All Amacrine Cells in the Mammalian Retina Show Disabled-1 Immunoreactivity

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ABSTRACT

Disabled 1 (Dab1) is an adapter molecule in a signaling pathway, stimulated by Reelin, which controls cell positioning in the developing brain. It has been localized to AII amacrine cells in the mouse and guinea pig retinas. This study was conducted to identify whether Dab1 is commonly localized to AII amacrine cells in the retinas of other mammals. We investigated Dab1-labeled cells in human, rat, rabbit, and cat retinas in detail by immunocytochemistry with antisera against Dab1. Dab1 immunoreactivity was found in certain populations of amacrine cells, with lobular appendages in the outer half of the inner plexiform layer (IPL) and a bushy, smooth dendritic tree in the inner half of the IPL. Double-labeling experiments demonstrated that all Dab1-immunoreactive amacrine cells were immunoreactive to antisera against calretinin or parvalbumin (i.e., other markers for AII amacrine cells in the mammalian retina) and that they made contacts with the axon terminals of the rod bipolar cells in the IPL close to the ganglion cell layer. Furthermore, all Dab1-labeled amacrine cells showed glycine transporter-1 immunoreactivity, indicating that they are glycinergic. The peak density was relatively high in the human and rat retinas, moderate in the cat retina, and low in the rabbit retina. Together, these morphological and histochemical observations clearly indicate that Dab1 is commonly localized to AII amacrine cells and that antisera against Dab1 is a reliable and specific marker for AII amacrine cells of diverse mammals. J. Comp. Neurol. 470:372–381, 2004. © 2004 Wiley-Liss, Inc.

Indexing terms: parvalbumin; calretinin; glycine transporter-1; protein kinase C; rod bipolar cells; immunocytochemistry

Amacrine cells constitute the most diverse group of cell types in the retina in terms of morphology, size, and distribution. They appear to make up approximately 40% of all the neurons of the inner nuclear layer (INL). By using several techniques, such as Golgi staining (Dowling and Boycott, 1969; Kolb and Nelson, 1981; Mariani, 1990), intracellular injection with Lucifer yellow (Tauchi and Masland, 1986; Sandell and Masland, 1984; Vaney, 1984, 1986; Menger et al., 1998), and photofilling (MacNeil and Masland, 1998; MacNeil et al., 1999), 20–30 distinct types of amacrine cells have been identified in the mammalian retina (Masland, 1988; Vaney, 1990; Wässle and Boycott, 1991; Strettoi and Masland, 1996; MacNeil and Masland, 1998; MacNeil et al., 1999). Among these cells, one of the best characterized amacrine cells is the AII amacrine cell, which is a bistratified rod amacrine cell that has been described previously (Famiglietti and Kolb, 1975; Kolb, 1984; Strettoi and Masland, 1996; MacNeil and Masland, 1998; MacNeil et al., 1999).
The AII amacrine cells form one part of a postsynaptic dyad at the ribbon synapse of rod bipolar cells in the inner plexiform layer (IPL), close to the ganglion cell layer (GCC; Kolb and Famiglietti, 1974; McGuire et al., 1984). They also make conventional chemical synapses with OFF-cone bipolar and OFF-ganglion cells and contact ON-cone bipolar cells via large gap junctions (Kolb and Famiglietti, 1974; Strettoi et al., 1992; Chun et al., 1993; Grünert and Wässle, 1996). AII amacrine cells are depolarized in response to light (Dacheux and Raviola, 1986; Boos et al., 1993), and stimulation of all AII amacrine cells results in the hyperpolarization of OFF-cone bipolar and OFF-ganglion cells via inhibitory chemical synapses and the depolarization of ON-cone bipolar cells via gap junctions. In this way, all amacrine cells play crucial roles in the rod-driven circuit in the retinas of various mammals.

Different methods have been used to identify AII amacrine cells in mammals. For example, in the cat and rabbit retinas, AII amacrine have been labeled by selective staining with a fluorescent dye (Vaney, 1985; Mills and Massey, 1991; Vaney et al., 1991). In immunocytochemical methods, different markers have been used across species. An antibody against parvalbumin has been used to label AII amacrine cells in the rat, cat, and rabbit retinas (Gabriel and Strauznick, 1992; Chun et al., 1993; Wässe et al., 1995; Casini et al., 1995). Antisera against calretinin also label AII amacrine cells in the rabbit (Mills and Massey, 1991; Massey and Mills, 1999), cat (Pastels et al., 1990), and macaque monkey (Wässe et al., 1995; Mills and Massey, 1999; Kolb et al., 2002) retinas. However, in addition to AII amacrine cells, additional types of amacrine cells were labeled by an antibody against parvalbumin or calretinin in these mammalian retina. Recently, antisera against Disabled 1 (Dab1), an adapter molecule in a signaling pathway stimulated by Reelin, which controls cell positioning in the developing brain (Rice and Curran, 1999), has been shown to label AII amacrine cells selectively in the mouse (Rice and Curran, 2000) and guinea pig (Lee et al., 2003) retinas.

In the present study, we describe Dab1 immunoreactivity patterns and characterize Dab1-immunoreactive amacrine cells both morphologically and quantitatively in different species. The Dab1 staining patterns and densities in human, rat, rabbit, and cat retinas were compared with previous markers for AII amacrine cell in various mammals to establish whether Dab1 antisera is a common, specific, and reliable marker for AII amacrine cells in these species.

MATERIALS AND METHODS

Tissue preparation

In total 12 mammalian retinas were examined, from Sprague-Dawley rat (six retinas), New Zealand white rabbit (two retinas), and human (two retinas). The animals were treated according to the regulations of the Catholic Ethics Committee of the Catholic University of Korea, Seoul, which conform to all NIH guidelines. The animals were deeply anesthetized by intraperitoneal injection of 4% chloral hydrate (1 ml/100 g body weight), and the eyes were enucleated. The anterior segments were then removed, and the eyecups were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4, for 2–3 hours. Human eyes were obtained through the eye bank of St. Mary’s Hospital of the Catholic University of Korea. The eyes were enucleated and fixed in 4% paraformaldehyde in PB and sent to our laboratory within 24 hours after the death of the donor. After fixation, the retinas were carefully dissected and transferred to 30% sucrose in PB for 24 hours at 4°C. They were then frozen in liquid nitrogen, thawed, and rinsed in 0.01 M phosphate-buffered saline (PBS; pH 7.4).

Immunocytochemistry

For fluorescence immunocytochemistry, 50-μm-thick vibratome sections were incubated in 10% normal goat serum (NGS) and 1% Triton X-100 in PBS, for 1 hour at room temperature, to block nonspecific binding sites. The sections were then incubated overnight with a rabbit polyclonal antibody directed against Dab1 [the anti-Dab1 antiserum was developed in rabbit by using the peptide C-terminal (CEPPSGGDNISPQDGS) that correspond to the mDab555 sequence beginning at residue 542 (Howell et al., 1997); kindly provided by Dr. B. Howell, NIH] at a dilution of 1:1,000 in PBS containing 0.5% Triton X-100 at 4°C. Retinas were washed in PBS for 45 minutes (3 × 15 minutes) and incubated for 2 hours in carboxymethylidocyanine (Cy3)-conjugated affinity-purified anti-rabbit IgG (1:100; Jackson, West Grove, PA) at room temperature. Sections were then washed for 30 minutes with 0.1 M PB and coverslipped with 10% glycerol in 0.1 M PB. For whole-mount immunostaining, the same immunocytochemical procedures as described above were used, but with longer incubation times. Images were imported into Adobe Photoshop 5.5 (Adobe Systems, Mountain View, CA), and, for presentation, all manipulations (brightness and contrast only) were carried out equally.

For double-label studies, sections were incubated overnight in a mixture of anti-Dab1 antibody (1:1,000) with the following antibodies: monoclonal antiparvalbumin (1:500 dilution; Sigma, MO), monoclonal anticalretinin (1:1,000 dilution; Chemicon, Temecula, CA), monoclonal antiprotein kinase C (PKC; 1:500 dilution; Sigma), and goat polyclonal anticytotoxic transporter-1 antibody (1:10,000 dilution; Chemicon) with 0.5% Triton X-100 in 0.1 M PBS at 4°C.

Sections were rinsed for 30 minutes with 0.1 M PBS and incubated with fluorescein isothiocyanate (FITC)-conjugated affinity-purified anti-mouse IgG or anti-goat IgG (1:100 dilution; Jackson) or with Cy3-conjugated anti-rabbit IgG (1:100 dilution; Jackson) for 1–2 hours at room temperature. Sections were then washed for 30 minutes with 0.1 M PB and coverslipped with 10% glycerol in 0.1 M PB. To confirm that the secondary antibody did not cross-react with an inappropriate primary antibody, some sections were incubated in rabbit and goat polyclonal primary antibody, followed by anti-mouse secondary antibody, and other sections were incubated in mouse primary antibody, followed by anti-rabbit secondary antibody. These sections showed no immunostaining.

Confocal laser scanning microscopy

Sections were analyzed with a Bio-Rad Radiance Plus (Bio-Rad, Hemel Hempstead, United Kingdom) confocal scanning microscope installed on a Nikon Eclipse E800 fluorescence microscope (Nikon, Tokyo, Japan). FITC and Cy3 signals were always detected separately. FITC labeling was excited using the 488-nm line of an argon ion laser and detected after passing an HQ513/30 (Bio-Rad) emis-
sion filter. For the detection of the Cy3 signal, the 543-nm line of a green HeNe laser was used in combination with the 605/52 (Bio-Rad) emission filter. Images were imported into Adobe Photoshop 5.5 (Adobe Systems) and photographed on slide film (Kodak Ektachrome 100; Eastman Kodak). For presentation, all manipulations (brightness and contrast only) were carried out equally.

**Topography and quantitation**

The topography of the Dab1-immunoreactive cell populations was analyzed, and these populations were quantified in well-stained retinas of human, rat, rabbit, and cat. The data for the density maps were plotted with conventional microscopy. For the density maps, a field of 200 × 200 µm² was sampled in 1-mm steps in the retina from superior to inferior. Nearest-neighbor analysis (Wässle and Riemann, 1978) was performed on the cells located in the midperipheral regions of the retina. The results were not corrected for shrinkage of the tissue during the mounting process, because this was negligible.

**RESULTS**

Dab1 immunoreactivity in the mammalian retina

In all mammalian retinas investigated, Dab1 immunoreactivity was found in a distinct subpopulation of amacrine cells (Fig. 1A–D). The somata of Dab1-immunoreactive cells were strongly immunostained and were found in the INL adjacent to the IPL border (Fig. 1A–D). These amacrine cells typically have a single primary dendrite that descends into the IPL and then gives off several side branches that run toward stratum 5 near the GCL. In sublamina a, short immunoreactive processes with large, irregular endings originate from the primary process. These processes resemble the lobular appendages previously described for AII amacrine cells of rabbit, cat, rat, monkey, mouse, and guinea pig retinas (Famiglietti and Kolb, 1975; Vaney, 1985; Dacheux and Raviola, 1986; Wong et al., 1986; Young and Vaney, 1990; Mills and Massey, 1991; Vaney et al., 1991; Strettoi et al., 1992; Wässele et al., 1993, 1995; Rice and Curran, 2000; Lee et al., 2003). Therefore, Dab1 immunoreactivity appeared in the same amacrine cell population in all species examined in the present study. However, in the human and cat retinas, Dab1 immunoreactivity was visible in other types of neurons in addition to amacrine cells. In the human retina (Fig. 1A), Dab1 immunoreactivity was visible in other types of neurons in addition to amacrine cells. In the human retina (Fig. 1A), Dab1 immunoreactivity was visible in the outer segments of photoreceptor cells, in the horizontal cells that form a fiber plexus in the outer plexiform layer (OPL), and in some bipolar cells with processes directed both to the IPL and to the OPL. In the cat retina (Fig. 1D), Dab1 immunoreactivity was seen in certain neurons in the GCL.

When we focused on the INL in whole-mount retinal preparations of each species, numerous Dab1-immunoreactive amacrine cell somata were distributed throughout the retina (Fig. 2A–D). These somata were round or pyriform in shape. In Figure 2A–D, the lobules are clearly visible in sublamina a of the IPL. A different focal plane at the inner aspect of the IPL shows a few processes ramifying in stratum 5 of the IPL. The terminal branches of labeled cells were difficult to trace, insofar as they intermingled with other labeled processes originating from other Dab1-labeled cells (data not shown).

Double immunofluorescence for Dab1 and AII amacrine cell markers

A few immunocytochemical markers that recognize AII amacrine cells, such as antisera against parvalbumin (Wässle et al., 1995) and calretinin (Wässle et al., 1995; Massey and Mills, 1999; Mills and Massey, 1999), were applied to the retinas of cat, rabbit, human, and rat. To identify whether Dab1 and calretinin or parvalbumin immunoreactivities are expressed within the same amacrine cells in different species, double-labeling experiments were performed. Figure 3 shows an example of vibratome sections doubly labeled with antisera against Dab1 (Fig. 3A,D,G,J) and calretinin (Fig. 3B) or parvalbumin (Fig. 3E,H,K) in human (Fig. 3A–C), rat (Fig. 3D–F), rabbit (Fig. 3G–I), and cat (Fig. 3J–L) retinas. In merged images (Fig. 3C,F,I,L), all Dab1-immunoreactive amacrine cells show calretinin (Fig. 3C) or parvalbumin (Fig. 3F,I,L) immunoreactivity. In the human, rat, and rabbit retinas, amacrine cells showing no colocalization of Dab1 and calretinin (Fig. 3C) or parvalbumin immunoreactivities were observed (Fig. 3F,I). The parvalbumin-labeled amacrine cells in the rat and rabbit retinas have been reported to belong to a class of wide-field amacrine cells (Wässle et al., 1993; Casini et al., 1995). Therefore, our results clearly demonstrate that antisera directed against Dab1 specifically labeled AII amacrine cells with lobular appendages and narrow-field dendrites in the mammalian retinas examined in the present study.

**Dab1-labeled amacrine cells show Glyt-1 immunoreactivity**

In the mammalian retina, most amacrine cells contain either glycine or γ-aminobutyric acid (GABA; Marc, 1989; Vaney, 1990; Marc et al., 1995; Vardi and Auerbach, 1995). Moreover, the Dab1 is known to label AII amacrine cells in the mammalian retina (Rice and Curran, 2000; Lee et al., 2003), which are glycnergic (Marc and Liu, 1985; Pourcho and Goebel, 1985; Grünert and Wässle, 1993; Goebel and Pourcho, 1997; Wright et al., 1997; Menger et al., 1998; Rice and Curran, 2000). Therefore, to confirm whether Dab1-labeled amacrine cells are glycnergic, we further characterized the Dab1-labeled amacrine cells neurochemically by using double labeling with antisera directed against Dab1 and glycine transporter 1 (Glyt-1). Figure 4 shows vibratome sections doubly labeled with antibodies against Dab1 (Fig. 4A) and Glyt-1 (Fig. 4B) in the human retina. All Dab1-labeled amacrine cells in human retina showed Glyt-1 immunoreactivity. Therefore, Dab1-labeled amacrine cells constitute a subpopulation of glycnergic amacrine cells.

**Double immunofluorescence for Dab1 and PKC**

The AII amacrine cell is one of two types of postsynaptic amacrine cells at rod bipolar ribbon synapses (Kolb and Famiglietti, 1974; Famiglietti and Kolb, 1975; Kolb, 1979; Pourcho, 1982; McGuire et al., 1984; Dacheux and Raviola, 1986; Raviola and Dacheux, 1987; Voigt and Wässle, 1987; Sterling et al., 1988; Kolb et al., 1990, 1991; Strettoi et al., 1990, 1995; Chun et al., 1993; Wässle et al., 1993). PKC is expressed primarily by rod bipolar cells in the mammalian retina (Negishi et al., 1988; Greferath et al., 1990), so we used PKC to identify rod bipolar cells in the mammalian retina. Figure 5 shows vertical sections
doubly labeled with antisera against Dab1 (Fig. 5A) or parvalbumin (Fig. 5D) and antisera against PKC (Fig. 5B,E) in rat retina. PKC immunoreactivity was found in cell bodies located in the outer part of the INL and in their axons, which terminated as two or three boutons in the IPL close to the GCL. In the merged image (Fig. 5C), it is apparent that the axon terminals of the rod bipolar cells make contacts with AII amacrine cell processes. However, parvalbumin-immunoreactive wide-field amacrine cell processes are not closely associated with the axon terminals of the rod bipolar cells, as shown in the Figure 5F. As expected from previous studies, there are contacts between the axon terminals of the rod bipolar cells and Dab1-immunoreactive cell processes. These results further confirm that the Dab1-labeled amacrine cells are indeed AII amacrine cells in the mammalian retina.
Quantitative analysis

Quantitative studies have characterized the Dab1-immunoreactive amacrine cell population. The density of Dab1-immunoreactive amacrine cells was measured from the superior to inferior retina of a whole mount (Fig. 6). The peak density was 4,400 ± 169 cells/mm² at 3-mm eccentricity in the human retina, 5,200 ± 139 cells/mm² in the central region around the optic disc in the rat retina, 3,250 ± 119 cells/mm² at 3-mm eccentricity in the rabbit retina, and 4,675 ± 120 cells/mm² at 3-mm eccentricity in the cat retina. The lowest density was at the retinal periphery of the inferior retina, with 1,275 ± 219 cells/mm² in the human retina, 1,000 ± 154 cells/mm² in the rat retina, 900 ± 191 cells/mm² in the rabbit retina, and 1,000 ± 112 cells/mm² in the cat retina.

To assess quantitatively the distribution of Dab1-immunoreactive amacrine cells, we used nearest-neighbor analysis (Wässle and Riemann, 1978). The distance from each soma to its nearest neighbor was measured in 0.25 × 0.25 mm² areas of the midperipheral retina. The resulting histogram for the midperipheral retinal region is shown in Figure 7. The histogram fits a Gaussian distribution fairly well in terms of mean distances and standard deviations (solid lines), indicating a statistically regular mosaic of labeled cells in this region.

DISCUSSION

In the present study, we demonstrated that antiserum directed against Dab1 can act as a specific marker for AII amacrine cells by comparing the morphologies of Dab1-labeled amacrine cells with those of AII amacrine cells labeled with other markers in human, rat, rabbit, and cat retinas. Dab1 encodes a cytoplasmic protein containing a motif known as a protein interaction/phosphotyrosine-binding domain. This domain was originally identified in the adaptor protein, Shc, as a region required for binding to the epidermal growth factor receptor, the insulin receptor, and other tyrosine-phosphorylated proteins (Margolis, 1996). Mammalian Dab1 was originally identified as an Src-binding protein in a yeast two-hybrid screen (Howell et al., 1997). It is expressed at high levels in the developing central nervous system and is phosphorylated on tyrosine residues during brain development. Tyrosine phosphorylation of Dab1 promotes its interaction with several nonreceptor tyrosine kinases, including Src, Fyn, and Ab1, through their SH₂ domains. This implies that Dab1 functions in kinase signaling cascades during development and plays an important role in the final positioning of migrating neurons in the mammalian central nervous system (Howell et al., 1997). In the present study, a certain population of amacrine cells was labeled by antisera di-

Fig. 2. Light micrographs of whole-mount human (A), rat (B), rabbit (C), and cat (D) retinas processed for Dab1 immunoreactivity. The focus is on the IPL close to the INL. Dab1-labeled amacrine cell bodies and lobular appendages (arrows) are visible. Scale bar = 50 μm.
rected against Dab1, in good agreement with previous reports on mouse and guinea pig retinas (Rice and Curran, 2000; Lee et al., 2003). These Dab1-labeled amacrine cells were located in a single row in the proximal INL, had thick primary processes with branched lobular appendages in sublamina a of the IPL, and ramified as bushy arbors in sublamina b of the IPL. They also showed positive immunoreactivity to antisera against parvalbumin or calretinin (i.e., markers for AII amacrine cells in the mammalian retinas examined so far), and their processes made close contacts with the axon terminals of the rod bipolar cells. These results clearly indicate that Dab1-labeled amacrine cells are AII amacrine cells in mammalian retinas. Different antisera have been used to identify AII amacrine cells in the mammalian retina, according to species. For instance, antisera against parvalbumin have been used in cat, rat, and rabbit retinas (Gabriel and Straznicky, 1992; Chun et al., 1993; Wässle et al., 1993; Casini et al., 1995).

Fig. 3. Confocal micrographs of a 50-μm-thick vertical vibratome section processed for Dab1 (A,D,G,J) and calretinin (B) or parvalbumin (E,H,K) immunoreactivity in human (A–C), rat (D–F), rabbit (G–I), and cat (J–L) retinas. Dab1 immunoreactivity was visualized by using a Cy3-conjugated secondary antibody. Calretinin and parvalbumin immunoreactivities were visualized by using an FITC-conjugated secondary antibody. Double exposure (C,F,I,L) shows colocalization (yellow) of Dab1 and calretinin or parvalbumin within the same amacrine cell bodies located in the proximal row of the INL (arrows). Note that Dab1 immunoreactivity is not visible in a parvalbumin-immunoreactive wide-field amacrine cell in F and I (arrowheads). Scale bar = 50 μm.
and antisera against calretinin in the primate retina (Wässle et al., 1995; Mills and Massey, 1999; Kolb et al., 2002). Why AII amacrine cells contain different calcium-binding proteins according to species is not known. However, the distribution of some markers in the vertebrate retinas tends to vary according to phylogenetic proximity (Marc, 1986; Mandell et al., 1990). In the present study, all AII amacrine cells were labeled by an antiserum directed against Dab1 regardless of species, clearly suggesting that Dab1 plays a role in AII amacrine cells of the mammalian retina. It has been suggested that Dab1 plays a role in the segregation of functionally distinct synapses in the IPL during development of the mouse retina (Rice and Curran, 2000). Reelin activates an intracellular signaling pathway by binding to lipoprotein receptors present on the surface of neurons in the developing brain and by stimulating tyrosine phosphorylation of Dab1 (D’Arcangelo et al., 1999; Hiesberger et al., 1999; Howell et al., 1999). Mutation of either Reelin or Dab1 results in abnormalities in laminar structures throughout the brain (Rice and Curran, 1999). These facts imply that AII amacrine cells and their processes express Dab1 so that they can be positioned properly in response to Reelin, which is produced by adjacent ganglion cells, as suggested by Rice and Curran (2000).

In the present study, all Dab1-immunoreactive cells in the human retina showed calretinin immunoreactivity, and those in rat, rabbit, and cat retinas showed parvalbumin immunoreactivity, whereas not all calretinin- or parvalbumin-labeled amacrine cells showed Dab1 immunoreactivity in human, rat, and rabbit retinas. These results are in good agreement with those of several investigators (Wässle et al., 1993; Casini et al., 1995, 1996; Massey and Mills, 1999; Kolb et al., 2002) who have shown that a class of wide-field amacrine cells expresses calretinin or parvalbumin immunoreactivity in the rat, monkey, and rabbit retinas. For example, Wässle et al. (1993) have reported that 1% of all parvalbumin-immunoreactive amacrine cells belong to a class of wide-field amacrine cells in the rat retina. Casini et al. (1995) noted the presence of a small population (4%) of non-AII amacrine cells stained for parvalbumin, and Massey and Mills (1999) also showed that 15% of the parvalbumin-labeled amacrine cells were non-AII amacrine cells in the rabbit retina. Furthermore, Kolb et al. (2002) identified that calretinin-immunoreactive amacrine cells in the fovea are diffuse amacrine cell types and that those in the extrafoveal region are AII amacrine cells and A19 amacrine cells in the monkey retina. With these findings taken together, it can be inferred that an antibody against parvalbumin or calretinin could not be a reliable marker for AII amacrine cells in the mammalian retina. Therefore, our results further demonstrate that antiserum against Dab1 that labels the pure morphology of AII amacrine cell is a reliable and specific AII amacrine cell marker in the retinas of a wide variety of mammals.

It is well known that AII amacrine cells accumulate glycine and that their synaptic function is blocked by strychnine (Pourcho and Goebel, 1985; Müller et al., 1998). Therefore, the Dab1-immunoreactive amacrine cells were further characterized by double-label immunocytochemistry with an antiserum directed against Glyt-1, which is known to label functionally glycinergic amacrine cells (Zafra et al., 1995; Vaney et al., 1998). Double immunocytochemical labeling with antisera directed against Dab1 and Glyt-1 showed that all Dab1-labeled cells expressed Glyt-1, demonstrating that Dab1-labeled amacrine cells are a subtype of glycinergic amacrine cells, which is consistent with previous reports (Menger et al., 1998; Rice and Curran, 2000; Lee et al., 2003). Therefore, AII amacrine cells of the retinas of mammals use glycine as an inhibitory neurotransmitter in visual processing.

In the present study, the density of Dab1-labeled amacrine cells varied greatly according to species. The peak density was relatively high (5,200–5,400 cells/mm²) in the...
human and rat retinas, moderate (4,600 cells/mm²) in the cat retina, and low (3,200–3,600 cells/mm²) in the rabbit retina. AII amacrine cells are important interneurons that transfer signals from rod bipolar cells to ON-type cone bipolar cells. The peak density of rod bipolar cells is 15,000–20,000 cells/mm² in the macaque monkey retina (Grünert and Martin, 1991), 5,450 cells/mm² in the rabbit retina (Strettoi and Masland, 1995), and 4,700 cells/mm² in the cat retina (Freed et al., 1987). The ratio of AII amacrine cells to rod bipolar cells is 0.6 in the rabbit retina and 1.0 in the cat retina. If we assume that the density of rod bipolar cells in the monkey retina is similar to that in the human retina, the human ratio of AII amacrine cells to rod bipolar cells will be 0.35. Therefore, the different densities of AII amacrine cells may be attributed to the different ratios of AII amacrine cells to rod bipolar cells in these species (Sterling et al., 1988).

To quantify the regularity of the Dab1-immunoreactive cell mosaic, a nearest-neighbor analysis was performed (Wassle and Riemann, 1978). Dab1-labeled amacrine cells form a nonrandom mosaic, as shown by the distribution of their nearest-neighbor distances. The regularity of a cell mosaic is expressed by the regularity index (r), which is the ratio between the mean of the nearest-neighbor dis-
stances and its standard deviation. A ratio of 1.0 indicates a random distribution and, the higher this ratio, the more regular the distribution (Eberhardt, 1967; Wässle and Riemann, 1978). In this study, the regularity index was 3.76 in the human retina, 5.1 in rat retina, 4.19 in the rabbit retina, and 3.55 in the cat retina. These are in good agreement with the AII amacrine cell mosaic of the cat, rabbit, and rat retinas (Vaney, 1985; Mills and Massey, 1991; Vaney et al., 1991; Wässle et al., 1993).

In conclusion, Dab1 immunoreactivity in different species is localized to a population of bistratified amacrine cells identical to the AII amacrine cells defined in other mammals by reactivity to calretinin or parvalbumin antibodies. Therefore, there is strong evidence, based on their morphology, distribution, and neurochemical content, that the Dab1-immunoreactive amacrine cells in the proximal INL of all the species examined here are indeed AII amacrine cells. Dab1 is therefore a reliable marker for the visualization of the AII amacrine cell population in mammalian retinas.

LITERATURE CITED


Howell BW, Gertler FB, Cooper JA. 1997. Mouse disabled (mDab1): an Src

Fig. 6. Density of Dab1-immunoreactive cells in whole mounts of human, rat, rabbit, and cat retinas. Dab1-immunoreactive amacrine cell densities measured in the proximal INL of different species. Sup, superior; Inf, inferior.

Fig. 7. Nearest-neighbor analysis of Dab1-immunoreactive amacrine cells in the inner nuclear layer of human, rat, rabbit, and cat retinas. Nearest-neighbor distance histograms for Dab1-immunoreactive amacrine cells in the midperipheral regions are shown. The histograms for Dab1-immunoreactive amacrine cells are relatively well matched by a Gaussian curve (solid line), which describes a regular cell distribution. Each histogram shows the number of cells in the sample (n), the mean distance between cells (m), the standard deviation (SD), and the regularity index (r).
AII AMACRINE CELLS IN THE MAMMALIAN RETINA


