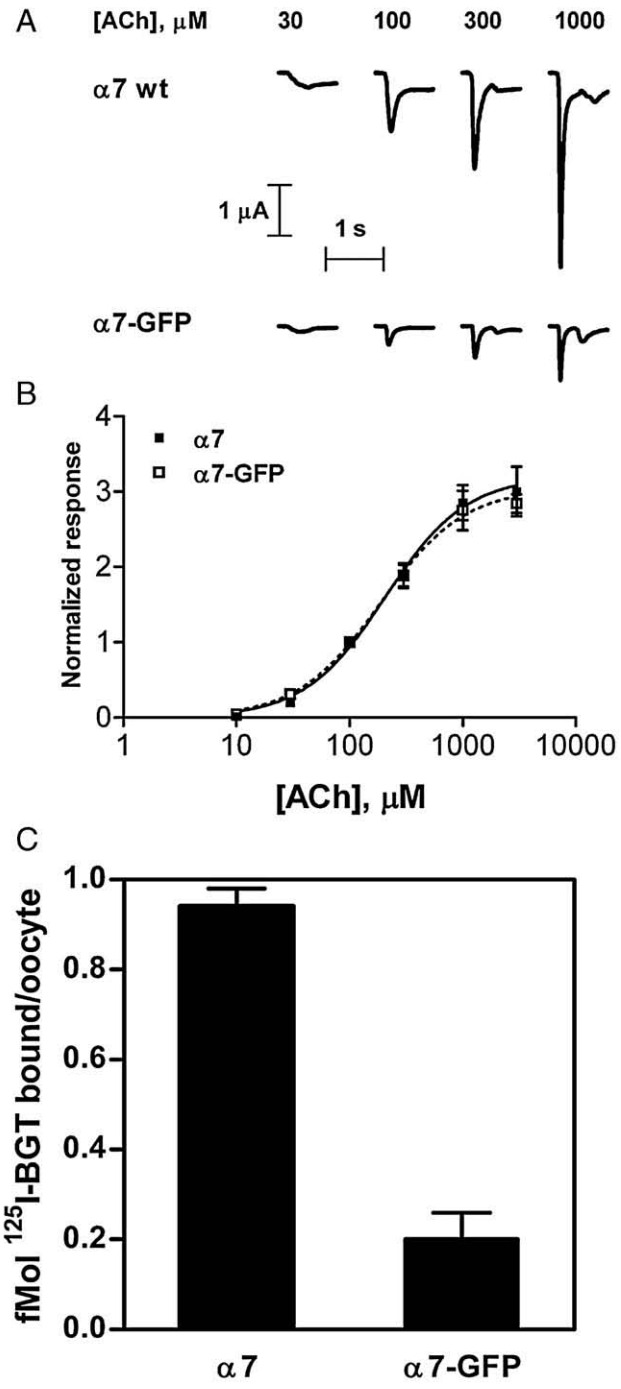

Fig. 1 – Expression of a7-GFP in *Xenopus* oocytes. Injection of α7 (top panel A) or α7-GFP mRNA (bottom panel A) gives rise to currents induced by application of varying concentrations of ACh. Note that expression of α7-GFP is approximately 30% of the wild-type receptor. Panel B shows the normalized response to ACh (100 μM=1), suggesting no gross change in the response of α7-GFP receptors compared to wild-type α7. Specific [^{125}I]α-BGT binding per oocyte is plotted in panel C. Note that α7-GFP binding is about 30% of α7 wild type.