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Glycinergic input of small-field amacrine cells in the retinas of wildtype and glycine receptor deficient mice

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Amacrine cells are known to express strychnine-sensitive glycine receptors (GlyRs), however, it is not known which of the four GlyRα subunits (α1–4) are expressed in this diverse group of cells. Herein, we studied the presence of glycine activated currents and spontaneous inhibitory postsynaptic currents (sIPSCs) of amacrine cells in the mouse retina. By recording glycinergic currents in retinal slices of wildtype mice and of mice deficient in GlyRα subunits (*Glra1*$^{spd\text{-}ot}$, *Glra2*$^{-/-}$, *Glra3*$^{-/-}$), we could classify AII and narrow-field amacrine cells (NF, Types 5, 6, 7) on the basis of their α-subunit composition. Glycinergic sIPSCs of AII cells displayed medium fast kinetics (mean decay time constant $\tau = 11 \pm 2$ ms), which were completely absent in the *Glra3*$^{-/-}$ mouse, indicating that synaptic GlyRs of AII cells mainly contain the α3 subunit. Glycinergic sIPSCs of NF cells had slow kinetics ($\tau = 27 \pm 6.8$ ms) that were significantly prolonged in *Glra2*$^{-/-}$ mice ($\tau = 69 \pm 16$ ms). These data show that morphologically distinct amacrine cells express different sets of GlyRs.

Keywords: Glycine receptors; sIPSCs; AII amacrine cells; *Glra1*$^{spd\text{-}ot}$ mice; *Glra2*$^{-/-}$ mice; *Glra3*$^{-/-}$ mice

Introduction

The mammalian retina contains more than 30 different types of amacrine cells (Masland, 2001) comprising two major functional classes, GABAergic and glycinergic amacrine cells. GABAergic amacrine cells are wide-field cells providing lateral interactions across the inner plexiform layer (IPL) (Lin and Masland, 2006). Glycinergic amacrine cells are small-field cells, whose dendrites are primarily involved in local interactions between the different sublaminas of the IPL, such as the OFF- and ON-sublaminas. The most prominent and best characterized glycinergic small-field amacrine cell is the AII amacrine cell, which is involved in signal transfer from the rod to the cone pathway (Kolb and Famiglietti, 1974; Famiglietti and Kolb, 1975). In addition, up to 11 further small-field amacrine cells have been identified in different species, of which at least 8 are glycine-positive (Pourcho and Goebel, 1985; Menger et al., 1998; MacNeil and Masland, 1998; Badea and Nathans, 2004).

Glycinergic amacrine cells receive input from bipolar cell axons through glutamatergic ribbon synapses. In addition, they also receive input from other amacrine cells, both glycinergic and GABAergic, through conventional chemical synapses. Glycinergic amacrine cells project their output synapses to bipolar cell axons, ganglion cell dendrites and amacrine cell processes (Dowling and Boycott, 1966; Pourcho and Owczarzak, 1991).

The glycine receptor (GlyR) is a pentameric ligand-gated chloride channel that is composed either of only α subunits (extrasynaptic GlyRs; for review see Betz and Laube, 2006), or of two α and three β subunits (synaptic GlyRs; see Grudzinska et al., 2005). To date, four GlyRα subunit isoforms (α1, α2, α3 and α4) have been identified in mammals, with alternative splicing providing further diversity (reviewed by Legendre, 2001; Lynch, 2004; Betz and Laube, 2006). In contrast, only a single GlyRβ subunit gene is known. GlyRs containing β subunits are concentrated at inhibitory synapses due to their interaction with gephyrin, a synaptic scaffolding protein that forms a submembraneous hexagonal lattice (reviewed by Kneussel and Betz, 2000). All GlyRα subunits have been localized immunocytochemically at mouse retinal synapses, with most synapses containing only a single type of α subunit (Haverkamp et al., 2003, 2004; Heinze et al., 2007).

In the present study, the glycinergic input of small-field amacrine cells was characterized in slices of mouse retinas. The cells were identified morphologically by the injection of a fluorescent tracer. Glycine induced currents and spontaneous inhibitory synaptic currents (sIPSCs) were recorded in wildtype mice and mice with specific GlyRα subunit deletions. The spontaneous mouse mutant *Glra1*$^{spd\text{-}ot}$ has a complete loss of the α1 subunit (Buckwalter et al., 1994; Kling et al., 1997). The knock out line *Glra3*$^{-/-}$ lacks GlyRα3 subunits (Haverkamp et al., 2003; Harvey et al., 2004). A GlyRα2

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subunit deficient mouse line was created for the present study. Our comparison of the kinetics of sIPSCs in wildtype and the different mutant mice indicates that morphologically distinct small-field amacrine cells express different sets of GlyRs.

Results

Characterization of a Glra2$^{-/-}$ mouse line

Mouse lines lacking the GlyR α1 and α3 subunits have been characterized previously (Buckwalter et al., 1994; Harvey et al., 2004). Mice deficient for the α2 subunit were not available when this project was initiated. Consequently, we obtained a newly generated *Glra2*$^{-/-}$ line from Lexicon Genetics Inc. (Texas, USA). As detailed in Fig. 1A, an embryonic stem (ES) cell line was produced in which exon 1 of the *Glra2* gene located on chromosome X was replaced with a lacZ/neomycin selection cassette. Positive clones were confirmed by Southern Blot and PCR analysis (data not shown). Subsequently, this ES cell line was used to generate mice carrying one copy of the modified allele. These mice and their offspring were bred to homozygosity to produce *Glra2*$^{-/-}$ mice. *Glra2*$^{-/-}$ mice displayed no overt phenotype, had normal weight and life expectancy and were fertile (data not shown). This is in agreement with results reported for another recently generated *Glra2*$^{-/-}$ mouse line (Young-Pearse et al., 2006). Animals were genotyped using two different PCR strategies, each being specific for either the wild-type or mutant allele (Fig. 1B). Reverse transcriptase PCR (RT-PCR) using two (5′ and 3′) different PCR primer sets demonstrated the absence of α2 transcripts in the *Glra2*$^{-/-}$ brain as compared to wildtype (Fig. 1C). However, mRNAs transcribed from the other *Glra* genes and the *Glrb* gene were readily detected in *Glra2*$^{-/-}$ brain cDNA (Fig. 1C). In addition, the expression of gephyrin as detected by RT-PCR also appeared to be unaffected in *Glra2*$^{-/-}$ tissue (Fig. 1C). As there are currently no specific antibodies that allow selective detection of the α2 subunit by Western blotting, it was not possible to demonstrate the absence of α2 protein biochemically. However, immunohistochemistry with an antibody that stains GlyRα2 in tissue sections (Haverkamp et al., 2004) revealed that GlyR α2 immunoreactivity is absent in the IPL of *Glra2*$^{-/-}$ retinas (Fig. 2B). In wildtype retinas, the GlyR α2 staining is uniformly distributed across strata 1–4 of the

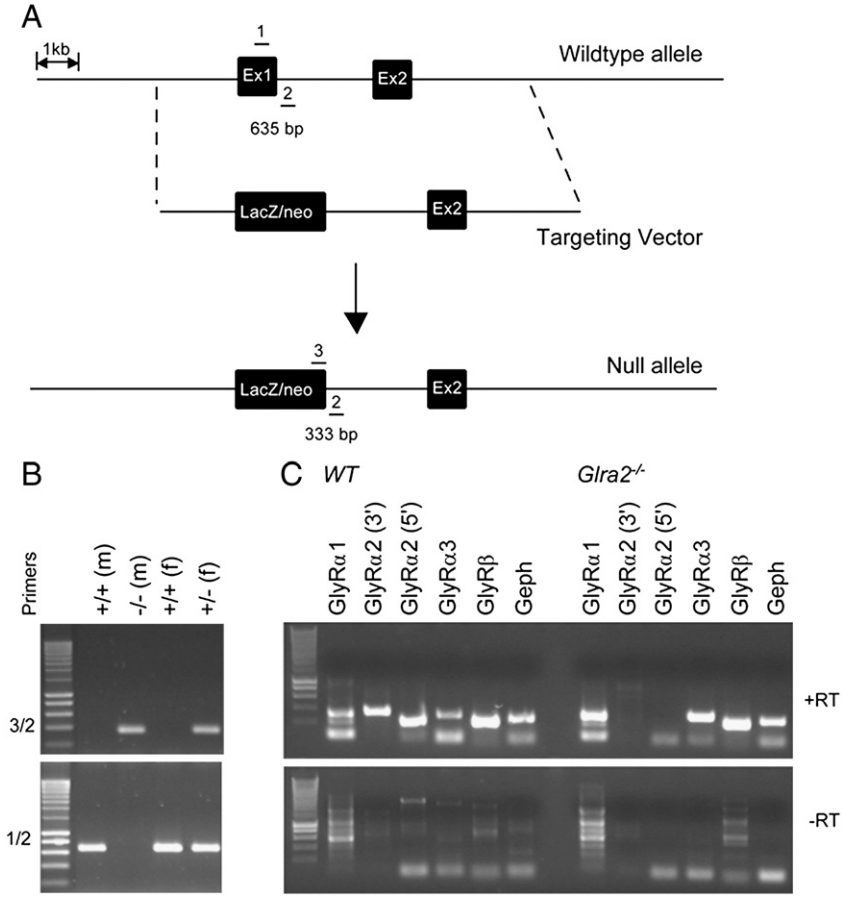

Fig. 1. Characterization of a *Glra2*$^{-/-}$ mouse line. (A) Targeting strategy used to generate a *Glra2* knockout allele. Exon 1 (Ex1) of the *Glra2* wildtype allele was replaced with a fused lacZ/Neomycine (LacZ/Neo) cassette via homologous recombination using a targeting vector to generate a *Glra2* Null allele. (B) Determination of mice *Glra2* genotype using PCR. Primers #1 and #2 produced as expected a 635 bp band in only +/+ and +/− mice but not in −/− mice. Primers #2 and #3 produced as predicted a 333 bp band in only +/− and −/− mice but not in +/+ mice; male (m), female (f). (C) RT-PCR analysis confirmed that this *Glra2*$^{-/-}$ mouse line lacks GlyRα2 subunit mRNA. Brain cDNA was generated from wildtype and *Glra2*$^{-/-}$ genotyped animals, for RT-PCR (+RT) with two different sets of primers matching the 5′ end (GlyRα2(5′)) and 3′ end (GlyRα2(3′)) of the GlyRα2 subunit mRNA. GlyRα1, GlyRα3, GlyRβ and gephyrin (Geph) transcripts were detected in both +/+ and −/− brains, whereas mRNA for the GlyRα2 subunit was present only in +/+ brain. Equivalent RT-PCR samples lacking the Reverse Transcriptase (−RT) confirmed that the PCR products in the +RT samples originated from their respective mRNAs.