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Effects of Histamine on Light Responses of Amacrine Cells in Tiger Salamander Retina

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Abstract

Using immunofluorescence, we showed that histamine receptor 1 is expressed by horizontal cell axons and a subset of amacrine cells in the tiger salamander retina. The effects of histamine on light responses of amacrine cells were studied in slice preparations. Histamine modulated the light responses of many salamander amacrine cells, depending upon the morphological type. The most pronounced effects of histamine were decreases in the light responses of broadly stratified amacrine cells, particularly those having medium-sized dendritic field diameters. To determine whether the effects of histamine were direct, Co^{++} was substituted for Ca^{++} in the extracellular medium to block synaptic transmission. Histamine still affected broadly stratified amacrine cells, but not narrowly stratified amacrine cells under these conditions. Taken together, these findings suggest that inhibitory interactions between strata of the IPL and within the classical receptive fields of the ganglion cells would be particularly sensitive to histamine released from retinopetal axons.

Keywords

Retinopetal; Centrifugal; Voltage Clamp; H_1 -receptor; Horizontal Cell

INTRODUCTION

In the retinas of guinea pigs (Airaksinen and Panula 1988), macaques, rats (Gastinger et al. 1999, 2001) and mice (Greferath et al. 2009), there are axons containing histamine that originate from perikarya in the posterior hypothalamus. Three lines of evidence suggest that amacrine cells, inhibitory local circuit neurons of the inner retina, are among their targets. First, these retinopetal axons project to the inner plexiform layer (IPL), where the amacrine cells also ramify and synapse with bipolar cells, ganglion cells and other amacrine cells. Second, histamine is known to modulate the efficacy of GABAergic synapses from amacrine cells onto other amacrine cells in the rat retina (Feigenspan and Bormann 1994). Finally, histamine receptors have been localized to dopaminergic amacrine cells in rat and mouse retinas (Gastinger et al. 2006; Greferath et al. 2009), and histamine inhibits the

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release of dopamine from guinea pig retinas (Weber and Schlicker 2001). There are many distinct morphological types of amacrine cells in mammals, but only a few of these types have well known functions (MacNeil and Masland 1998).

We selected the tiger salamander retina for these experiments, instead, because their amacrine cells are equally diverse, if not more so, and their light responses have been well characterized (Pang et al. 2002). We showed that histamine H_1 -receptors (HR1) were expressed by a subset of amacrine cells. We then recorded the light responses from amacrine cells in slice preparations of salamander retina using patch electrodes. We found that histamine modulated the light responses of many, but not all, amacrine cells and that the effects varied according to the morphological type. We also localized HR1 to horizontal cell axon terminals but did not investigate their function.

It is particularly appropriate that we dedicate this paper to Dr. Dianna Johnson because the approach we have adopted is one that we learned from her, namely using the retina as a model system, classifying specific populations of neurons by the neurotransmitter they contain and identifying their targets based on the localization of receptors for that neurotransmitter. Dianna's pioneering work in this area influenced vision researchers all over the world, but no more so than in Houston. She was a mentor and role model for many of us, and she has contributed in many other ways to the community of neuroscientists here. She was the first Principal Investigator of the Core Grant that established the microscopy facility used in this work, and she has made many other contributions to the research infrastructure. But her most important contribution was to foster collaborations among vision researchers at the various institutions in Houston, of which this paper is an example.

EXPERIMENTAL PROCEDURE

Retinal preparations

Larval tiger salamanders (*Ambystoma tigrinum*) were purchased from Charles D. Sullivan, Co. (Nashville, TN). The animal protocol was approved by Baylor College of Medicine, and all animals were treated in accordance with the institutional and NIH guidelines. They were maintained on a daily 12 h light-dark cycle and were dark-adapted for 1–2 h before the experiment. To maintain the retina in the fully dark-adapted state, all further procedures were performed under infrared illumination with dual-unit Prowler infrared scopes (B.E. Meyers, Redmond, WA). The dissection procedures have been described previously (Werblin 1978; Wu 1987). In brief, the animals were decapitated and the eyes were enucleated and hemisected. The cornea, lens and vitreous were carefully removed. The retinas were removed from the posterior eyecups and flattened, photoreceptor side up, onto a filter (pore size: 0.45 μ m, Millipore, Billerica, MA). The retina and filter paper were sectioned into 200 μ m thick slices in a chamber and fixed onto the chamber with high vacuum grease (Dow Corning Corporation, Midland, MI). The chamber was then mounted on the stage of an upright, fixed stage fluorescence microscope (BX51WI, Olympus Optical Co., Tokyo, Japan) within a light-tight Faraday cage. The retinal slices were continuously superfused with Ringer's solution at 22°C containing (in mM): 108 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 2 $CaCl_2$, and 5 HEPES, adjusted to pH 7.7 with NaOH. All chemicals were dissolved in Ringer's solution and applied in the superfusate.

Electrophysiological recordings

Amacrine cells were visualized in the slices using an infrared CCD camera (Rolera-XR, Q-image, Surrey, BC, Canada) mounted on the microscope and then were monitored on a laptop PC screen with Q-Capture Suite Software (Q-image). Whole-cell recordings were obtained with a double patch clamp EPC10 amplifier (HEKA Elektronik, Lambrecht,

Germany) in voltage- or current-clamp mode. The electrodes were pulled from borosilicate glass (BF 150-86-10, Sutter Instrument, Novato, CA) using a Flaming/Brown P-97 puller (Sutter Instrument Company, Novato, CA). The electrodes had a resistance of 8–12 M Ω when filled with an intracellular solution containing the following (in mM): 118 Cs methanesulfonate, 12 CsCl, 5 EGTA, 0.5 CaCl₂, 4 ATP, 0.3 GTP, 10 Tris, 0.8 Lucifer yellow, adjusted to pH 7.2 with CsOH.

Current and voltage signals were filtered at 3 kHz and digitized at 5 kHz using a Pentium computer equipped with LIH 1600 AD/DA board (HEKA). Patchmaster software (HEKA) was used to generate voltage and current commands.

Light stimulation

Uniform, full-field light stimuli were projected via the microscope condenser using a light-emitting diode (LED) lamp with a 630 nm peak wavelength (Lighting Components, Brooklyn, NY). Photon flux at the surface of the retina was measured with a Li-Cor (Lincoln, NE) quantum sensor (model Li-190SB) and radiometer/photometer (model Li-185B). The intensity was 8.28×10^{18} photons/m²/s, which saturates rods and activates cones (Yang and Wu 1996).

Visualization of cell morphology

After recording electrophysiological data, the electrode was carefully pulled out from the cell soma, and the retinal slice was immediately fixed in fresh 4% paraformaldehyde/phosphate buffered saline (PBS), pH 7.8, for 2 h at room temperature. Three-dimensional cell morphology was visualized via Lucifer yellow fluorescence with a confocal microscope (Zeiss 510; Carl Zeiss, Thornwood, NY). Images were acquired with a 40 $\times$ water immersion objective (numerical aperture, 0.75), using the 458 nm excitation line of an argon laser and a high-pass 505 nm emission filter. Consecutive optical sections were superimposed to form a single image using Zeiss Laser Scanning Microscope-PC software, and these compressed image stacks were further processed in Adobe Photoshop 6.0 (Adobe Systems, San Jose, CA) to improve the signal-to-noise ratio. Because signal intensity values were typically enhanced during processing to improve the visibility of smaller processes, the cell bodies and larger processes of some cells appear saturated because of their higher concentration of fluorophore. The background images of the retinal slices were acquired simultaneously with the fluorescent cells, and they were imaged using transmitted light. We selected cells in the proximal row of the inner nuclear layer (INL) with somas situated beneath the surface of the slice, and they usually had relatively intact processes, as assessed by rotation of the stacked images.

Data analysis

Data analysis was carried out using Igor 5.0 (WaveMetrics, Lake Oswego, OR), Sigmaplot 9.0 (Systat Software Inc., San Jose, CA). Firing rates and bursting activity were analyzed with Mini Analysis software (Synaptosoft Inc., Fort Lee, NJ). To compare values from two conditions, paired t tests were used. The level of significance was set at $p < 0.05$. All values are given as the mean $\pm$ SEM.

Immunolabeling

In some experiments, one eye was enucleated and hemisected, and the posterior half of the eyecup was immersion fixed in fixed in 0.1M phosphate buffer (PB) pH 7.4 containing 4% paraformaldehyde for 15–60 minutes at 20°C. Following an overnight rinse in 30% sucrose, the eyecups were embedded in OCT compound (Ted Pella, Redding, CA), frozen and sectioned at 12 μ m with a Microm HM 505E (Thermo Fisher, Walldorf, Germany). The

frozen sections were collected on Superfrost plus slides (Fisher, Pittsburgh, PA), air dried, and stored at -80°C until use. For the whole mount preparations, the anterior chamber and lens were removed, and the eyecups were fixed in 4% paraformaldehyde in 0.1M PB for 5–10 minutes at 20°C . The fixed retinas were removed from the posterior eyecups, flattened onto a coverglass and fixed in fresh 4% paraformaldehyde in PB pH 7.4 for 1 hour at 20°C . The retinas were briefly rinsed in phosphate-buffered saline (PBS, pH 7.4) and stored in 30% sucrose for 24 hours at 4°C . To facilitate penetration of the antibodies, retinal whole mounts were rapidly frozen with dry ice and thawed two to three times at 37°C on a tissue warming tray, then rinsed three times with 0.1M PBS pH 7.4 for 10 min per rinse. Retinal frozen sections and whole mounts were then incubated in a blocking solution of 5% Chemiblock (Millipore, Billerica, MA) + 0.3% tritonX-100 (Sigma-Aldrich, St. Louis, MO) for a minimum of 2 h at 20°C .

The tissue was rinsed three times in PBS after this and all other steps. Sections were incubated in primary antiserum or purified IgG at 20°C with 1:100 donkey serum in PBS with sodium azide (PBSa) in a humidified chamber. In some experiments, 5% Chemiblock was substituted for the donkey serum. Sections were incubated with goat antibodies against a synthetic peptide corresponding to the extracellular loop between the 4th and 5th transmembrane domains of human HR1 (1:1,000; Vila et al., submitted for publication) overnight or longer at 4°C or 20°C . In two experiments, another antiserum raised against a synthetic peptide corresponding to a sequence within the cytoplasmic loop #3 of human HR1 (AB5654P, Millipore) was used. Following blocking in 5% Chemiblock for 8 hours, flat mounts were rinsed in PBS for 2 hours and incubated in primary antiserum (1:1000) for 5 days at 4°C . Retinal sections were rinsed three times for 10 min per rinse and flat mounts for 30 min per rinse with PBS to remove excess primary antibody. Some sections were also labeled using rabbit polyclonal antibodies against calretinin (1:1000, Millipore) and GABA (1:1,000, a gift of Dr. David Pow), or else mouse monoclonal antibodies against tyrosine hydroxylase (TH16 1:1,000, Sigma), or somatostatin (1:250, Millipore).

Retinal sections were incubated in secondary antibodies for 2 hours at 20°C in 0.1M PB containing 5% Chemiblock and 0.3% tritonX-100. Whole-mount preparations were incubated in secondary antibodies for 1 day at 4°C . Secondary antibodies were all raised in donkeys and affinity-purified. These included: anti-goat IgG conjugated to Cy3 (1:1000 Jackson ImmunoResearch, West Grove, PA), anti-rabbit IgG conjugated to Alexa 488 (1:750; Molecular Probes, Eugene, OR) and anti-mouse IgG conjugated to Cy5 (1:1000 Jackson ImmunoResearch). All secondary antibodies were used at dilutions of 1:1,000. Tissue was coverslipped in Vectashield mounting medium (Vector Laboratories, Burlingame, CA) with 4,6-diamino-2-phenylindole dihydrochloride (DAPI).

RESULTS

Anatomy

High levels of immunoreactive HR1 were found in the outermost row of the inner nuclear layer (INL) and in the outer plexiform layer (OPL). A dense network of tortuous HR1-immunoreactive processes was uniformly labeled and found throughout the retina (Fig. 1A). The labeled processes were thick, 2–4 μm in diameter with occasional swellings along their length, and they varied in length from 50 to at least 200 μm . HR1 immunoreactive processes in the OPL were similar to those of peroxidase-injected (Lasansky and Vallergera 1975; Lasansky 1978) and Neurobiotin-injected horizontal cell axon terminals in salamanders (Zhang et al. 2006). The horizontal cell perikarya and dendrites were not labeled with antibody to HR1, however. Occasionally, descending processes were labeled with antibody to HR1, but they were difficult to follow.

To confirm that HR1 was expressed by horizontal cell axons and not perikarya or dendrites, sections were double labeled with antibodies to calretinin (CR), a calcium binding protein used as a marker for horizontal cells in salamanders (Jiang et al. 2007). Antibodies to HR1 and CR both labeled horizontal cell processes in the INL and OPL (Fig. 1B). Antibody to HR1 labeled thick processes, whereas antibody to CR labeled perikarya and finer neuronal processes. Both types of labeled processes were found in the same layers and were often in contact, but the two markers were not colocalized. HR1 immunoreactive processes were not found in the outer nuclear layer (ONL), did not have terminals or other specializations and did not make specialized contacts with cones or rods. HR1 immunoreactivity was never found in perikarya of horizontal cells, and there were no nuclei in the processes labeled with antibodies to HR1.

A few cells that expressed HR1 had perikarya in the innermost row of the INL. They were classified as amacrine cells because they had labeled dendrites in the inner plexiform layer (IPL, Fig. 2). HR1-immunoreactive amacrine cells were very sparse; there were never more than two cells in a 12 μ m vertical section through the entire eyecup. Whole-mount preparations of tissue fixed the same way did not contain labeled amacrine cells. Amacrine cell labeling in sections was variable; HR1-immunoreactive amacrine cell bodies were only found in 3 out of 10 retinas processed identically. The perikarya were ovoid in shape, with the long axis vertically, and never displaced to the ganglion cell layer (GCL). The labeling was found exclusively in the cytoplasm and large dendrites. HR1-immunoreactive dendrites typically descended to the inner half of the IPL and sometimes branched. No dendritic terminals were labeled, however, making classification of these amacrine cells difficult. Several ON amacrine cells described previously by Pang et al (2002) fit this description, particularly monostratified amacrine cells A8 and A9 types and also the broadly monostratified A19 type. In double labeling experiments, HR1-expressing cells did not contain immunoreactive calretinin (Deng et al. 2001), somatostatin (Yazulla 1986), GABA (Zhang et al. 2006) or tyrosine hydroxylase (Morona et al. 2007). Both primary antisera against HR1 yielded similar patterns of labeling; no labeling was detected using antisera directed against HR2 or HR3, even those that reliably label neurons in retinas of other species.

Electrophysiology

All of the amacrine cells in the electrophysiological study had perikarya in the most proximal row of the INL. They were classified according to the depth at which their processes ramified using criteria developed for salamander amacrine cells previously (Pang et al. 2002). The IPL was divided into 10 equal strata, with 1 beginning at the lower border of the INL and 10 ending at the upper border of the ganglion cell layer (Fig. 3A). For the analysis of histamine effects, the diffuse amacrine cells, whose dendrites occupied 5 or more contiguous strata were combined with the bistratified and tristratified amacrine cell types into the broadly stratified group. The amacrine cells whose dendrites occupied fewer than 5 contiguous strata were combined into the narrowly stratified group. Only 3 cells in this group were narrowly monostratified; most occupied 2–4 contiguous strata. The two groups are compared in Fig. 3B. The range of dendritic field arbors in the dataset is also indicated in Fig. 3B. Because the measurements were made in slice preparations, they are likely to be underestimates, particularly for the wide field cells.

Light responses

Most amacrine cells in the sample responded at both the onset and offset of the stimulus. Fig. 3A shows the currents elicited by a 2 sec light stimulus at a range of holding potentials from -100 to $+60$ mV. In this example, the responses to the onset and the offset of the stimulus were approximately equal in amplitude. All of the broadly stratified amacrine cells

in our sample ($n = 33$) had ON-OFF responses like these, but they varied in the absolute size of the light responses and in the relative sizes each component. Most of the narrowly stratified cells ($n = 14$) also had responses at light onset and offset, and the rest ($n = 7$) had only one component in their light responses. The light responses also varied in their kinetics, as described previously (Pang et al. 2002).

Effects of histamine on amacrine cells

The light responses of a subset of amacrine cells were influenced by histamine applied in the superfusate at a concentration of 5 μM . The effect of histamine on a broadly stratified cell with a medium sized dendritic arbor is illustrated in Fig. 4. The membrane potential was held at -60 mV and then was stepped to a range of voltages from -100 mV to $+80$ mV (Fig. 4A). Histamine had no effect on the current voltage relationship in the dark (Fig. 4B, left panel). This control experiment was repeated on 12 other amacrine cells, and no effects of histamine on the membrane properties were observed under these conditions. The currents elicited at both the onset and the offset of a 2 sec light stimulus were clearly reduced by histamine (Fig. 4A). The effect on the peak current was more pronounced for the ON component of the response than the OFF component.

Fig. 5 shows other examples of histamine effects on amacrine cell light responses at holding potentials of -60 mV. In some cases, histamine increased the light-induced current or else had no effect. In addition, histamine sometimes acted selectively on one component of the light response. There was no apparent correlation between the light response kinetics and the magnitude of the histamine effects. Fig. 5 also shows that the effects of histamine on amacrine cells varied according to their morphological types.

Fig. 6A shows group data for the effects of histamine on light responses of broadly stratified amacrine cells. Histamine acted selectively on broadly stratified cells whose dendritic field diameters ranged in size from 150 to 275 μm . Histamine had very little or no effect on broadly stratified cells with larger or smaller arbors. When broadly stratified cells with medium sized arbors responded well to histamine, the effect was always a reduction in their light responses. The ON responses of broadly stratified cells were reduced significantly more than the OFF responses (Fig. 6B).

The effects of histamine on the narrowly stratified amacrine cells were more variable and different than the responses of broadly stratified cells in a number of respects. Fig. 6C shows the effects of histamine on the narrowly stratified amacrine cells. There was no difference between ON and OFF responses in the magnitude of the histamine effects, and there was also no apparent relationship between the sizes of the dendritic fields and the sensitivity to histamine. Narrowly stratified amacrine cells having responses at both light onset and offset were generally more sensitive to histamine than cells responding to only one phase of the stimulus. A majority of the ON-OFF cells, 64%, had responses to histamine larger than 20% compared with only 43% of the cells with ON or OFF responses.

To determine whether or not the effects of histamine were direct, Co^{++} was substituted for Ca^{++} in the extracellular medium to block synaptic transmission, and amacrine cells were recorded in current clamp mode (Fig. 7). The effects of histamine on broadly stratified amacrine cells ($n = 16$) were direct. The mean resting potential in Co^{++} was -41.3 ± 4.7 mV and was depolarized to -29.0 ± 3.8 mV with 5 μM histamine ($p = 0.0005$). Three broadly stratified amacrine cells began to fire action potentials after histamine was applied (Fig. 7B). There were no statistically significant effects of histamine in the presence of Co^{++} on narrowly stratified amacrine cells ($n = 11$). Therefore, the effects of histamine on these cells were likely to be indirect.

We also studied 11 broadly stratified amacrine cells and 6 narrowly stratified cells using pipettes containing a potassium gluconate solution. The effects of histamine on the light responses were qualitatively similar under these conditions. Using these electrodes in current clamp mode, it was also possible to observe effects of histamine on the maintained activity of amacrine cells (Fig. 8). In the cell illustrated here, a small hyperpolarizing current (-50 pA) was injected in order to increase the amplitude and frequency of the spikes; presumably by reducing inactivation of voltage gated sodium channels. Histamine slowly decreased the maintained firing rate of this unidentified amacrine cell. Similar results were obtained from a narrowly stratified type of amacrine cell (not illustrated).

DISCUSSION

There is evidence suggesting a hypothalamic origin for retinopetal axons in tiger salamanders (Reperant et al. 2006), and our results suggest that histamine is among the neurotransmitters released from these axons. The major finding in our anatomical experiments was that HR1 were expressed by horizontal cell axon terminals, which we identified by morphological criteria (Lasansky and Vallergera 1975). These originate from the smaller, more abundant type of horizontal cell, now called B-type (Zhang et al. 2006). The cell bodies and dendrites of these horizontal cells, which we identified with antibody to calretinin (Deng et al. 2001) were unlabeled.

HR1 were also expressed in a small number of amacrine cells, but because their processes were not well-labeled, they were difficult to classify morphologically. In our physiological experiments in which synaptic transmission was blocked, however, a large proportion of the broadly-stratified amacrine cells responded to histamine. Taken together, these findings suggest that broadly stratified amacrine cells express other types of histamine receptors that were not detectable in our anatomical experiments. It was not possible to label salamander retinas with antisera directed against mammalian forms of HR2 and HR3, but these other histamine receptors might mediate the direct effects of histamine on other broadly-stratified amacrine cells.

Another major finding in this study was that light responses of broadly stratified amacrine cells were reduced by histamine. In general, the function of these amacrine cells is to convey information between strata of the IPL (Baccus 2007). There are ten strata in the IPL of tiger salamanders, and each is different in the polarity, kinetics and the ratio of rod to cone signals in the excitatory inputs from bipolar cells (Wu et al. 2000; Pang et al. 2004). The inhibitory inputs in each stratum are more variable in these respects as a result of the actions of broadly stratified amacrine cells (Pang et al. 2002; Pang et al. 2007). Inhibition between strata has also been reported in earlier studies of salamander retina (Belgum et al. 1982; Arkin and Miller 1988). The findings reported here suggest that, when histamine is present, there would be fewer such inhibitory interactions between strata. Instead, the inhibitory inputs would tend to have the same kinetics as the excitatory inputs, with a brief delay and the polarity reversed, and there would be fewer inhibitory inputs with faster or slower kinetics. The inhibitory and excitatory inputs would also have similar ratios of rod to cone input, and there would be fewer disinhibitory interactions.

The effects of histamine on the light responses of broadly stratified amacrine cells were selective. The cells whose light responses were most sensitive to histamine were those with dendritic field diameters between $100\text{ }\mu\text{m}$ and $275\text{ }\mu\text{m}$; broadly stratified amacrine cells with larger or smaller arbors were unresponsive. The dendritic field diameters of retinal ganglion cells in tiger salamander retina range from less than $100\text{ }\mu\text{m}$ to more than $700\text{ }\mu\text{m}$ (Toris et al. 1995). The receptive field centers of ON-center ganglion cells, the types with the smallest receptive field centers in the tiger salamander retina, range between $100\text{ }\mu\text{m}$ and $800\text{ }\mu\text{m}$

(Cook and McReynolds 1998). Thus, the broadly stratified amacrine cells sensitive to histamine would be expected to exert their effects over distances smaller than the receptive field centers of most ganglion cells and within the classical receptive fields of the smallest ganglion cells.

Histamine also modulated the light responses of a subset of narrowly stratified amacrine cells. In general, these types of amacrine cells mediate lateral interactions within a single stratum of the IPL, often over relatively long distances. For example, effects of stimuli outside the classical receptive fields are mediated by narrowly stratified amacrine cells (Baccus 2007). The narrowly stratified cells most sensitive to histamine were those that depolarized at both the onset and offset of the stimulus. Unlike the broadly stratified amacrine cells, the effects of histamine on narrowly stratified did not depend on the sizes of their dendritic arbors. Other differences between the two types of amacrine cells were that the narrowly stratified cells responded to histamine with both increases and decreases in their light responses and the effects were indirect. Histamine also decreased the rate of maintained firing in a subset of spiking amacrine cells.

The dopaminergic amacrine cell is a particularly well-characterized type of narrowly stratified amacrine cell. Like the cell illustrated in Fig. 5Cf, it ramifies almost entirely in the most distal stratum of the IPL and depolarizes at both the onset and offset of light stimuli (Yang and Wu 1991). Histamine enhanced the ON component of the response by 33% and decreased the size of the OFF component by 14%. Although a larger sample of recordings from this type of cell with confirmation of its identity by immunolabeling would be required to be certain, these results suggest that dopaminergic amacrine cells in tiger salamanders are sensitive to histamine, as they are in mammals (Weber and Schlicker 2001). In this respect, retinopetal axons containing histamine in the salamander are similar to those containing gonadotropin releasing hormone in fish, whose targets are also dopaminergic neurons (Zucker and Dowling 1987).

Many questions about the functions of histaminergic retinopetal containing axons in salamanders remain unanswered, however, particularly the functions of histamine receptors on horizontal cell axon terminals. In this respect, the horizontal cells of salamanders resemble those of macaques, whose horizontal cells also express HR1 (Vila et al., submitted for publication). Because these axons make conventional chemical synapses onto bipolar cells in the outer plexiform layer of salamanders (Lasansky 1980), many types of cells ramifying in the inner retina would be affected by any changes in their membrane properties, light responses, or synaptic outputs. We hope to investigate these possibilities in future experiments.

In summary, we have shown that histamine influences the light responses of a subset of amacrine cells in the tiger salamander retina and identified two sites of histamine receptors mediating these effects, amacrine cells and horizontal cell axon terminals.

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Figure 1.

HR1 in horizontal cell axons. **A.** This is a stack of 3 optical sections through the outer INL from a whole-mount labeled with HR1 antibody. Immunoreactive HR1 (red) is found in long, tortuous processes with occasional swellings. **B.** Calretinin (green) and HR1 (red) immunoreactivity in a single optical section of the outer INL and OPL. Both antibodies labeled processes that co-stratify and contact each other, but the two markers were not colocalized. Immunoreactive calretinin was found in fine dendrites (arrow) and perikarya of horizontal cells, and immunoreactive HR1 was found in axons. Scale bar = 20 μm

Figure 2.

HR1 in an amacrine cell. The cells containing immunoreactive HR1 (red) had perikarya in the innermost row of the inner nuclear layer. Only the larger dendrites were labeled; these branched in the inner half of the IPL. DAPI staining (blue) indicates the nuclear layers (see text for abbreviations). Scale bar = 20 μ m

Figure 3.

A. A representative amacrine cell (a) A Lucifer yellow stained amacrine cell. Blue is the DIC image of the retinal slice. (b) A drawing of the same cell on a schematic diagram of the retina with the IPL divided into ten equal strata. (c) The light-evoked current responses of this cell to a two sec light step [630 nm, 8.28×10^{18} photons/m²·s] at various holding potentials. R, Rod; C, cone; W_D , width of dendritic field. Scale bar = 20 μm . **B.** Distribution of dendritic stratification levels in the IPL of 54 amacrine cells. These cells are grouped into broadly stratified amacrine cells ramifying in 5 or more strata, (a) and narrowly stratified amacrine cells ramifying in 1–4 strata (b). The vertical bars indicate the presence of processes in the strata. The dendritic field width of each cell is given on the X axis.

Figure 4.

Histamine effects on an amacrine cell of the broadly-stratified group, a diffuse cell with a medium sized dendritic field. **A.** The light-evoked current responses to a 2 sec light step [630 nm, 8.28×10^{18} photons/m²·s] from a voltage clamped amacrine cell in normal Ringer's, in 5 μ M histamine and after washing with normal Ringer's. Currents were recorded at 10 holding potentials from -100 mV to 80 mV for 6 sec. Before these experiments, the membrane potential was held at 0 mV. Light evoked light responses were recorded at light onset (ON- response) and light offset (OFF- response). **B** A plot of step pulse-induced current as a function of holding potential. **C.** A plot of ON- response (peak) as a function of holding potential. **D.** A plot of OFF- response (peak) as a function of holding potential.

Figure 5.

A. Stacked optical sections of broadly stratified amacrine cells (a–e) which vary in dendritic field width. The ten strata of the IPL in each image are marked with white lines. Scale bars = $20\text{ }\mu\text{m}$. **B.** The current responses (a–e) recorded from the amacrine cells (A, a–e) were evoked by a 2 sec light step at a -60 mV holding potential in Ringer's (black line) and in $5\text{ }\mu\text{M}$ histamine (gray lines). **C.** Stacked optical sections of narrowly stratified amacrine cells (f–j). Conventions are the same as above.

Figure 6.

Comparison of the effects of 5 μM histamine on light responses of broadly stratified and narrowly stratified amacrine cells. **A.** The scatter plot shows of effects histamine on ON (open circles) and OFF (closed circles) light responses at a holding potential of -60 mV as a percentage of the control response versus the width of dendritic field (μm) of each amacrine cell. The broadly stratified cells whose light responses were decreased by histamine generally had dendritic field diameters ranging from 150–275 μm (vertical bars). **B.** Within this range of dendritic field diameters, histamine affected ON responses significantly more than OFF responses. **C.** Histamine produced both increases and decreases in the light responses of narrowly stratified amacrine cells. There was no statistically significant difference between the effects on the ON and OFF responses of these cells.

Figure 7. Effects of histamine with synaptic transmission blocked. **A.** The bar indicates the duration of histamine application (5 μM) onto this broadly stratified amacrine cell. **B.** Another broadly stratified amacrine cell began firing action potentials when 5 μM histamine was applied.

Figure 8.

Effects of histamine on the maintained activity of a spiking amacrine cell. **A.** Application of histamine (5 μ M) onto a spiking amacrine cell decreased spiking frequency. **B.** Maintained activity was recorded in current clamp mode from the same amacrine cell in normal Ringer's, in 5 μ M histamine and after washing with normal Ringer's. **C.** The time course of the effect of 5 μ M histamine on the maintained activity of the spiking amacrine cell.