Group I Metabotropic Glutamate Receptors mGluR1 α and mGluR5a: Localization in Both Synaptic Layers of the Rat Retina

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We examined the distribution of the group I metabotropic glutamate receptors, mGluR1 α and mGluR5a, in the adult rat retina and during postnatal development using receptor-specific antisera. In contrast to the restricted localization of group II and group III mGluRs to either the outer plexiform layer (OPL) or the inner plexiform layer (IPL), group I mGluRs are present in both synaptic layers in the rat retina. Double-labeling experiments and electron microscopy showed that in the OPL the two receptors are localized on the dendritic tips of bipolar cells postsynaptic to photoreceptor terminals. In the IPL the

two mGluRs are localized on amacrine cell processes postsynaptic to bipolar cell terminals. These results suggest that group I mGluRs are involved in synaptic processing in both plexiform layers and in both the scotopic and photopic pathways in the rat retina. We propose that mGluR1 α and mGluR5a play an important modulatory role in the responses of retinal neurons to inhibitory and excitatory neurotransmitters.

Key words: mGluR1 α ; mGluR5a; bipolar cells; amacrine cells; outer plexiform layer; inner plexiform layer; rat retina; retinal development; immunocytochemistry

In the mammalian retina glutamate is the major excitatory neurotransmitter used in visual information processing. The flow of visual information in the vertical direction from the photoreceptors to bipolar cells and horizontal cells in the OPL and from bipolar cells to amacrine cells and ganglion cells in the IPL is mediated via glutamate (for review, see Massey, 1990). This flow of information is modulated by the laterally connecting inhibitory interneurons, horizontal cells and amacrine cells, that preferentially use GABA or glycine as their neurotransmitters (for review, see Brecha, 1983; Yazulla, 1986; Massey and Redburn, 1987; Marc, 1989).

Glutamate acts on two different classes of receptors, ionotropic and metabotropic glutamate receptors. Ionotropic glutamate receptors mediate fast excitatory synaptic transmission (for review, see Monaghan et al., 1989; Seeburg, 1993; Hollmann and Heinemann, 1994), whereas metabotropic glutamate receptors (mGluRs) are G-protein-coupled proteins that bind the ligand, influence a variety of intracellular second messenger systems, and modulate neuronal activity (for review, see Pin and Duvoisin, 1995). Glutamate can act in an excitatory or inhibitory manner via the action of mGluRs.

So far eight different members of the mGluR family have been cloned. Based on sequence homologies, pharmacological properties, and second messenger systems, mGluRs can be subdivided into three groups (for review, see Hollmann and Heinemann, 1994; Nakanishi, 1994; Pin and Duvoisin, 1995). mGluR2, 3 (group II) and mGluR4, 6, 7, 8 (group III) are functionally coupled to adenylyl cyclase and inhibit the formation of cAMP in artificial expression systems (Tanabe et al., 1992, 1993; Nakajima et al., 1993; Nakanishi, 1994;

Okamoto et al., 1994; Saugstad et al., 1994; Duvoisin et al., 1995). mGluR1 and mGluR5 (group I), are activated most strongly by quisqualate. They are functionally coupled to stimulation of phospholipase C, increased synthesis of inositol 1,4,5-trisphosphate (IP₃), and Ca²⁺ release from internal stores (Masu et al., 1991; Abe et al., 1992; Aramori and Nakanishi, 1992).

The presence of mGluR1 and mGluR5 in the rat retina was shown with *in situ* hybridization at the mRNA level (Hartveit et al., 1995) and for mGluR1 α with immunocytochemistry at the protein level (Peng et al., 1995). Several components of a possible pathway involving glutamate and IP₃ signaling, e.g., phosphatidylinositol diphosphate (PIP₂), IP₃, and IP₃ receptors, also were shown to be present in the rat retina (Anderson et al., 1985; Das et al., 1986; Milani et al., 1990; Peng et al., 1991). However, knowledge on the exact distribution of group I mGluRs in the retina with respect to cellular and subcellular localization and their possible function in retinal synaptic circuitry is still lacking.

We have examined light and electron microscopically the cellular and subcellular distribution of mGluR1 α and mGluR5a in the rat retina using receptor-specific antisera. We show that group I mGluRs are used in synaptic processing in both synaptic layers in the rat retina and suggest that these receptors play an important role in the modulation of the responses of retinal neurons to inhibitory and excitatory neurotransmitters.

MATERIALS AND METHODS

Antisera against mGluR1 α and mGluR5a. The affinity-purified polyclonal antiserum against mGluR1 α was obtained from Chemicon (AB1551, Chemicon, Temecula, CA) and was generated in rabbit against a C-terminal peptide of rat mGluR1 α (PNVTYASVILRDYKQSSSTL) conjugated to keyhole limpet hemocyanin. The affinity-purified polyclonal antiserum against mGluR5a also was raised in rabbit against a peptide corresponding to the C-terminal amino acid sequence of rat mGluR5a (PSSPKYDTLIIRDYQSSSSL) conjugated to ovalbumin (Vidnyánszky et al., 1994).

Animals and tissue preparation. Retinas of adult albino rats, 6–8 weeks of age, and of different postnatal stages, 1–30 d old, were investigated. For the developmental studies, only retinas from the same litter mates

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Figure 1. Specificity of the antiserum against mGluR1 α . Membrane proteins of retina (80 μ g/lane) were separated on a 7.5% SDS-PAGE gel and transferred onto nylon membrane. *Numbers and arrowheads in A* indicate the position and weight (kDa) of marker bands. *A*, The antiserum labeled a protein band at $\sim$ 140 kDa. *B*, Labeling was prevented by coincubation of the antiserum with the antigenic peptide. *C*, *D*, On retina sections preadsorption of the antiserum with its antigenic peptide resulted in the loss of specific immunoreactivity (*C*) compared with the specific mGluR1 α immunostaining (*D*). *ONL*, Outer nuclear layer; *OPL*, outer plexiform layer; *INL*, inner nuclear layer; *IPL*, inner plexiform layer; *GCL*, ganglion cell layer. Scale bar, 25 μ m (shown in *D* for *C*, *D*).

and only retinal pieces with the same eccentricity were compared. The rats were anesthetized deeply with halothane and decapitated. A detailed description of the preparation of the retinal tissue for light and electron microscopic immunocytochemistry is given in Brandstätter et al. (1996).

Light microscopic immunocytochemistry. In addition to the antisera against mGluR1 α (0.2 μ g/ml) and against mGluR5 α (1 μ g/ml), we used the monoclonal antibody MC-3A against PKC α (1:100; Seikagaku, Tokyo, Japan). Immunocytochemical labeling was performed by the indirect fluorescence method. To characterize the cellular distribution of mGluR5 α in more detail, we combined the mAb MC-3A against PKC α , known to stain rod bipolar cells (Greferath et al., 1990), in double-labeling experiments with the specific anti-peptide antiserum against mGluR5 α . The binding sites of the primary antibodies were revealed by secondary antibodies: goat anti-mouse or goat anti-rabbit IgG coupled either to carboxymethylindocyanine (Cy3, red fluorescence; Dianova, Hamburg, Germany) diluted 1:1000 or fluorescein isothiocyanate (FITC, green fluorescence; Sigma-Aldrich, Deisenhofen, Germany) diluted 1:100 or to Texas Red (red fluorescence; Amersham, Braunschweig, Germany) diluted 1:100. In the double-labeling experiments, sections were incubated in a mixture of the primary antibodies, followed by a mixture of the secondary antibodies.

Preembedding immunoelectron microscopy. After blocking, vibratome sections (50 μ m thick) were incubated in primary antisera against mGluR5 α and mGluR1 α for 4 d at 4°C. The primary antisera were used at the same concentration and diluted in the same medium, but without Triton X-100, as used for light microscopy.

Detection of the immunostaining and microscopic analysis were performed as previously described (Brandstätter et al., 1996).

Characterization of the antisera against mGluR1 α and mGluR5 α . The antiserum against mGluR5 α has been fully characterized and used previously for light and electron microscopic distribution studies (Vidnyánszky et al., 1994). The specificity of the antiserum against mGluR1 α was assessed by immunoblotting of rat retina membrane proteins (Fig. 1*A,B*). Albino rats were deeply anesthetized and decapitated. The retinas were dissected and homogenized in lysis buffer containing 4 mM HEPES, 220 mM D(+)-mannose, 70 mM sucrose, 1 mg/ml benzamidine hydrochloride, 0.5 mg/ml aprotinin (Merck, Darmstadt, Germany), and 0.25 mg/ml benzethonium chloride (Sigma-Aldrich) at pH 7.5, centrifuged for 3 min

at 1000 $\times$ g and 4°C. Subsequently, the supernatant was centrifuged for 15 min at 15,000 $\times$ g. The pellet was resuspended in lysis buffer to obtain a crude retinal membrane protein fraction. After denaturation with SDS and 2-mercaptoethanol, crude retinal membrane proteins (80 μ g/lane) and biotinylated SDS molecular weight markers were electrophoresed on 7.5% SDS-polyacrylamide gels. Proteins were transferred onto cationized nylon membranes by standard Western blotting technique. After incubation with blocking buffer [5% (v/v) normal goat serum (NGS) and 0.05% (w/v) Tween 20 in PBS (0.01 M), pH 7.4] for 1 hr at room temperature, blots were incubated with primary (0.1 μ g/ml) and secondary antibodies for 1 hr each in blocking buffer. Binding of the antiserum against mGluR1 α to polypeptides was detected by goat anti-rabbit IgG antibodies coupled to alkaline phosphatase (diluted 1:100; Dianova) with 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Boehringer Mannheim Biochemica, Mannheim, Germany) as substrates.

The antiserum against mGluR1 α detected a single protein band with a molecular weight of $\sim$ 140 kDa in membrane preparations of rat retina (Fig. 1*A*). This is in agreement with the published molecular weight of mGluR1 α deduced from its cDNA sequence (Masu et al., 1991). Preincubation of the antiserum against mGluR1 α with a 10-fold excess (w/w) of the antigenic peptide led to no labeling in the subsequent immunoblot analysis (Fig. 1*B*).

For staining of retina sections, controls were made by preincubating the antisera against mGluR1 α and mGluR5 α with a 10-fold excess of their respective antigenic peptides for 1 hr at room temperature, resulting in a complete loss of specific staining (Figs. 1*C*, 3*B*). In double-labeling experiments, controls were prepared by omitting one of the two primary antibodies during the incubation, and in this case, only the immunoreactivity for the remaining primary antibody was detected.

In addition, we tested the specificity of the antiserum against mGluR5 α and mGluR1 α by staining sections of adult rat brain. Unfixed rat brains were sectioned on a cryostat at 18 μ m thickness, collected on gelatin-coated slides, postfixed for 5 min in 4% (w/v) paraformaldehyde in phosphate buffer, and immunostained as described for the retina sections. Immunolabeling was detected with a biotinylated goat anti-rabbit IgG (1:100; Sigma-Aldrich) and extravidin-coupled peroxidase (1:100; Sigma-Aldrich). After preincubation with 0.05% (w/v) 3, 3'-diaminobenzidine (DAB; Sigma-Aldrich), the sections were reacted by adding 0.01% (v/v)

Figure 2. Control stainings of cryostat sections of rat cerebellum with the antiserum directed against mGluR1 α and mGluR5 α . *A*, Strong labeling for mGluR1 α was found in the molecular layer (ML) and the Purkinje cell layer (arrowheads point to individual Purkinje cell somata). Weak mGluR1 α immunolabeling was found in the granular cell layer (GCL). *B*, mGluR5 α immunolabeling was weak in all three layers, the molecular layer (ML), the Purkinje cell layer, and the granule cell layer (GCL). Scale bar, 100 μ m.

H₂O₂. The staining patterns obtained in rat brain (Fig. 2) were compared with published data of the distribution of mGluR1 α and mGluR5 α to ensure the specificity to the antibody reactivity. As has been shown for mGluR1 α (Baude et al., 1993) and mGluR5 (Romano et al., 1995), mGluR1 α immunoreactivity in the cerebellum was strong in the molecular layer and the Purkinje cell layer and weak in the granule cell layer (Fig. 2*A*), whereas mGluR5 α immunoreactivity was weak in the molec-

ular layer and Purkinje cell layer and stronger in the granule cell layer (Fig. 2*B*).

RESULTS

Distribution of mGluR5 α in the adult rat retina

In the adult retina, mGluR5 α immunoreactivity showed a distinct labeling pattern in the IPL, the OPL, and in the inner nuclear layer (INL) (Fig. 3*A*). Patches of punctate immunoreactivity were seen throughout the IPL with reduced staining in the outer half of the IPL (OFF sublamina) and intense staining in the inner half of the IPL (ON sublamina). A narrow immunoreactive band of processes in the outermost part of the IPL was labeled more strongly. Intense immunoreactivity also was detected in the OPL in putative dendrites of bipolar cells (Fig. 3*A*). In the INL, somata and occasionally axons of putative bipolar cells were diffusely labeled (Fig. 3*A*). Punctate distribution of the mGluR5 α receptor staining at the light microscopic level indicated synaptic localization, as was shown for several other types of neurotransmitter receptors in the retina (Pourcho and Owczarzak, 1991; Yazulla and Studholme, 1991; Grünert and Wässle, 1993; Hartveit et al., 1994; Brandstätter et al., 1996). The diffuse labeling on putative bipolar cell somata in the INL, however, also showed extrasynaptic localization of mGluR5 α immunolabel. In control experiments, preadsorption of the anti-mGluR5 α antiserum with the respective antigenic peptide before applying to retina sections resulted in complete absence of specific staining (Fig. 3*B*).

To determine whether bipolar cell somata in the INL and their dendrites in the OPL are immunolabeled for mGluR5 α , we performed double-labeling experiments with the antiserum against mGluR5 α and an antibody against an isoform of protein kinase C (PKC), shown to stain rod bipolar cells in the rat retina (Greferath et al., 1990) (Fig. 4). These experiments clearly demonstrated that at least rod bipolar cell somata in the INL and their dendrites in the OPL were immunolabeled for mGluR5 α (Fig. 4). In the IPL there was no detectable colocalization between the mGluR5 α staining and the labeling of rod bipolar cell terminals by the anti-PKC antibody (Fig. 4). The micrographs in Figure 4 were printed as mirror images, and the symmetry of staining that can be detected along the midline (arrows in Fig. 4) shows colocalization.

Subcellular distribution of mGluR5 α

Immunoreactivity for mGluR5 α was found intracellularly because of the epitope specificity of the anti-mGluR5 α antiserum.

mGluR5 α is localized to bipolar cell dendrites in the OPL

In the OPL, dendrites of bipolar cells postsynaptic to photoreceptor terminals were mGluR5 α -immunoreactive (Fig. 5). In the majority of cases the immunolabeled bipolar cell dendrites were detected postsynaptic to the terminals of rod photoreceptors (rod spherules), therefore belonging to rod bipolar cells (Dowling and Boycott, 1966) (Fig. 5*A,B*). This corroborates the light microscopic finding of mGluR5 α -immunoreactive rod bipolar cell somata and dendrites. Horizontal cells, the two lateral postsynaptic elements at the rod photoreceptor synapse in the OPL (Dowling and Boycott, 1966), were never found to be mGluR5 α -immunoreactive (Fig. 5*A,B*). Occasionally, we also found the dendrite of a cone bipolar cell stained for mGluR5 α that made contact at the terminal of a cone photoreceptor (cone pedicle) but was not associated directly with the synaptic complex (Fig. 5*C*).

mGluR5 α is localized to amacrine cell processes in the IPL

In the IPL, mGluR5 α immunoreactivity was found on amacrine cell processes postsynaptic to bipolar cell ribbon synapses, consis-

Figure 3. mGluR5a immunostaining of a vertical cryostat section through the rat retina. *A*, Labeling for mGluR5a was present in both synaptic layers, the OPL and the IPL, and in the INL. Throughout the IPL diffuse and punctate labeling was present, with stronger staining in the inner half of the IPL. In addition, bipolar cell somata in the INL and their dendrites in the OPL were mGluR5a-immunoreactive. *B*, Preadsorption of anti-mGluR5a antiserum with the immunogen resulted in a complete loss of specific immunoreactivity. *C*, The retinal layers are shown with Nomarski optics. Abbreviations are as in Figure 1. Scale bar, 25 μ m (shown in *C* for *A–C*).

tent with the light microscopic finding of punctate receptor staining in the IPL (Fig. 3). We detected mGluR5a immunoreactivity on amacrine cell processes postsynaptic to OFF-cone bipolar cell terminals (Fig. 5*D*), ON-cone bipolar cell terminals (Fig. 5*E*), and rod bipolar cell terminals (Fig. 5*F*). Whereas at the cone bipolar cell synapses the two postsynaptic elements are preferentially a process of an amacrine cell and a dendrite of a ganglion cell (Dowling and Boycott, 1966), at the rod bipolar cell synapse both postsynaptic elements belong to amacrine cells (Famiglietti and Kolb, 1975; Chun et al., 1993). At the bipolar cell synapses only one of the postsynaptic processes was labeled.

Distribution of mGluR1 α in the adult rat retina

mGluR1 α , the second member of group I mGluRs, like mGluR5a was expressed in both synaptic layers, the OPL and the IPL, of the adult rat retina (Fig. 6*A*). In the IPL, staining for mGluR1 α revealed several distinct immunoreactive bands with a mixture of diffuse and punctate staining separated by bands of reduced or no immunoreactivity (Fig. 6*A*). Whereas the outer half of the IPL (OFF sublamina) was subdivided into three smaller mGluR1 α -immunoreactive bands, the complete inner half of the IPL (ON sublamina) showed a more or less homogenous distribution of mGluR1 α immunoreactivity (Fig. 6*A*). Clear labeling of a narrow band of mGluR1 α -immunoreactive processes also was found in the OPL. In the INL, occasionally labeled somata of putative bipolar cells were detected (Fig. 6*A*).

Subcellular distribution of mGluR1 α

As with mGluR5a, the reaction product of the mGluR1 α immunostaining was found intracellularly because of the epitope specificity of the antiserum.

mGluR1 α is localized to processes postsynaptic to ribbon synapses in the OPL and IPL

Like the labeling for mGluR5a, mGluR1 α immunoreactivity in the OPL was found in rod bipolar cell dendrites postsynaptic at ribbon synapses of rod photoreceptor cells (Fig. 7*A*). Only rarely did we find a cone bipolar cell dendrite, postsynaptic to the terminal of a cone photoreceptor cell, stained for mGluR1 α (data

not shown). Again, the two lateral horizontal cell processes at the photoreceptor synapse were never found to be mGluR1 α -immunoreactive (Fig. 7*A*).

In the IPL amacrine cell processes postsynaptic to OFF-cone bipolar cell terminals (Fig. 7*B*), ON-cone bipolar cell terminals (Fig. 7*C*) and rod bipolar cell terminals (Fig. 7*D*) were labeled for mGluR1 α . Like mGluR5a immunoreactivity, mGluR1 α staining was present in only one of the two postsynaptic amacrine cell processes at the rod bipolar cell ribbon synapse. From our material we are not able to say whether ganglion cell dendrites also were stained for mGluR1 α .

Postnatal development of mGluR5a and mGluR1 α expression

During postnatal development, mGluR5a-immunoreactive processes of neurons stratifying in the IPL were detected first at approximately postnatal day 3 (P3) (Fig. 8*B*). At around P5 the homogenous mGluR5a labeling pattern in the IPL changed to a more stratified appearance, with stronger staining in the inner part and weaker staining in the outer part of the IPL (Fig. 8*C*). In addition, the mGluR5a staining was no longer restricted to the IPL but appeared in the somata of putative bipolar cells in the INL and their dendrites in the OPL (Fig. 8*C*). The adult labeling pattern showing a pronounced stratification of mGluR5a immunostaining in the IPL and clear labeling of bipolar cell somata in the INL and of their dendrites in the OPL was observed already at approximately P8 (Fig. 8*D*).

In contrast to mGluR5a, low mGluR1 α immunoreactivity was present already at birth (P1) (Fig. 9*A*). The intensity of mGluR1 α immunoreactivity and the level of stratification of the staining in the IPL increased during the first and second postnatal week, P7–P12 (Fig. 9*B,C*), and reached the adult staining pattern at approximately P19 (Fig. 9*D*). Like mGluR5a, the antiserum against mGluR1 α also stained bipolar cell somata in the INL and their dendrites in the OPL, but to a lesser extent (Fig. 9*B,C*). During later postnatal development, P12–P19, this staining increasingly became restricted to the dendrites of bipolar cells in the OPL (Fig. 9*D*).

Figure 5. High-power electron micrographs showing the ultrastructural localization of mGluR5a immunoreactivity in the two synaptic layers, the OPL and the IPL, in the retina. *A–C*, mGluR5a staining in the OPL was found in the dendrites of rod bipolar cells (*star*) postsynaptic to rod spherules (*A, B*) and occasionally in dendrites of cone bipolar cells (*star*) postsynaptic to cone pedicles (*C*). The two lateral elements at the photoreceptor synapses, the horizontal cells (*hc*), were not found to be mGluR5a-immunoreactive. *D–F*, mGluR5a staining in the IPL was found in the processes of amacrine cells (*star*) postsynaptic to OFF-cone (*D*), ON-cone (*E*), and rod bipolar cells (*F*). The presynaptic ribbon in the terminals of the photoreceptor and bipolar cells is marked with an *arrowhead*. Scale bars, 0.2 μ m (shown in *B* for *A, B*; shown in *F* for *D–F*).

Figure 6. MGlur1 α immunostaining of a vertical cryostat section through the rat retina. *A*, Labeling for mGluR1 α was found in both synaptic layers, the OPL and the IPL. Throughout the IPL diffuse and punctate labeling was present with bands of reduced mGluR1 α immunoreactivity subdividing the IPL into several strata. In addition, somata of bipolar cells (arrowheads) were weakly stained in the INL, as compared with the stronger staining of bipolar cell dendrites in the OPL. *B*, The retinal layers are shown with Nomarski optics. Abbreviations are as in Figure 1. Scale bar, 25 μ m (shown in *B* for *A*, *B*).

Involvement of group I mGluRs in synaptic processing in the OPL

MGlur1 α and mGluR5a were found in the OPL of the rat retina preferentially localized to the dendritic tips of rod bipolar cells postsynaptic to the terminals of rod photoreceptor cells. Only on rare occasions staining was found in dendrites of cone bipolar cells postsynaptic to the terminals of cone photoreceptor cells. Rod bipolar cells are ON bipolar cells that are active under scotopic conditions. One of their functions is the transmission of the light ON signal from the rod photoreceptor cells to the ON ganglion cells via the AII amacrine cells.

To date, mGluR6 is the sole mGluR found on the dendrites of ON bipolar cells in the OPL (Nomura et al., 1994). The function of mGluR6, sensitive to L-2-amino-4-phosphonobutyrate (L-AP4), is the transmission of the light signal from the photoreceptor cells to the ON bipolar cells; the photoreceptors are hyperpolarized by light, and this hyperpolarization is converted via the action of mGluR6 to a depolarization of the ON bipolar cells that convey the light ON signal to the ON ganglion cells [Masu et al. (1995); for review, see Nakanishi (1995)].

Because of the physiology of group I mGluRs, it is unlikely that they are involved in the direct transmission of synaptic signals in the OPL. Our immunocytochemical data showing group I mGluRs postsynaptic in the dendrites of bipolar cells in the OPL could explain, however, the modulatory action of mGluR agonists on the GABA responses of bipolar cells reported by Feigenspan and Bormann (1994). They showed that extracellular application of 1-amino-cyclopentane-1,3-dicarboxylate (ACPD), an agonist for group I mGluRs (for review, see Pin and Duvoisin, 1995), to retinal bipolar cells in culture stimulated the decline of GABA_C responses. They suggested that glutamate released from photore-

ceptor cells in the OPL could act on mGluRs in bipolar cells, which in turn could decrease the response to inhibitory GABAergic inputs from horizontal or amacrine cells. Stimulation of phospholipase C, increased accumulation of IP₃ and diacyl glycerol (DAG), and activation of PKC could lead to phosphorylation of the GABA_C receptor channels. Group I mGluRs in dendrites of bipolar cells (present study), PKC in dendrites of bipolar cells (Greferath et al., 1990), and IP₃ and GABA_C receptors also found in the OPL in the rat retina (Peng et al., 1991; Enz et al., 1996) could be the anatomical substrates for such an action. For Purkinje cells it has been shown that the action of IP₃ on the release of Ca²⁺ from intracellular stores remains restricted locally to a few micrometers within their dendrites and also is highly regulated temporally (Wang and Augustine, 1995).

Involvement of group I mGluRs in synaptic processing in the IPL

Both mGluR1 α and mGluR5a were found in amacrine cell processes postsynaptic to bipolar cell ribbon synapses in the IPL. Amacrine cells do not express GABA_C receptors in the rat retina (Enz et al., 1995), and therefore it is very unlikely that group I mGluRs in the IPL should have the same mode of action as that suggested for mGluR1 α and mGluR5a in bipolar cell dendrites in the OPL. Most amacrine cells are inhibitory interneurons releasing either GABA or glycine (for review, see Wässle and Boycott, 1991). For group II and group III mGluRs present in amacrine cell processes in the IPL, it was suggested that they could influence the release of inhibitory neurotransmitter at reciprocal synapses back onto bipolar cells. Such a mechanism could modulate the release of glutamate from bipolar cells (Brandstätter et al., 1996; Koulen et al., 1996).

Figure 7. High-power electron micrographs showing the ultrastructural localization of mGluR1 α immunoreactivity in the two synaptic layers. *A*, mGluR1 α staining in the OPL was found in the dendrites of rod bipolar cells (*star*) postsynaptic to rod spherules. The two lateral elements at the photoreceptor synapses, the horizontal cells (*hc*), were not found to be mGluR1 α -immunoreactive. *B–D*, mGluR1 α staining in the IPL was found in the processes of amacrine cells (*star*) postsynaptic to OFF-cone (*B*), ON-cone (*C*), and rod bipolar cells (*D*). The presynaptic ribbon in the terminals of the photoreceptor and bipolar cells is marked with an arrowhead. The arrow in *D* marks a reciprocal synapse in the unlabeled amacrine cell process. Scale bars, 0.2 μ m.

indirectly. Because of the pharmacology of group I mGluRs, characterized by stimulation of PLC, increase of inositol phosphates, and release of Ca^{2+} from intracellular stores (for review, see Pin and Duvoisin, 1995), we think that a mechanism like that suggested for the action of group II and group III mGluRs in the IPL does not hold true for group I mGluRs. We suggest that group I mGluRs located in the processes of amacrine cells in the IPL could have a general effect on the activity of the cells, which in turn would influence their release probability for neurotransmitter.

Postnatal retinal development of group I mGluR expression

During postnatal development the expression patterns of mGluR1 α and mGluR5a differed temporally and spatially. Although mGluR1 α was expressed before mGluR5a, both receptors

were first present in the IPL, where the earliest synapses form during retinal development (Horsburgh and Sefton, 1987). Only later in development both mGluRs were expressed in neuronal somata in the INL and their processes in the OPL, where synapse formation occurs later than in the IPL. In contrast to different brain regions, where a downregulation was observed for mGluR5 (Romano et al., 1996), mGluR1 α and mGluR5a were upregulated during postnatal retinal development. It has been shown that intracellular levels of free Ca^{2+} influence neurite elongation and growth cone movement during development (Cohan et al., 1987) and that group I mGluRs are important for neuronal survival during development (Nicoletti et al., 1996; Pizzi et al., 1996). Because of the early appearance of group I mGluRs before synaptogenesis in the rat retina, they could influence synaptic differentiation during postnatal development and also might play

Figure 8. Vertical cryostat sections of rat retinas showing the postnatal development of mGluR5a immunoreactivity. The retinal layers are shown with Nomarski optics accompanying each micrograph (abbreviations as in Fig. 1). *CBL*, Cytoblast layer. *A*, At postnatal day 1 (P1), no mGluR5a-specific staining is found. Only a diffuse background staining in the *CBL* can be seen. *B*, Diffuse staining of processes in the *IPL* is seen at P3. *C*, At P5 the staining in the *IPL* differentiates into strong labeling in the inner half of the *IPL* and weak staining in the outer half of the *IPL*. In addition, intense labeling of bipolar cell somata in the *INL* and their dendrites in the *OPL* can be seen. *D*, At approximately P8 the expression of mGluR5a reaches the adult labeling pattern, with distinct stratification of the immunolabeling in the *IPL*. Stars indicate unspecifically labeled blood vessels. Scale bar (shown in *D*), 25 μ m.

Figure 9. Vertical cryostat sections of rat retinas showing the postnatal development of mGluR1 α immunoreactivity. The retinal layers are shown with Nomarski optics accompanying each micrograph (abbreviations as in Fig. 1). *CBL*, Cytoblast layer. *A*, At postnatal day 1 (P1) weak and diffuse staining for mGluR1 α can be seen in the *IPL*. *B*, At approximately P7, the diffuse staining in the *IPL* becomes more intense, and the staining changes from a homogeneous to a stratified pattern. Faint staining of somata in the *INL* and of processes in the *OPL* also can be seen. *C*, At P12 the staining in the *IPL* is strong and more punctate, and the labeling in the *INL* and *OPL* shows the somatodendritic staining of bipolar cells. *D*, At approximately P19 the expression of mGluR1 α reaches the adult labeling pattern, with strong labeling in the *IPL* and weak labeling in the *OPL*. Scale bar (shown in *D*), 25 μ m.

a role in the final consolidation of synaptic connections in the retina (Redburn and Rowe-Rendleman, 1996).

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