Voltage- and Transmitter-gated Currents of All-Amacrine Cells in a Slice Preparation of the Rat Retina

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All-amacrine cells are crucial interneurons in the rod pathway of the mammalian retina. They receive input synapses from rod bipolar cells and make electrical output synapses into the ON-pathway and glycinergic chemical synapses into the OFF-pathway. Whole-cell currents from more than 50 voltage-clamped All-amacrine cells were recorded in a slice preparation of the rat retina. The recorded cells were identified by intracellular staining with Lucifer yellow. Spike-like potentials could be elicited upon depolarization by current injection. A voltage-activated, fast, TTX-sensitive, inward Na^+ current was identified. A prominent outward K^+ current could be suppressed by tetraethylammonium. GABA as well as glycine activated Cl^- channels, which could be blocked by bicuculline and strychnine, respectively. Four agonists of excitatory amino acid receptors—kainate (KA), AMPA, 2-amino-4-phosphonobutyrate (APB), and NMDA—were tested. Inward currents at holding potentials of $V_h = -70$ mV were found by application of KA and AMPA but not by application of APB and NMDA. These currents could be blocked by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). ACh did not evoke any current responses.

[Key words: mammalian retina, amacrine cells, retinal slice, GABA, glycine, glutamate, neurobiotin coupling]

In the photopic pathway of the mammalian retina, cones feed into cone bipolar cells, which comprise two basic physiological types: ON-bipolar cells that are depolarized and OFF-bipolar cells that are hyperpolarized by a light stimulus. Cone bipolar cells synapse onto ganglion and amacrine cells. In the scotopic pathway rods are connected to rod bipolar cells, but instead of these synapsing directly onto ganglion cells, a distinctive type of narrow-field amacrine cell, the so-called AII-amacrine cell, is interposed (Kolb and Famiglietti, 1974; Famiglietti and Kolb, 1975; Kolb, 1979; Sterling, 1983; Strettoi et al., 1992). AII-amacrine cells make conventional chemical synapses with OFF-cone bipolar and OFF-ganglion cells and contact ON-cone bipolar cells via large gap junctions. In this way they could produce signals of opposite polarity in ON- and OFF-ganglion cells.

This wiring diagram is mainly based on ultrastructural observations in cats (Kolb and Famiglietti, 1974; Famiglietti and

Kolb, 1975; Sterling, 1983), rabbits (Dacheux and Raviola, 1986; Strettoi et al., 1990, 1992), and rats (Chun et al., 1993). However, AII-amacrine cells are constituents of all mammalian retinas looked at so far, and it is very likely that the rod pathway is essentially identical in all mammals (Daw et al., 1990; Vaney, 1990; Wässle and Boycott, 1991).

The morphology and topographic distribution of AII-amacrine cells have been elaborated in the cat (Vaney, 1985), rabbit (Mills and Massey, 1991; Vaney et al., 1991), and rat retina (Chun et al., 1993; Wässle et al., 1993). The AII-amacrine cells are small, distinctly bistratified cells, with so-called lobular appendages in sublamina *a* of the inner plexiform layer (IPL) and bushy dendritic fields in sublamina *b* (Perry and Walker, 1980). Their cell bodies are about 7–10 μm in diameter; in the vertical view they protrude slightly into the IPL and give rise to one or several stout processes that descend into the IPL. In the cat and rabbit retina they comprise about 10% of all amacrine cells (Vaney, 1985; Vaney et al., 1991); in the rat retina their percentage is approximately 8–9% (Wässle et al., 1993). The AII-amacrine cells are probably the most numerous amacrine cell type in these retinas (Vaney, 1990).

AII-amacrine cells of the cat retina have been shown to accumulate ^3H -glycine (Pourcho and Goebel, 1985) and to express glycine immunoreactivity (Pourcho and Goebel, 1987). Hence, they are very likely glycinergic interneurons.

Light responses of AII-amacrine cells are depolarizing in the cat and in the rabbit retina (Nelson, 1982; Dacheux and Raviola, 1986). In the rabbit retina they produce small action potentials, and little spike-like potentials were also observed in cat AII-amacrine cells. Light responses of rod bipolar cells have been reported to be hyperpolarizing in cat (Kolb and Nelson, 1983; Nelson and Kolb, 1983) and depolarizing in the rabbit (Dacheux and Raviola, 1986). However, recent evidence from the dark-adapted *in vivo* cat retina (Müller et al., 1988) and from dissociated rod bipolar cells (Yamashita and Wässle, 1991b; Kaneko et al., 1992) suggests that rod bipolar cells are depolarizing bipolar cells. A single AII-amacrine cell receives synaptic input from as many as 30 rod bipolar cells (Sterling et al., 1988; Vaney et al., 1991).

Voltage- and transmitter-gated currents of rod bipolar cells have been studied from dissociated cells of the cat, mouse, and rat retinas (Kaneko et al., 1989, 1992; Karschin and Wässle, 1990; Yeh et al., 1990; Yamashita and Wässle, 1991a,b). This has been possible because the characteristic morphology of rod bipolar cells is not lost during the dissociation procedure and they can be recognized by immunocytochemical markers (Negishi et al., 1988; Greferath et al., 1990). Although we searched through many Petri dishes of dissociated retinal cells, we did

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not observe AII cells. Apparently they lose their processes during dissociation, and since they cannot be labeled by other means—such as ganglion cells by retrograde transport of fluorescent tracers—they could not be identified for electrophysiological recording.

In order to study the voltage- and transmitter-gated currents of AII-amacrine cells, a thin slice preparation for patch-clamp recordings from neurons of the mammalian retina was established (Werblin, 1978; Wu, 1987; Edwards et al., 1989). The electrodes contained Lucifer yellow (LY), which made identification of AII-amacrine cells possible. Whole-cell currents were recorded in response to voltage steps and the currents were identified as Na^+ or K^+ currents. Transmitter-gated currents were studied by application of agonists and antagonists through a multibarrel puffer pipette. No precautions were taken to preserve light responses, which were recorded only occasionally in this slice preparation.

Materials and Methods

Preparation of slices. Adult albino rats were deeply anesthetized with halothane and decapitated. The eyes were quickly enucleated and transferred into a plastic Petri dish containing physiological saline (E1 of Table 1) at 0–4°C. The eye was opened by an encircling cut and the retina was carefully dissected free. The retina was then cut into four pieces. One of these pieces was held with a pair of forceps and, by “rolling” the curved surface of a scalpel blade over the edge, thin slices of 100–200 μm thickness were cut. We find that this procedure, with some practice, gives better slices than vibratomes. Approximately 10 slices were cut in this way and transferred into a recording chamber. The other three pieces of retina were transferred into a holding chamber containing physiological saline (solution E2 of Table 1) at room temperature, where they were continuously oxygenized with carbogen (95% O_2 , 5% CO_2). The retinas survived in good condition up to 8 hr in the holding chamber.

Recording chamber and optical setup. The recording chamber was placed on the fixed stage of a microscope (Zeiss ACM). The microscope was equipped with a focusing system, which moved the objectives, epifluorescence illumination, and differential interference contrast (Nomarski) optics. A water-immersion objective with a working distance of 1.6 mm was used (Zeiss, 40/0.75 W), the metal parts of which were coated with Epon to avoid corrosion and contact voltages. The recording chamber was made out of Perspex and was continuously perfused with extracellular solution. The volume in the chamber was 600 μl and the perfusion rate was 2 ml/min. The bottle containing extracellular solution was kept at room temperature (18–22°C) and continuously bubbled with carbogen.

In the recording chamber the slices were held in place with a grid of parallel nylon threads (Konnerth et al., 1987; Edwards et al., 1989). The electrode was mounted on a Leitz micromanipulator and approached the slices at a shallow angle (15° to the horizontal plane). Fine adjustment was possible with the aid of a piezoelectric manipulator (Physik Instrumente, Waldbronn, Germany). Patch pipettes were pulled from borosilicate glass (Clark, Redding, UK; outer diameter, 1.5 mm; wall thickness, 0.3 mm) on a horizontal electrode puller (DMZ-Universal Puller, Zeitz Instrumente, Augsburg, Germany). After heat polishing, their tip diameter was approximately 1 μm and their resistance with standard intracellular solution was 8–13 M Ω . All recordings were made with an EPC-7 patch-clamp amplifier (List, Darmstadt, Germany). The recording electrode was connected with Ag/AgCl wire to the preamplifier; the reference electrode was an Ag/AgCl pellet in the recording chamber. Liquid junction potentials of the patch pipette were measured as described by Fenwick et al. (1982). The holding potentials were corrected for junction potentials. Capacitative currents caused by the electrodes were canceled by the circuit of the EPC-7 amplifier. Additional capacitative currents appeared after breaking into the cells, reflecting the capacitance of the cells. The series resistances were estimated from the peak amplitudes of the capacitative currents. They were approximately 20 M Ω . The capacitative currents could not be fitted by a single exponential, indicating the cells are not isopotential. Early attempts to cancel the series resistance by the circuit of the EPC-7 amplifier were not successful, since cells were lost easily due to the instabilities introduced

Table 1. Summary of the bath solutions (E1–E4) and of the electrode solutions (I1, I2) used in the present study

	E1	E2	E3	E4	I1	I2
NaCl	137	110	110	137	10	10
KCl	5	5	5	5	130	
CaCl_2	2	2	2	2	1	1
MgCl_2					2	1
MgSO_4	1	1				
Na_2HPO_4	1	1				
NaHCO_3		25	25			
CsCl_2						120
CoCl_2			3	2		
Glucose	15	15	15	15		
HEPES	10	10	10	10	10	10
TEA-Cl						15
ATP					2	2
EGTA					1.1	1.5
Glycine				0.5 μM		
pH	7.4	7.4	7.4	7.4	7.2	7.2

All numbers are millimolar concentrations, with the exception of glycine, where the concentration is micromolar.

by the feedback circuitry. Hence, in later experiments the series resistance of the electrode was not compensated for. Series resistance was measured and steady state (DC) series resistance errors were accounted for numerically when quoting command voltages.

The voltage protocols were generated on an IBM-compatible personal computer and applied through an interface (Labmaster/TL-1, pCLAMP software 5.5, Axon Instruments). The data were low-pass filtered between 3 and 10 kHz by a variable frequency filter (AP-255-5, A.P. Circuit Corp.). Depending upon the time resolution needed, the sampling interval could be varied from 24 μsec to 40 msec.

Whole-cell recording. Both conventional (Hamill et al., 1981) and perforated-patch techniques (Horn and Marty, 1988) were used. In the conventional mode electrodes were filled with standard intracellular solution (solutions I1, I2 of Table 1). The seal resistance between the electrode and the cell was routinely greater than 10 G Ω . Since Lucifer yellow (LY) was added to the electrode solution (0.2 mg/ml), rapid loading of the cell was achieved after rupturing the patch and establishing the whole-cell configuration. Within less than 1 min dendrites were well filled (Fig. 1).

In the perforated-patch method (Horn and Marty, 1988) the electrode was filled with the solution I1 (Table 1). Nystatin (Serva, Heidelberg, Germany) was dissolved in dimethyl sulfoxide (Sigma, Deisenhofen, Germany) at a concentration of 100 mg/ml and added to the electrode solution at a final concentration of 300 $\mu\text{g}/\text{ml}$. The electrode tip was positioned on the cell surface and a gigaohm seal was made by gentle suction. No suction was applied to the pipette after the initial seal formation. As described in detail by Horn and Marty (1988) and Yamashita and Wässle (1991a,b), within 2 min after the seal formation, nystatin made the membrane of the cell-attached patch electrically permeable without rupture (“perforated patch”). No filling of the cells with LY was observed, indicating that the patch was not broken. At the end of the recordings, gentle suction ruptured the patch, LY diffused into the cells, and their morphology became apparent.

Drug application and solutions. The different extracellular and electrode solutions are listed in Table 1 and will be specified in Results. Drugs were dissolved in the bath solution and applied from six-barrelled pneumatic puffer pipettes. The tip diameter of each pipette was about 10 μm and the pipettes were placed close (20–25 μm) to the cell (Fig. 1). In control experiments all six barrels were filled with the same solution, for example, 2 μM GABA, to make sure that each barrel was positioned correctly to allow the test solutions to engulf the recorded cells maximally. Differences of less than 20% were found in whole-cell currents when the different barrels were activated. Movements of the puffer pipette by 20–30 μm did not significantly change the responses. Air pressure (0.3–0.5 bar) was applied under computer control. Without the pressure, bath solution continuously flowed into the pipettes by capillary attraction. This effect prevented the test solutions from leaking

Figure 1. Slice preparation of a rat retina during patch-clamp recordings from an AII-amacrine cell. *A*, In this Nomarski micrograph the retinal layers are indicated (*OS*, rod outer segments; *IS*, rod inner segments; *ONL*, outer nuclear layer; *OPL*, outer plexiform layer; *INL*, inner nuclear layer; *IPL*, inner plexiform layer; *GCL*, ganglion cell layer). The patch electrode, of which only the tip is in focus, approaches an AII-amacrine cell body from the left side. The puffer pipette can be seen in the lower right corner. *B*, Using translucent light and epifluorescence one can see the cell body of the AII-amacrine cell, whose membrane was ruptured by the patch electrode, filled with LY. *C*, With this focal plane the dendritic tree of the LY-filled AII-amacrine cell and its stratification in the IPL can be seen. *D*, Using only epifluorescence illumination the typical bistratified morphology of the AII-amacrine cell and the patch electrode become apparent. In the outer part of the IPL the AII has the so-called lobular appendages; in the inner part, a bushy dendritic tree. Scale bar, 50 μ m.

out. The concentration of drugs (given in parentheses) refers to the concentrations in the barrel of the puffer pipette. The actual concentrations at the cell membranes are lower and not known. Glutamate (2 mM), kainate (KA; 0.2 mM), GABA (2 μ M), glycine (0.2 mM), acetylcholine (1 mM), taurine (0.5 mM), and tetrodotoxin (TTX; 1 μ M) were obtained from Sigma (Deisenhofen, Germany). L-2-Aminophosphonobutyric acid (L-APB; 0.2 mM), *N*-methyl-D-aspartate (NMDA; 1 mM), and 6-cyanoquinoline-2,3-dione (CNQX; 20 μ M) were obtained from Cambridge Research Biochemicals (Cambridge, UK). Muscimol (10 μ M) was purchased from Research Biochemical Incorporated (Natick, MA). AMPA (0.1 mM) was obtained from Tocris Neuramin (Bristol, UK). The following drugs were dissolved in extracellular solution and bath applied: strychnine (1 μ M; Serva, Heidelberg, Germany), bicuculline (20 μ M; Cambridge Research Biochemicals, Cambridge, UK), tetraethylammonium (TEA; 10–25 mM; Merck, Darmstadt, Germany), and 4-aminopyridine (4-AP; 10 mM; Merck, Darmstadt, Germany). HEPES and nystatin were purchased from Serva (Heidelberg, Germany), Lucifer yellow, from Sigma (Deisenhofen, Germany); and all other chemicals, from Merck (Darmstadt, Germany).

The level of light adaptation of the retinal slice. The room lights were switched on during all experimental procedures. For dissection of the retina and during the slicing procedure the Petri dish was illuminated by the light beam of the dissection microscope (OMI, Zeiss, Oberkochen,

Germany). When approaching individual cells the translucent illumination of the microscope was switched on, and for identification of the Lucifer yellow-filled cells epifluorescent illumination was applied. Hence, the general status of the retinal slice is very likely light adapted.

Tracer coupling and immunostaining of AII-amacrine cells. In some experiments patch electrodes were filled with standard intracellular solution to which Lucifer yellow (0.2 mg/ml) and neurobiotin (15 mg/ml) (Vector, Burlingame, CA) were added (Horikawa and Armstrong, 1988). Neurobiotin has recently been reported to show tracer coupling of AII-amacrine cells (Vaney, 1991; Hampson et al., 1992). Within less than 1 min LY filled all the dendrites of the AII cell recorded in the whole-cell mode; hence, rapid filling with neurobiotin can also be expected. After varying recording times, usually 10–20 min, the slices were fixed for 40 min in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.4. They were washed three times for 10 min in PBS, incubated for 30 min in 0.5% Triton X-100 in PBS, and then reacted with 1:100 extravidin (Sigma) in PBS for 2–3 hr. The slices were incubated for 10 min in 0.05% 3,3'-diaminobenzidine (Sigma) in PBS with 0.01% hydrogen peroxide. They were washed three times for 10 min in PBS and then coverslipped in 90% glycerine in phosphate buffer.

Details of the immunocytochemical staining of frozen sections of the rat retina with antibodies against parvalbumin are given elsewhere (Röhrenbeck et al., 1987, 1989; Wässle et al., 1993).

Results

Identification of AII-amacrine cells

The layers of the retina and individual neurons can be recognized in the slices using Nomarski optics (Fig. 1*A*). Since AII-amacrine cells are the most numerous amacrine type and because their cell body protrudes into the IPL (Perry and Walker, 1980; Voigt and Wässle, 1987), with some experience one can select them for recording with a rather high success rate. However, every cell recorded was filled with LY to make sure that it was an AII-amacrine cell. The presence of the dye in the cell had no obvious effect on the currents recorded (Stewart, 1978; Eliasof et al., 1987; Werblin et al., 1988; Edwards et al., 1989). Altogether, 53 AII-amacrine cells were successfully recorded from and stained in the present study. Figure 1*B–D* shows micrographs of an AII-amacrine cell with the patch electrode attached during the recordings in the slice chamber, and the typical bistratified AII morphology becomes apparent. The cell body protrudes slightly into the IPL and a stout single process leaves the cell body (Fig. 1*D*). In the outer third of the IPL the lobular appendages are found; in the inner third a densely branched dendritic plexus is stained. It was often difficult to remove the recording electrode from the cell body without pulling the cell apart. Therefore, most cells were photographed with the electrode attached to the cell body. Sometimes cell processes were damaged by the slicing and such cells are not included in the present analysis. Recordings from individual cells were usually stable for about half an hour.

Coupling of AII-amacrine cells

Electron microscopical reconstructions of AII-amacrine cells have demonstrated extensive gap junctions with ON-bipolar cells and with other AII-amacrine cells (Kolb and Famiglietti, 1974; Famiglietti and Kolb, 1975). More recently it has been shown that AII-amacrine cells of the rabbit retina show tracer coupling when injected with biocytin or neurobiotin (Vaney, 1991; Hampson et al., 1992). The extent of coupling could be modulated by dopamine; hence, it may depend on the level of light adaptation. In order to find out whether AII-amacrine cells in the slice preparation used for this study are coupled, patch electrodes were filled with LY and neurobiotin. The cells recorded from could be identified during the experiment by the LY filling, and afterward the slice was fixed and reacted for neurobiotin (Horikawa and Armstrong, 1988; Vaney, 1991). Figure 2, *A* and *B*, shows an AII-amacrine cell in a rat retinal slice preparation filled with neurobiotin. The staining is restricted to the injected cell and did not diffuse into neighboring AII cells. Two other cell bodies of the inner nuclear layer (INL), possible bipolar cells, are weakly labeled (arrowhead, Fig. 2*B*); however, we think this is nonspecific uptake from the extracellular space (Kenan-Vaknin et al., 1992). Altogether, eight AII cells were filled with neurobiotin; of these, six were positively stained and in no case did we find clear tracer coupling with other AII cells or with bipolar cells. We observed one instance of extensive coupling between amacrine cells; however, neither the filled cell nor the coupled cells are AII cells (Fig. 2*D*). The micrograph in Figure 2*C* shows the AII-amacrine population in the rat retina immunostained with antibodies against parvalbumin (Wässle et al., 1993). The cell bodies of AII cells form a single row along the INL/IPL border. The outer half of the IPL is well filled with their lobular appendages; their arboreal dendrites terminate close to the ganglion cell layer. Ganglion

cells are also weakly labeled. It is apparent from Figure 2*C* that AII cells are frequent and there is a good chance of hitting an AII cell body even without prelabeling.

Viability of the mammalian retinal slice

The dissection of the eye and the slicing (Werblin, 1978; Wu, 1987) causes a lot of stress to this *in vitro* preparation of the retina. However, we routinely found synaptic potentials and in some cases AII cells even showed light responses. Figure 3*A* shows a whole-cell patch-clamp recording from the same AII cell that was injected with neurobiotin in Figure 2, *A* and *B*. When the microscope light was turned on for approximately 1 sec, a prominent inward current was induced (Mittman et al., 1990). Continuous spontaneous current fluctuations were also observed (Stern et al., 1992). These as well as the light response could be blocked by superfusing the slice preparation with 3 mM Co^{2+} in the bathing medium. Figure 3*B* shows such spontaneously occurring currents of another cell, and Figure 3*C* shows that they can be blocked by Co^{2+} . It is therefore likely that the current fluctuations represent postsynaptic currents evoked by spontaneous Ca^{2+} -dependent release of transmitter from neurons that are presynaptic to amacrine cells. To avoid indirect influences from other neurons, caused by vesicular release of neurotransmitter, most of the following measurements were made in the presence of Co^{2+} .

Voltage-gated currents of AII-amacrine cells

Voltage-gated currents were measured by the conventional patch-clamp technique (Hamill et al., 1981). Having established whole-cell configuration in the voltage-clamp mode, the patch-clamp amplifier was set to the current-clamp mode to measure the resting potential (zero current potential) of the cell. The range of resting potentials found was -30 mV to -90 mV, and the histogram in Figure 4*A* shows their distribution. The majority of the cells had a resting potential more negative than -50 mV.

When depolarizing current pulses were applied, small transient depolarizations could be elicited (Fig. 4*B*). The cell in Figure 4*B* was current clamped to a potential of -68 mV. When a current of 25 pA was injected, the cell depolarized and spike-like potentials were elicited. They could be blocked by superfusion of the slice with TTX (Fig. 4*C*).

This indicates that voltage-dependent Na^{+} channels are surely involved in the generation of the spike-like potentials. However, for true Na^{+} action potentials they were too small and too slow (duration at half-peak > 10 msec). In order to show that this is not due to a systematic error of our recording setup and a failure of the slice preparation in general, we used the perforated-patch method (nystatin) (Horn and Marty, 1988) to record from a ganglion cell (Fig. 5*A*) and an AII-amacrine cell (Fig. 5*B*) in the same slice. Under these conditions it would be expected that washout of second messenger systems or of Ca^{2+} -activated currents is minimized. The ganglion cell was depolarized from a resting potential of -65 mV by injection of a depolarizing current of 20 pA. At a threshold between -50 and -40 mV, rather large action potentials of unitary size were elicited. We have also measured the activation kinetics of the Na^{+} currents of ganglion cells (not illustrated) and, in agreement with previous reports from solitary dissociated ganglion cells of the mammalian retina, we find an activation at about -45 mV (Lipton and Tauck, 1987; Kaneda and Kaneko, 1991). The AII-amacrine cell (Fig. 5*B*) was depolarized from the resting potential of -63 mV by injection of a depolarizing current of 5 pA. At

Figure 2. *A*, Micrograph of a rat retinal slice following neurobiotin injection into an AII-amacrine cell. The filled cell is strongly labeled and shown at higher magnification in *B*. A putative bipolar cell body (*arrowhead*) is weakly labeled. We think that the dye uptake of this bipolar cell is through damage of the axon, which passes by the AII cell body and does not represent tracer coupling. *C*, Micrograph of a frozen section through a rat retina stained for parvalbumin using the peroxidase-antiperoxidase method. The stained cells have the characteristic morphology of AII-amacrine cells: a stout descending primary dendrite, thin, short branches with lobular appendages in the outer half of the IPL and arboreal dendrites close to the ganglion cell layer (*GCL*). Their positive identification as AII cells is presented elsewhere (Wässle et al., 1993). The micrograph demonstrates that AII-amacrine cells are found at a relatively high proportion at the INL/IPL border. *D*, Micrograph of a rat retinal slice following neurobiotin injection into the amacrine cell marked by the *arrow*. Many other amacrine cells are labeled by tracer coupling. Neither the filled cell nor the coupled cells are AII-amacrine cells. Scale bar: 100 μ m for *A*, 50 μ m for *B–D*.

a threshold close to -60 mV, spike-like potentials are elicited and a sustained discharge rate is observed during depolarization. Further depolarization by larger currents increased the number of spike-like potentials. The spike-like events differ in three parameters from ganglion cell action potentials: they have a

lower threshold of -55 to -60 mV in contrast to the -45 mV of ganglion cells; the spike-like potentials are smaller and slower than ganglion cell action potentials; and finally, spike-like potentials are found superimposed.

In order to study the ionic currents generating the spike-like

potentials, the voltage-gated currents of AII-amacrine cells were measured (Fig. 6). The cell was voltage clamped close to the resting potential at -70 mV and the voltage was stepped to more positive values for 60 msec (Fig. 6A). A small fast inward current was followed by a large outward current. Figure 6B shows the inward current in isolation. The voltage was changed from a holding potential of -70 mV to -60 mV and only the fast inward current was activated. When TTX was applied to the cell (Fig. 6B) the fast inward current was completely blocked; hence, it is likely to be a voltage-dependent Na^+ current (Hille, 1992). The same fast transient currents were also recorded when 3 mM Co^{2+} was added to the bathing medium in order to block Ca^{2+} currents and synaptic activity. They were found in 45 out of 49 AII cells tested. TTX was applied to nine cells and always blocked the fast inward currents.

The characteristics of this current were measured from the current-voltage curves. However, as mentioned in Materials and Methods, the input capacitance of AII cells is large and the capacitive transients do not decay along a simple exponential time course. Moreover, since many cells were lost when the series resistance compensation was turned on, the following measurements were done without that compensation. Due to the series resistance, the voltage clamp might not be as effective as needed, and because the cell has many processes, these might not be isopotential. Hence, there is an obvious problem of space clamp in the following measurements. Figure 7A shows the current-voltage curve of the TTX-sensitive current in an AII-amacrine cell, which was voltage clamped at a holding potential of $V_h = -90$ mV. At different command voltages the peak amplitude of the fast transient inward current was measured. K^+ outward currents were suppressed by TEA and Cs^+ in the patch pipette. The TTX-sensitive current activated between -65 and -60 mV; however, the steep slope of activation of the current and the gentle slope of the fully activated current in Figure 7A are affected by the problem of space clamp. The activation at low voltages (< -60 mV) seems to be a characteristic of the voltage-dependent Na^+ current of the AII cells. In ganglion cells, as mentioned before, we consistently found an activation at -45 mV for the Na^+ currents.

It was possible to inactivate this current by different prepotentials. This is shown in Figure 7B for another AII-amacrine cell. The cell was held at -90 mV, then prepotentials (V_p) of 60 msec duration and variable height were applied. After a brief reset pulse (duration, 1 msec) the command voltage (duration, 30 msec) was set at $V_c = -40$ mV and the peak of the fast transient inward current was measured. It can be seen in Figure 7B that steady state inactivation occurs already at $V_p = -80$ mV, and at $V_p = -30$ mV this current is completely suppressed.

The prominent outward current of Figure 6A quite likely is caused by activation of voltage-dependent K^+ channels. In Figure 8 the influence of TEA and of 4-AP on these channels is illustrated. The outward current (Fig. 8A) has a phasic/tonic characteristic. Bath application of TEA strongly reduces the current (Fig. 8B); bath application of 4-AP has only little effect (Fig. 8C). When TEA and 4-AP are applied together (Fig. 8D) most of the current is blocked. With Cs^+ and TEA in the electrode solution (not shown) a complete block of the outward current could be achieved.

The voltage dependence of the outward current is displayed separately for the peak (Fig. 9A) and the sustained component (Fig. 9B). The original data and the data corrected for the series resistance are shown. The currents activate at approximately

Figure 3. *A*, Whole-cell currents recorded from an AII-amacrine cell during voltage clamp. This is the same AII-cell that was injected with neurobiotin and is shown in Figure 2A. The cell was clamped close to the resting potential at $V_h = -60$ mV. Many spontaneously occurring putative postsynaptic currents can be seen. At the time indicated by the arrow the translucent illumination of the microscope was turned on for approximately 1 sec, and a distinct inward current was elicited. *B*, Whole-cell currents of another cell that was voltage clamped at $V_h = -60$ mV. Many spontaneous putative postsynaptic currents can be detected. These are blocked when 3 mM Co^{2+} is added to the bathing medium, as shown in *C*.

-50 mV and the peak component shows a steeper slope than the sustained component. Because of the relatively high uncompensated series resistance, at a given command voltage the phasic component is more strongly attenuated than the tonic component, and is underestimated in Figures 6 and 8.

The steady state inactivation of the currents is shown in Figure 9C. The tonic component does not seem to inactivate by the prepotentials, while the transient component is inactivated by prepotentials of -30 mV.

Although application of 4-AP and TEA did not dissect two such components, steady state inactivation mainly influenced the transient peak. Hence, it is possible that two separate types of K^+ channel contribute to the outward current (Klumpp et al., 1991; Salkoff et al., 1992).

In summary one can conclude that AII-amacrine cells show spike-like potentials when their membrane is depolarized. It is very likely that voltage-dependent Na^+ and K^+ currents are

Figure 4. *A*, Distribution of resting potentials of AII-amacrine cells measured at the beginning of the recordings in the current-clamp mode (zero current). The mean resting potential was found to be $-59.1 \pm 13.3 \text{ mV}$ ($N = 44$). *B*, Voltage response of an AII-amacrine cell upon injection of current. The resting potential of the cell was -48 mV , and the holding potential was $V_H = -68 \text{ mV}$. A depolarizing current of 25 pA (horizontal line above the abscissa) elicited spike-like potentials at a threshold of -60 to -55 mV . The amplitude of the spikes was quite small ($<10 \text{ mV}$). When more current was injected, the frequency of the spikes increased and a sustained discharge rate was observed. Small currents depolarized the cell without eliciting spike-like potentials. *C*, When the slice was superfused with TTX, applied at a concentration of $1 \mu\text{M}$ from the puffer pipette, the spike-like potentials were blocked. Current injection was 30 pA (bath solution E2, electrode solution I1).

involved in the generation of these small spike-like potentials. However, the precise kinetics and relative sizes of the underlying currents, and where on the AII cells they are localized, need further investigation.

No attempts were made in the present study to isolate or investigate voltage-activated Ca^{2+} currents.

Ligand-gated currents of AII-amacrine cells

It is very likely that bipolar cells of the mammalian retina use glutamate as their transmitter (Bloomfield and Dowling, 1985; Massey and Miller, 1988; Massey, 1990). It has been shown that glycine and GABA are transmitter substances of the majority of mammalian amacrine cells (for review, see Massey and

Redburn, 1987; Vaney, 1990), but there is also a distinct population of cholinergic amacrine cells (Masland, 1986; Voigt, 1986). In order to test whether AII-amacrine cells have receptors in their membranes for these four transmitter substances, whole-cell currents were measured upon application of related compounds. Cobalt was added to the bathing medium, which was shown in Figure 3C to block vesicular synaptic release of transmitter from presynaptic neurons. The perforated-patch method (nystatin) was used to minimize washout of second messenger systems that are critical for activation of APB-gated currents (Nawy and Jahr, 1991; Yamashita and Wässle, 1991a,b; Shiells and Falck, 1992a,b). Figure 10 shows whole-cell currents elicited by application of KA, muscimol (MUSC), glycine (GLY), L-APB,

Figure 5. Recordings from a ganglion cell (*A*) and from an AII-amacrine cell (*B*) that were current clamped at their resting potentials. *A*, Rather large action potentials are elicited by injecting a depolarizing current of 20 pA through the patch pipette into a ganglion cell. *B*, Small spike-like potentials were elicited when a current of 5 pA was injected into an AII-amacrine cell. Please note the superposition of spike-like potentials (bath solution E2, electrode solution I1 + nystatin).

Figure 6. *A*, Whole-cell currents recorded from an AII-amacrine cell during voltage clamp. From a holding potential of -70 mV the voltage was stepped to -40 , -20 , 0 , $+20$, $+40$, and $+60$ mV (electrode solution I1, bath solution E2). *B*, The same AII-amacrine cell is depolarized from -70 mV to -60 mV, which activated only the fast transient inward current. *C*, Superfusion of the cell with TTX (concentration in the puffer pipette, $1 \mu\text{M}$) blocked the transient inward current. The bottom trace shows the voltage protocol (no leakage subtraction applied).

and ACh. A prominent inward current was induced by the glutamate agonist KA, but no response was measured when APB was applied. Muscimol, a GABA_A-receptor agonist, and glycine induced strong inward currents, while ACh application had practically no effect. The currents upon application of muscimol and glycine were recorded after perfusion of the cell with the Cl⁻ solution of the electrode, that is, under symmetrical Cl⁻ concentrations of 137 mM inside and outside. Under these conditions (E_{Cl} near 0 mV) an inward current was measured at $V_H = -70$ mV. The transmitter effects were also tested in the current-clamp mode (Fig. 11). A cell clamped at the resting potential (zero current) was strongly depolarized by KA and glutamate (GLU), while APB had no effect. Muscimol and glycine, as well as taurine (TAU), also caused depolarizations of the membrane potential (symmetrical Cl⁻). At the limited time resolution of Figure 11, no spike-like potentials can be detected upon depolarization of AII cells by, for instance, glutamate. However, at better resolution spike-like potentials similar to those elicited by current injection could be detected (not shown).

Characterization of ligand-gated currents of AII-amacrine cells

The results upon application of muscimol, glycine, and taurine shown in Figures 10 and 11 were recorded with symmetrical Cl⁻ concentrations. Under those circumstances the currents reversed at 0 mV holding potential. When the Cl⁻ concentration in the electrode was lowered, the reversal potential followed the prediction of the Nernst equation for Cl⁻ and we conclude that muscimol as well as glycine and taurine gate Cl⁻ conductances. The equilibration of Na⁺ and K⁺ ions through the perforated patch is about 10 times faster than that of Cl⁻ ions (Horn and Marty, 1988). Therefore, the interior Cl⁻ concentration is close to the intact value just after the pore formation by nystatin. When muscimol or glycine were applied during this initial period they caused hyperpolarization of AII-amacrine cells in the current-clamp mode and an outward current when the cells were voltage clamped at the resting potential (not shown). After a few minutes the polarities reversed because of Cl⁻ equilibration

Figure 7. Current-voltage curves for the voltage-dependent Na⁺ current. *A*, Peak amplitude of the inward current in response to voltage steps of 60 msec duration ($V_H = -90$ mV). The open circles and the dashed curve show the amplitudes measured. The solid circles and the solid line show the current-voltage relation corrected for the measured series resistance ($10 \text{ M}\Omega$). The inward current activates between -65 and -60 mV (electrode solution I2, bath solution E2). *B*, The abscissa gives the voltage of the conditioning prepotential (duration 60 msec). The ordinate shows the peak amplitude of the fast transient inward current measured after this prepotential at a command voltage of -40 mV. Between -85 and -80 mV steady state inactivation starts and at -30 mV this inward current is completely inactivated.

Figure 8. Voltage-dependent K^+ currents of AII-amacrine cells. *A*, The cell was voltage clamped at -70 mV and voltage was stepped to -60 , -40 , -20 , 0 , $+20$, $+40$, and $+60$ mV for 60 msec (electrode solution I1, bath solution E2). An outward current with a phasic/tonic characteristic was elicited. *B*, Application of TEA from the puffer pipette (25 mM) effectively reduced this outward current. *C*, Application of 4-AP (10 mM) had only a slight effect. *D*, Application of TEA and 4-AP strongly suppressed the outward current. There was no need to apply TTX to this cell, because the Na^+ inward current was very small. In other cells where TTX was applied, it blocked the fast inward current without significant effects on the outward currents. The traces at the bottom show the voltage protocols applied.

(Yamashita and Wässle, 1991a). From this result we conclude that for intact AII-amacrine cells the Cl^- reversal potential is more negative than the resting potential. GABA and glycine thus would hyperpolarize AII-amacrine cells *in situ*. The actions of glycine and taurine (not shown) could be blocked by coapplication of strychnine (STRY), suggesting that both act on a conventional strychnine-sensitive glycine receptor (Fig. 12A). The glycine-induced currents showed a fast peak followed by a sustained plateau (Figs. 10, 12), which suggests a rapid desensitization of the receptor (Bormann, 1990). The glycine effects were not blocked by coapplication of bicuculline (BIC) (Fig. 12B).

Muscimol, the $GABA_A$ -receptor agonist, induced a prominent current in all AII-amacrine cells. The current showed a transient/sustained characteristic. Figure 12B shows the actions of muscimol and glycine and their interaction with bicuculline. Only the muscimol-induced current was effectively blocked by bicuculline. When GABA was used instead of muscimol, a comparable reduction was observed by application of bicuculline. We therefore conclude that GABA activates a Cl^- conductance in AII-amacrine cells through $GABA_A$ -type receptors (Ishida, 1992).

The following agonists at excitatory amino acid (EAA) receptors (Watkins et al., 1990) were tested on AII-amacrine cells: glutamate, KA, quisqualate, NMDA, and APB. While glutamate, KA, and quisqualate induced prominent currents, NMDA and APB were ineffective. Because NMDA receptors are blocked by Mg^{2+} ions, show strong voltage dependence, and are augmented by coapplication of glycine, different conditions were used to test for the effects of NMDA (Mayer and Westbrook, 1987; Ascher et al., 1988). Low $[Mg^{2+}]$, addition of $0.5 \mu M$ glycine, and/or more positive holding potentials did not unmask an NMDA-gated current in AII-amacrine cells (Fig. 13A,B). It is therefore unlikely that NMDA receptors make an important contribution to the EAA-induced currents of AII cells. Under comparable conditions we found NMDA responses in other types of amacrine cells and in ganglion cells; hence, we exclude the possibility that a methodological problem might be responsible for not finding them in AII-amacrine cells.

Glutamate, KA, and AMPA induced prominent responses when applied to AII-amacrine cells (Fig. 13A,B). As shown in Figure 13D, the KA- and AMPA-induced inward currents could be blocked completely by coapplication of CNQX. The reversal potential of these currents was close to 0 mV. Hence, KA and AMPA very likely gate nonselective cation channels with Na^+ ions as the main charge carrier at the holding potential of -70 mV. Since CNQX is an antagonist at both the KA- and AMPA-type of receptors (Watkins et al., 1990), we could not further characterize these EAA receptors of AII cells.

As described before (Figs. 10, 11), APB had no effect on the majority of AII-amacrine cells. Altogether, 10 cells were tested for APB responses, all with nystatin in the electrode. In only three cases were APB responses measured; however, these disappeared when Co^{2+} was added to the bathing medium (Fig. 14). Hence, we conclude that APB has no direct effect on AII-amacrine cells. In contrast, all cells tested showed prominent inward currents ($V_H = -70$ mV) when KA, glutamate, or AMPA were applied. Therefore, if glutamate is the transmitter released by rod bipolar cells, it will cause depolarization of AII-amacrine cells, and the synapse between rod bipolars and AII-amacrine cells is a sign-conserving synapse.

Discussion

Electrical coupling of AII-amacrine cells

AII-amacrine cells of the cat, rabbit, and rat retinas have been reconstructed from electron microscopic serial reconstructions and it has been reported that they make two types of gap junctions (Famiglietti and Kolb, 1975; Kolb, 1979; Sterling, 1983; Strettoi et al., 1992; Chun et al., 1993). On the descending shaft they have large gap junctions with ON-cone bipolar cells. These gap junctions display an asymmetric structure with a layer of fluffy material underlying the cytoplasmic aspect of the AII cell membrane (Kolb, 1979; Strettoi et al., 1992). The vitral dendrites of neighboring AII-amacrine cells are connected by small, symmetrical gap junctions.

What is known about the functional role of these gap junctions, how are they modulated by the light or dark adaptation of the eye, and how do they influence the interpretation of the

present results? Extensive coupling using neurobiotin as a tracer was recently observed *in vitro* between rabbit AII-amacrine cells as well as between AII-amacrine cells and cone bipolar cells (Vaney, 1991). Coupling could be greatly reduced by dopamine acting on D₁ receptors (Hampson et al., 1992). Similar uncoupling by dopamine has been shown previously for horizontal cells of the vertebrate retina (for review, see Dowling, 1991; Witkovsky and Dearth, 1991). AII-amacrine cells receive many synapses from dopaminergic amacrine cells. Most of these synapses are at the cell body or the primary dendrites (Pourcho, 1982; Voigt and Wässle, 1987; Kolb et al., 1990), and dopaminergic processes form ring-like structures surrounding the perikarya of AII cells. Hence, it is conceivable that *in vivo* AII cell coupling is under dopaminergic control. Dopamine release in the mammalian retina is greater in light-adapted tissue (Parkinson and Rando, 1983; Godley and Wurtman, 1988; Witkovsky and Dearth, 1991); therefore, it can be expected that AII cells are uncoupled in light-adapted retinas.

The retinal slice preparation of the present study was light adapted and this might be the reason why AII cells were not coupled (Fig. 2A). The weak staining of a few bipolar cell bodies close to the injected AII cell body very likely is caused by uptake of neurobiotin through their axons passing by the AII cell body. Labeled bipolar cell bodies are restricted to a narrow column above the injected AII cell body and do not form the regular, widely spaced mosaic described by Vaney (1991) for tracer-coupled ON-cone bipolar cells. However, there is also a pharmacological reason why we think AII cells and ON-cone bipolar cells are not electrically coupled in our preparation. It is well known that APB acts as an agonist of the photoreceptor transmitter when applied to ON-bipolar cells (Slaughter and Miller, 1981; Massey et al., 1983; Bolz et al., 1984) and hyperpolarizes the cells by closing nonspecific cation channels (Nawy and Jahr, 1991; Shiells and Falck, 1992b). Hence, were the ON-cone bipolar cells electrically coupled to AII-amacrine cells, application of APB should hyperpolarize AII-amacrine cells. This seems not to be the case in the slices of the present study. APB was applied onto 10 AII cells. In only three cases were APB responses measured; however, they were blocked by Co²⁺ added to the bathing medium. It is very likely that these effects of APB are due to the rod bipolar input into AII cells, and Co²⁺ blocks the vesicular, Ca²⁺-dependent synaptic transmission between rod bipolars and AII cells (Tachibana and Okada, 1991). Although this APB experiment does not exclude the possibility of unidirectional current flow from AII cells into ON-cone bipolars, the lack of tracer coupling makes this unlikely. In conclusion, AII cell gap junctions seem to be closed in our slice preparation and, more generally, they seem to be closed in the light-adapted retina.

Voltage-gated currents of AII-amacrine cells

When Cajal introduced the name "amacrine cell," he wanted to direct attention to the fact that such cells have no axon. This is certainly true for AII-amacrine cells, but might not be so for many wide-field amacrine cells (reviewed in Vaney, 1990). AII-amacrine cells in the rat have small dendritic fields and the maximum distance from the cell body to the finest dendritic tips is less than 100 μ m. In the inner part of the IPL, where they receive input from rod bipolar cells, they have a bushy dendritic field; in the outer part of the IPL, AII-amacrine cells have thin, short dendrites that terminate in rather large swellings, the so-called lobular appendages (Fig. 1D). Given this rather com-

Figure 9. Current-voltage curves for the voltage-dependent K⁺ currents elicited in response to voltage steps of 60 msec duration ($V_H = -90$ mV). **A**, Voltage dependence of the peak response. The open circles and the dashed line show the amplitudes measured. The solid circles and the solid line show the current-voltage relation corrected for the measured series resistance (23 MΩ). **B**, Voltage dependence of the sustained response, which was measured 55 msec after depolarization (other conventions as in **A**). **C**, Steady state inactivation of the peak current (solid squares) and of the sustained current (open squares). After a conditioning prepotential of 60 msec duration (shown on the abscissa), the currents elicited by a test potential of +40 mV were measured. The sustained current is not inactivated by the prepotential, while the peak current decreases. At a prepotential of -30 mV the curves meet, indicating that the phasic component of the response is completely inactivated.

pact dendritic field, there is no need to conduct impulses for long distances, and it is very likely that the voltage-gated channels described in the present study subserve a different function.

As mentioned before, we faced great difficulties in characterizing these voltage-gated channels because of problems with the space clamp. Although AII-amacrine cells are rather small neurons, we measured an input capacitance up to 10 pF, which is more than twice that of other amacrine cells in rat retina slices. In addition, the capacitive currents did not decay along a single exponential. We also had difficulties in compensating the series resistance. It is possible that the voltage-activated channels of AII cells are found *not* on the soma but on the dendrites, such

Figure 10. Whole-cell currents recorded from an AII-amacrine cell that was voltage clamped at $V_h = -70$ mV. The bars show the time when the drugs were applied through the puffer pipette (bath solution E3, electrode solution I1 + nystatin). See Results for more details.

as the lobular appendages, which may act as isolated compartments. TTX-sensitive dendritic spikes have also been reported for amacrine cells of the mudpuppy retina (Miller and Dacheux, 1976) and in neocortical pyramidal cell dendrites (Stuart and Sakmann, 1992). Such a distribution of TTX-sensitive I_{Na} in isolated compartments might be responsible for our problems with the space clamp and for the superposition of the spike-like potentials. There is some circumstantial evidence to support this. Sometimes we recorded from AII-amacrine cells from which most of the dendrites were cut off during the slicing procedure. Such cells appeared to be healthy with a normal input resistance but did not show spike-like potentials.

Another unusual feature of the voltage-gated channels found on AII cells is their low activation at -55 to -60 mV, in contrast

to ganglion cells, where sodium currents first appeared at -45 mV. The experiment where we depolarize a ganglion cell (Fig. 5A) and an AII cell from identical resting potentials by current injection shows this difference in activation threshold quite convincingly. It is therefore possible that two different types of Na^+ channels are expressed in AII and ganglion cells (Stühmer et al., 1989; Patlak, 1991).

What might be the function of the voltage-gated channels in AII cells? Intracellular recordings of AII-amacrine cell light responses are available from the cat (Nelson, 1982) and from the rabbit retina (Dacheux and Raviola, 1986). These studies noted that the time course of the initial phase of AII light responses is much faster than that of rod bipolar cells, which provide the input. In rabbit retina (Dacheux and Raviola, 1986), small spike-

Figure 11. Voltage changes induced in an AII-amacrine cell by application of different drugs. The cell was current clamped at the resting potential (bath solution E2, electrode solution I1 + nystatin). See Results for more details.

Figure 12. *A*, Whole-cell currents recorded from an AII-amacrine cell, which was voltage clamped at $V_h = -70$ mV. Application of glycine (GLY) alone induced a strong inward current. When the same concentration of glycine was coapplied with strychnine (STRY), this current was strongly suppressed. *B*, When muscimol (MUSC) or glycine were applied, prominent inward currents were elicited. Bicuculline (BIC), when coapplied with muscimol or glycine, only suppressed the muscimol-induced current, while not reducing the glycine-induced current. The glycine-induced current was actually larger during coapplication with bicuculline. This does not mean potentiation of glycine action by bicuculline, but reflects limitations of the application techniques: different barrels of the puffer pipette were not equally effective (bath solution E2, electrode solution I1).

like potentials were found in the initial phase of AII light responses. We think that the opening of the voltage-dependent Na^+ channels is responsible for the acceleration of the light response. The spike-like potentials recorded in rabbit AII cells

might be the result of the voltage-dependent Na^+ and K^+ channels described in the present study. In conclusion, we interpret the voltage-gated channels found in AII-amacrine cells as "local signal boosters." They may be absent from the cell body and mainly found on dendrites where AII cells make their synaptic connections.

During our search for AII-amacrine cells, we occasionally also recorded and stained other types of amacrine cells. Although these cells were not studied in great detail, it was noticed that certain types of wide-field amacrine cells show a different spiking pattern when depolarized by current injection. Such cells fired

Figure 13. Whole-cell currents induced in AII-amacrine cells by EAA agonists and antagonists (electrode solution I1, bath solution E4). *A*, This AII cell was voltage clamped at -60 mV. Glutamate (GLU) induced a strong inward current, while NMDA had no effect. *B*, Responses of another AII cell to KA and AMPA; no current was induced by NMDA. The bath solution E4 is Mg^{2+} -free and contains glycine ($0.5 \mu\text{M}$); hence, NMDA currents, if present in AII-amacrine cells, should be recorded in *A* and *B*. *C*, This AII cell was current clamped at the resting potential of -50 mV. Application of glutamate caused a strong depolarization of the cell (electrode solution I1, bath solution E4). *D*, Whole-cell currents recorded from an AII cell, voltage clamped at -60 mV (bath solution E4, electrode solution I1). KA and AMPA were first applied on their own and induced prominent inward currents. Then, KA and AMPA were coapplied with CNQX, which completely blocked the responses. Finally, KA and AMPA were once more applied without CNQX and the currents were measured again.

Figure 14. *A*, APB-activated whole-cell current of an AII-cell, voltage clamped at $V_h = -70$ mV. APB induced an outward current in this cell. *B*, When 3 mM Co^{2+} was added to the bathing medium, APB responses were blocked. This suggests APB action was not directly at the AII cell membrane, but indirectly through activation of a presynaptic neuron (intracellular solution E1, extracellular solution I1).

a single spike at the beginning of the depolarization and were refractory during the sustained depolarization. The voltage-gated Na^+ channels in these cells were activated at a potential more positive than that of AII-amacrine cells. In the mudpuppy retina, Barnes and Werblin (1986) and Eliasof et al. (1987) described and analyzed the voltage-gated currents that generate single spike activity in amacrine cells. They showed that steady state inactivation of the voltage-dependent Na^+ channels blocks these channels at holding potentials more negative than the activation threshold of voltage-dependent K^+ channels. Therefore, the membrane cannot quickly repolarize and activation of a second spike is not possible. Further experiments have to show whether the same mechanism is responsible for single-spike activity in amacrine cells of the mammalian retina. AII-amacrine cells certainly show a sustained firing behavior and, in light of this, it is worth noting that the steady state inactivation of Na^+ channels and activation of K^+ channels have overlapping ranges (see Figs. 7, 9).

Glutamate receptors of AII-amacrine cells

There is evidence from immunostaining that rod bipolar cells, which provide the major input into AII-amacrine cells, use glutamate as their transmitter (for review, see Massey, 1990). In fish retina the release of glutamate from bipolar cells has been shown more directly (Tachibana and Okada, 1991). The presence of three types of glutamate receptors on AII-amacrine cells has been investigated in the present article. It was found that the KA/AMPA receptor is expressed while NMDA and APB receptors seem not to be expressed by AII-amacrine cells.

The APB receptor is coupled to a second messenger system (Nawy and Jahr, 1991; Yamashita and Wässle, 1991b; Shiells and Falck, 1992a,b); therefore, possible APB responses were measured with the nystatin method (Horn and Marty, 1988). Since binding of glutamate on the APB receptor closes nonselective cation channels, such synapses are sign reversing. On the contrary, binding of glutamate on the KA/AMPA receptor opens such channels, and the synapses are sign conserving. Our finding that AII cells express the KA/AMPA receptor therefore suggests that the rod bipolar/AII-amacrine synapse is sign conserving. In other words, the depolarization by light of rod bipolar cells results in the release of glutamate, which in turn depolarizes AII-amacrine cells. This is an important result, since a sign-reversing synapse had been posulated between rod bipolars and AII-amacrine cells in the cat retina (for review, see Daw et al., 1990).

Despite several efforts, such as recording in low Mg^{2+} , at holding potentials of about -40 mV, or with added glycine, we did not observe NMDA responses in AII-amacrine cells. We had no problems recording NMDA responses in ganglion cells; hence, it is unlikely that we missed NMDA-gated channels for methodological reasons. In a recent study of mudpuppy amacrine cells, Dixon and Copenhagen (1992) found that light responses of sustained amacrine cells do not involve an NMDA-type of receptor; they are blocked by CNQX, suggesting a KA/AMPA-type of receptor. AII-amacrine cells also give sustained light responses (Nelson, 1982; Dacheux and Raviola, 1986) and express the KA/AMPA receptors, but not the NMDA type.

The recent isolation of at least five different cDNAs encoding functional subunits of glutamate receptors (Hollmann et al., 1989; Keinänen et al., 1990) might introduce further diversity. In the rat retina all five glutamate receptor genes are expressed, and in the amacrine layer at least four of these receptors seem to be expressed (Hughes et al., 1992; Müller et al., 1992).

GABA, glycine-, and ACh receptors on AII-amacrine cells

Reconstructions of AII-amacrine cells from electron microscopic serial sections have revealed many conventional synapses along their dendritic branches (Sterling et al., 1988; Kolb et al., 1991; Strettoi et al., 1992). Therefore, in addition to the rod bipolar input, other amacrine cells provide synaptic input into AII-amacrine cells. Using immunocytochemical labeling, both glycinergic and GABAergic synapses were found (Grünert and Wässle, 1991; Kolb et al., 1991). AII-amacrine cells were also labeled with antibodies against glycine receptors (Grünert and Wässle, 1991).

The present study has shown that AII-amacrine cells have GABA- as well as glycine-gated channels in their membrane. Both transmitters open Cl^- channels. Although the precise concentration of Cl^- in AII-amacrine cells is not known, the perforated-patch method applied in the present study allows some conclusions with respect to the Cl^- reversal potential. Using nystatin in the patch electrode, GABA or glycine produced hyperpolarizing voltage responses during the first few minutes. Gradually the Cl^- in the electrode diffused into the cell and the response became depolarizing. This shows that the Cl^- reversal potential of intact AII cells is more negative than the resting potential. Hence, GABAergic or glycinergic synapses hyperpolarize AII-amacrine cells and the amacrine cells providing this input might cause the lateral inhibition found in AII cells (Nelson, 1982; Dacheux and Raviola, 1986). Since glycinergic amacrine cells have small dendritic fields (Pourcho and Goebel, 1985) they might provide the antagonistic surround, which is not much wider than the AII receptive-field center (Nelson, 1982). Most GABAergic amacrine cells have large dendritic fields (Pourcho and Goebel, 1983; Wässle and Boycott, 1991) and they may provide more extended lateral interactions. This remains to be tested in further experiments where the spatial extents of the GABAergic or glycinergic inputs can be measured (Barnes and Werblin, 1987; Lukasiewicz and Werblin, 1990).

AII-amacrine cells apparently lack ACh receptors. Although cholinergic amacrine cells are a prominent cell type in all vertebrate retinas (for review, see Masland, 1986) and have been investigated in great detail, we are not aware of any evidence that they might be specifically involved with the rod pathway.

In summary, the mammalian retinal slice preparation can be used to study individual neurons and their pharmacological properties. No precautions were taken in the present study to rescue light responses; actually, we were surprised to find any such responses given the strong bleaching of the retina and the lack of pigment epithelium. We are therefore optimistic that they can be measured routinely when the dissection and microscopy are done under infrared light illumination (Mittman et al., 1990). Such a preparation would be of great potential for further studies of mammalian retinal function.

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