The Actions of Serotonergic Agonists and Antagonists on the Activity of Brisk Ganglion Cells in the Rabbit Retina

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Extracellular electrophysiological recordings were obtained from rabbit retinal ganglion cells in either a superfused eyecup or an *in vivo* preparation. Selective antagonists or agonists of serotonin at the 5-HT₂ or 5-HT_{1A} receptors were applied, and the changes in light-evoked and spontaneous activity were studied. Both 5-HT_{1A} agonists and 5-HT₂ antagonists reduced the ON-components of the light-evoked responses of all classes of brisk ganglion cell; spontaneous activity was reduced in these cells as well. These results suggest that the indoleamine-accumulating amacrine cells of the rabbit retina serve to facilitate the output of the depolarizing rod bipolar cell and thereby increase the efficacy of transmission between this and other cells in the rabbit retina, and that this process is mediated by 5-HT₂ receptors. On the basis of the similarity of the actions of the 2 classes of drug studied, we hypothesize further that 5-HT_{1A} receptors mediate an inhibitory process that serves to terminate the indoleamine-induced facilitation. This process may be located either in the bipolar terminal or presynaptic to it in the terminal of the putative indoleaminergic cells.

The vertebrate retina has proven to be a useful model system in which to study the function of specified neurotransmitters. Its utility comes from the fact that it can be perfused with pharmacological agents at the same time that natural stimuli are applied. The interpretation of the results of such experiments is aided by the retina's highly stereotyped structure and the localization of putative neurotransmitters to anatomically identified cells, which has been made possible by recent technical advances. The neurons of the retina are divided into 6 basic types: photoreceptors, bipolar, horizontal, amacrine, interplexiform, and ganglion cells (Dowling and Dubin, 1984). Most of the conventional transmitters and many of the neuroactive peptides have been found localized to specific anatomical classes of amacrine cell (reviewed by Brecha, 1983). By combining pharmacological studies of the retina with these anatomical data, we can assess directly the function of neurochemically specified ensembles of neurons in visual processing (Daw et al., 1982; Wässle et al., 1986). Generalizations that have emerged from such studies in the retina have proven to hold true for other regions of the visual system, as well as for other sensory regions

of the CNS. For example, inhibitory input from GABAergic neurons is necessary to elaborate direction and orientation selectivity in rabbit retinal ganglion cells (Wyatt and Daw, 1976; Caldwell et al., 1978); similar results have been obtained in cat visual cortex (Sillito, 1977; Sillito et al., 1980).

Biochemical and histochemical data suggest that serotonin or a related indoleamine is a neurotransmitter in the mammalian retina (reviewed by Ehinger, 1982; Osborne, 1982; and Redburn, 1984). Specific mechanisms have been identified for the uptake (Ehinger and Floren, 1978; Osborne and Richardson, 1980) and depolarization-dependent release (Osborne, 1980; Thomas and Redburn, 1980; Mitchell and Redburn, 1985) of serotonin. A serotonin-sensitive adenylate cyclase (Blazynski et al., 1985) and several classes of serotonin receptors have also been demonstrated (Osborne, 1981; Mitchell and Redburn, 1985). While direct visualization of endogenous serotonin-containing neurons in mammalian retinas has not been successful using either histofluorescence or immunohistochemical techniques, indoleamine-accumulating amacrine cells have been identified in several species, including rabbit (Ehinger and Floren, 1976; Ehinger et al., 1982). More recently, several anatomically distinct classes of indoleamine-accumulating amacrine cells in the rabbit retina have been demonstrated (Sandell and Masland, 1986; Vaney, 1986).

Despite this body of biochemical and anatomical information, our understanding of the function of the indoleamine-accumulating neurons is still rudimentary. Early electrophysiological studies implicated serotonin in retinal function but did little to elucidate its role in visual processing (Ames and Pollen, 1969). More recently, Thier and Wässle (1984) demonstrated that serotonin had reciprocal effects on the excitability of ON-center and OFF-center ganglion cells in the cat. Their study employed only agonists of serotonin, and thus the effects of the light-evoked release of transmitter were not addressed. In contrast, we have employed specific antagonists of serotonin receptors to study the function of the light-evoked release of the endogenous indoleamine.

Multiple classes of serotonin binding sites have been demonstrated in the CNS and are grouped as either 5-HT₁ or 5-HT₂ on the basis of the affinities of a variety of pharmacological agents (Peroutka and Snyder, 1979; reviewed in Leysen, 1985). Biochemical evidence suggests that there are 2 different effector mechanisms coupled to these receptors: an adenylate cyclase linked to some 5-HT₁ receptors (Markstein et al., 1986) and phosphoinositide turnover linked to 5-HT₂ receptors (Conn and Sanders-Bush, 1985; DeCourcelles et al., 1985). Evidence supporting the assertion that these binding sites are true receptors mediating neurotransmission comes from a variety of behav-

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Table 1. Summary of the effects of serotonergic drugs on brisk ganglion cells in the rabbit retina

Cell type	Increased	Decreased	No effect
5-HT₂ antagonists			
OFF-center cells			
ON-response	0/27	23/27	4/27
OFF-response	9/27	4/27 ^a	14/27
Spontaneous activity	0/27	18/27	6/27 3/27 ^c
ON-center cells			
ON-response	0/22	15/22	7/22
OFF-response	1/22	4/22 ^b	14/22 ^c 3/22 ^d
Spontaneous activity	1/22	12/22	6/22 3/22 ^c
5-HT_{1A} agonists			
OFF-center cells			
ON-response	1/28	24/28	3/28
OFF-response	9/28	2/28	17/28
Spontaneous activity	0/28	17/28	10/28 1/28 ^c
ON-center cells			
ON-response	0/14	12/14	2/14
OFF-response	4/14	1/14	9/14 ^c
Spontaneous activity	4/14	6/14	4/14

^a In these cells, all large-field units, the ON-response was blocked prior to the reduction of the OFF-response.

^b In 2 of these cells, the ON-response was decreased prior to the OFF-response.

^c No spontaneous activity was found in these cells.

^d An active OFF-response was recorded, but there was no effect of the drugs.

^e No active OFF-response was recorded.

ioral, electrophysiological, and pharmacological data (see Lysen et al., 1984, and Peroutka, 1984, for reviews). Moreover, 5-HT₂ antagonists tend to affect the excitatory effects of serotonin, but not its inhibitory effects (Aghajanian, 1981; Jones, 1982; VanderMaelen, 1985). While the 5-HT₂ class appears to be homogeneous, 5-HT₁ receptors have been subdivided into 3 types (A, B, and C) on the basis of binding studies (Pedigo et al., 1981; Pazos et al., 1984), but the specificity of agonists and antagonists at the various sites for neurons within the CNS is still being determined. Consequently, we have concentrated in this study on drugs with a well-determined selectivity.

Materials and Methods

Preparation. Two preparations of the rabbit retina were employed in this study: an *in vitro* superfused eyecup and an *in vivo* intact eye. These preparations have been described in detail elsewhere (Jensen and Daw, 1984, 1986). Briefly, pigmented rabbits of either sex or, occasionally, New Zealand white rabbits were anesthetized by an intraperitoneal injection of urethane (0.8 gm/kg) and alpha-chloralose (0.08 gm/kg), supplemented by 0.5–1.5% halothane in a 2:1 mixture of N₂O and O₂ during surgical procedures. The eyecup preparation was made by enucleating the eye, removing the anterior chamber and vitreous body, and then inverting the eyecup over a Lucite dome. The preparation was superfused with an artificial saline composed of 120 mM NaCl, 23 mM NaHCO₃, 6 mM dextrose, 3.1 mM KCl, 0.5 mM KH₂PO₄, 1.2 mM MgSO₄, 1.15 mM CaCl₂, 0.1 mM L-ascorbate, 0.5 mM L-glutamine, 0.15 mM myo-inositol. The flow rate of the superfusate was maintained at 7–10 ml/min; the bath temperature was held between 37 and 32°C, and the pH

was adjusted to 7.4 by equilibrating the solution with 95% O₂ and 5% CO₂. Drugs, dissolved in either saline or distilled water, were infused into the line feeding the chamber at flow rates of 0.059–0.206 ml/min. Stimuli were focused on the surface of the retina with the aid of an adjustable final lens. Extracellular spikes were recorded with the use of tungsten-in-glass electrodes, which had 12–20 μm exposed tips and were 1.5–2.5 μm at their base.

For the *in vivo* preparation, after surgical levels of anesthesia were achieved (as above), a cannula for drug infusion was inserted into the common carotid via the external maxillary artery. To maximize the amount of the drug entering the retinal supply, the lingual branch of the external carotid was ligated. Following installation in a stereotaxic head-holder, the animal was paralyzed and artificially respired. Throughout the course of the experiment, the animal's expired CO₂ was monitored and the respirator adjusted to keep the end-tidal levels at 4–5%. The electrocardiogram was also monitored; if an increase of more than 10% of the initial heart rate was observed, then a second injection of chloralose and urethane was given.

The eye was fixed by attachment to a scleral ring, and a guide tube was inserted into the vitreous. The electrode was passed into the vitreous through this tube and positioned in the visual streak under visual inspection with the aid of a Keeler ophthalmoscope. The electrodes used were similar in construction to those used in the eyecup experiments, but were smaller (7.5–10 μm in length and 1.25–2.0 μm at their base). Stimuli were presented on a tangent screen positioned 57 in. in front of the animal. The background level of illumination was generally 14 cd/m², and the stimuli were 86 cd/m² (unattenuated).

Pharmacological agents. The "classical" antagonists of serotonin, those which antagonize many behavioral effects of the injection of tryptophan, are more potent and selective for the 5-HT₂ site. These include ketanserin, ritanserin, methysergide, and LY 53857. Of these, ketanserin is the most potent and selective for 5-HT₂ sites, but, unfortunately, ketanserin also has marked effects on histamine and alpha-adrenergic receptors (Lysen et al., 1981, 1982). LY 53857 has none of these disadvantages, although it is somewhat less potent than ketanserin at 5-HT₂ sites (Cohen et al., 1985). The selective 5-HT₂ drugs employed in this study were obtained as gifts, as follows: ketanserin and pipamperone, Janssen Pharmaceutica; LY 53857, The Lilly Laboratories; methysergide, Sandoz Pharmaceuticals.

No selective antagonists exist for the 5-HT₁ class of receptors. However, there are several agonists that are highly selective for 5-HT₁ receptors. Many of these drugs show a marked selectivity among the various subtypes of 5-HT₁ receptors. In this study, we have concentrated on 2 agonists that demonstrate higher selectivity for the 5-HT_{1A} receptor, namely, 5-methoxy *N,N*-dimethyltryptamine (5-MeODT; Sigma Chemical Co.) and 8-hydroxy-dipropylaminotetralin (8-OH-DPAT; Research Biomedicals) (Gozlan et al., 1983; Middlemiss and Fozard, 1983; Hamon et al., 1984; Sills et al., 1984; Tricklebank et al., 1985).

Results

5-HT₂ antagonists

OFF-center brisk cells. The 3 classes of OFF-center brisk cells (Caldwell and Daw, 1978), transient (Y-like), sustained (X-like), and large-field units, were all affected similarly by antagonists active at the 5-HT₂ receptor. Table 1 summarizes the results obtained from all cells to date. The results obtained in both preparations were identical in all respects, and thus a single description is given. Ketanserin, LY 53857, and methysergide all produce similar effects on OFF-center brisk cells; in general, the ON-component of the light-evoked response of these cells is reduced, and in some cases the OFF-component of the response is concomitantly increased during drug perfusion. These effects are reversible, returning to control levels in 30 min to 2 hr. A reduction of OFF-responses in OFF-center cells was found in only 4 cases and only after a complete blockage of the ON-component had been achieved. The spontaneous activity of OFF-center ganglion cells was also inhibited by 5-HT₂ antagonists.

Results typical of those obtained from OFF-center brisk cells are shown in Figure 1. The light-evoked responses of all cells

Figure 1. Effects of the specific 5-HT₂ receptor antagonist, LY-53857, on the light-evoked activity of an OFF-transient ganglion cell recorded in the eyecup preparation. Control and drug records are clearly marked in the first 4 traces. On this and all subsequent figures, the *bottom trace* is a stimulus marker, an *upward deflection* indicates onset of light, *downward* indicates light offset. Note the prominent reduction of the ON-responses to both diffuse and annular illumination, as well as the production of a period of ON-inhibition in the *third row* of traces, indicating an increase in the relative strength of the central mechanism.

to spot, diffuse, and annular stimulation (see Fig. 1, left to right columns, respectively) are presented as peristimulus-time histograms. It can be seen that the ON-response elicited by diffuse illumination is abolished during perfusion with 1 μM LY 53857. The response to annular illumination is also reduced (Fig. 1, right column). Continued perfusion with a slightly higher concentration of the drug produced (1) a further reduction in the transient ON-response (see Fig. 1, annulus record, third row), (2) more sustained ON-inhibition during illumination (see Fig. 1, spot and diffuse records, third row). Although it is not as clear in this cell as in other cases, spontaneous activity was also reduced by perfusion with 5-HT₂ antagonists (see Fig. 2, Table 1).

In many cells a profound decrease in surround antagonism was evident during drug perfusion. Figure 2 documents such a result from an OFF-center transient cell. The OFF-response during spot illumination increased significantly over that in the control case; furthermore, diffuse illumination produced a center-type response during drug perfusion, whereas a response was barely evident under control conditions. At the same time, the drug reduced the ON-response and increased the OFF-response elicited by an annulus. The reduction of spontaneous activity observed with 5-HT₂ antagonists can be readily seen in Figure 2.

ON-center brisk cells. The effects of these drugs on ON-center ganglion cells were similar to those on OFF-center cells in that the ON-components of light-evoked responses to all stimuli were diminished (Table 1). In some cases, continued perfusion with the antagonists, and thereby an increased concentration in

the preparation, led to a decrease of the OFF-component as well. The effects on the light-evoked responses of ON-center cells appear to be more subtle and required higher concentrations of the drugs than did the effects on OFF-center cells.

Figure 3 shows the results of an experiment with an ON-transient brisk cell, recorded in the eyecup preparation. In this cell a concentration-dependent decrease of the ON-response of the cell was obtained. At relatively lower concentrations (25 μM), the ON-response of the cell is reduced and the OFF-component increased (Fig. 3, second trace). Further reduction of the ON-response was obtained during the period when the drug concentration was changed from 25 to 64 μM (Fig. 3, third trace); after 5 min at the higher concentration (64 μM), the ON-response was reduced further (Fig. 3, fourth trace). Recovery records demonstrate that the effects are reversible and gradually return to control levels. The spontaneous activity of this cell, although low, was reduced as well during LY 53857 application. Recovery of the spontaneous activity lagged behind the effects on the light-evoked response.

The selective 5-HT₂ antagonist, ketanserin, produced effects similar to those of LY 53857. Figure 4 documents an experiment performed on an ON-center transient cell. Application of 12.5 μM ketanserin increased the OFF-response and decreased the ON-response of this cell, while having little effect on the spontaneous activity (Fig. 4, second trace). Continued perfusion with the drug produced a further decline in the cell's responsivity, abolishing the response altogether (Fig. 4, third trace). As the drug was washed out of the chamber, the sensitivity and re-

Figure 2. This OFF-transient cell was recorded *in vivo*. Methysergide, another 5-HT₂ antagonist, when infused into the carotid blood supply, produced a dramatic increase in the OFF-response of this OFF-center transient cell. The spontaneous activity and the surround response of this cell was decreased by drug infusion, but the cell was lost before recovery records were obtained. The concentration of the drug in the syringe before reaching the retina was 12.5 mM; assuming a 100-fold dilution (Caldwell et al., 1978), this yields a blood concentration of 125 μ M. A total of 15 mg of methysergide was injected into this 4 kg rabbit.

sponsivity of the cell returned to control levels (Fig. 4, fourth trace).

5-HT₁ agonists

OFF-center brisk cells. Since no selective antagonists of the 5-HT₁-like receptors are available, we employed 2 selective agonists, 5-MeODT and 8-OH-DPAT, for these studies. Both of these are more selective for the 5-HT_{1A} receptor than for the other subtypes; however, 8-OH-DPAT shows far greater selectivity than does 5-MeODT (Middlemiss and Fozard, 1983; Tricklebank et al., 1985).

The application of these agonists generally had the same effects on the light-evoked responses of OFF-center ganglion cells as did the 5-HT₂ antagonists. That is, they produced a reduction of the surround or ON-input to OFF-center cells and, in some cases, an increase in the OFF-responses (Table 1). Figure 5 documents the results from a large-field unit during application of 5-MeODT. The decrease in the spontaneous activity and in the ON-components is clear. The recovery records demonstrate that this effect was reversible.

In order to compare the effects of 5-HT_{1A} agonists and 5-HT₂ antagonists more directly, both drugs were applied to the same cell in several experiments. As is seen in Figure 6, the application of either 8-OH-DPAT or LY 53857 in similar concentrations produced identical results for this cell. Both the ON-response and the spontaneous activity were reduced, with little increase

in the amplitude of the OFF-response. Recovery from both drugs was rapid and complete in 20–30 min.

ON-center brisk cells. 5-HT_{1A} agonists also produced effects that were similar to those of 5-HT₂ antagonists in ON-center brisk cells (Table 1). Application of 5-MeODT to ON-sustained cells resulted in a reversible decrease of the light-evoked response (Fig. 7). In this case 5-MeODT was applied twice and produced the same reversible effect. On the other hand, no effect in any trial was noted on the spontaneous activity of the cell. While the transient component of this cell was relatively unaffected by 5-MeODT, both sustained and transient components were reduced in other ON-sustained cells. Application of 8-OH-DPAT produced effects similar to those of 5-MeODT on ON-center cells (Fig. 8) with one exception, namely, the spontaneous activity was increased by this drug in 4 cells (Fig. 8, Table 1). In the majority of cases, however, the 5-HT_{1A} agonists either reduced spontaneous activity or had no effect on it.

Both 5-HT₂ antagonists and 5-HT_{1A} agonists were applied to the same cell in several experiments. In general, as with OFF-center cells, these drugs produced similar results in that ON-center responses were reduced. Figure 8 illustrates one such experiment. In this case, LY 53857 and 8-OH-DPAT affected the light-evoked responses similarly but had different effects on the spontaneous activity (compare Fig. 8, second and fourth traces). That is, both drugs reduced the light-evoked response,

Figure 3. LY 53857 produced a dose-dependent decrease (see text for details) of the ON-response of this ON-center cell when it was illuminated by a small spot of light centered on its receptive field (*in vitro* recording). Note also the enhancement of the OFF-response that occurred.

whereas 8-OH-DPAT increased the spontaneous activity and LY 53857 decreased it.

Comparison of 5-HT₂ antagonists and dopamine (D₁) antagonists

The effects of perfusion with 5-HT₂ antagonists are similar in several respects to those reported for antagonists at the D₁ receptor: in both cases the primary effect is a reduction of ON-responses. We therefore decided to make a direct comparison of the effects of SCH 23390, a selective D₁ antagonist, with LY 53857 and ketanserin. One cell type in which the reported effects are different is the ON-sustained ganglion cell. SCH 23390 and other D₁ antagonists convert the sustained ON-response of these cells to a sustained inhibitory response (Jensen and Daw, 1986), but we have not seen such an effect with 5-HT₂ antagonists. Thus we concentrated on this cell type in our comparisons.

Two examples are shown in Figure 9. Perfusion with a 5-HT₂ antagonist reduced both the sustained and transient components of the response, as well as the spontaneous activity (Fig. 9, second trace). In this experiment, no enhancement of the OFF (surround)-response of the cells was seen. Recovery from the drug's effect was rapid and often complete in 20–30 min (Fig. 9, third trace). In both these cells the effect of SCH 23390 on

Figure 4. Ketanserin reduced the ON-response of this ON-center cell recorded in the eyecup preparation (*second and third traces*). In this cell a bimodal effect on the OFF-response can be seen. At first, the response is enhanced (*second trace*) and then reduced when the response of the cell is abolished altogether. Recovery records demonstrate that these effects are reversible.

the light-evoked properties was different from that of LY 53857. SCH 23390 reduced the light-evoked response more than it did the spontaneous activity. Indeed, in the cell in the right-hand column of Figure 9, SCH 23390 almost abolished the light-evoked response, while spontaneous activity was increased. The conversion of a sustained excitatory response into a sustained inhibitory response by SCH 23390 can be seen in the left-hand column of Figure 9. Neither LY 53857 nor any other 5-HT₂ antagonist produced this effect. In other experiments with direction-selective cells, SCH 23390 and LY 53857 also had differing effects: SCH 23390 reduced only the leading-edge response, while LY 53857 reduced both the leading and trailing edges (W. J. Brunken and N. W. Daw, unpublished observations).

Discussion

The generalization that has emerged from our investigations of the function of the indoleaminergic neurons of the rabbit retina in the brisk cell pathway is that interference with indoleaminergic neurotransmission primarily affects ON-responses (see Table 1) (Brunken and Daw, 1986). Perfusion of the rabbit retina with 5-HT₂ antagonists reduces the ON-responses of both ON- and OFF-center brisk ganglion cells. These drugs also reduce the spontaneous activity of all brisk cells. OFF-responses are frequently increased in OFF-center cells, but this is presumably the result of a decrease in the surround antagonism. Occasionally, a transient increase in the OFF-responses of ON-center cells was noted (cf. Fig. 3).

The second, somewhat surprising, result is that agonists selective for the 5-HT_{1A} site produce responses that are similar in

Figure 5. Effect of a 5-HT_{1A} agonist (5-MeODT) on an OFF large-field unit recorded *in vitro*. 5-MeODT reduced the spontaneous activity and the ON-response of this cell, while the OFF-response was unaffected.

Figure 6. The effects of a 5-HT_{1A} agonist (8-OH-DPAT) and a 5-HT₂ antagonist (LY 53857) were directly compared on this OFF large-field unit recorded in the eyecup preparation. Perfusion with 8-OH-DPAT reduced both the ON light-evoked and the spontaneous activity (*second trace*). A similar concentration of LY 53857 produced identical results in this cell (compare *fourth and second traces*). The cell was lost before recovery from the effects of LY 53857 was complete.

Figure 7. ON-sustained cells respond with both a transient and maintained discharge (*Control*); in these cells, 5-HT_{1A} agonists reduced the light-evoked response. In this cell, 5-MeODT decreased only the sustained component, leaving the transient component relatively unimpaired. Two applications of the drug to this same cell demonstrate that this effect is easily reversible and reproducible.

most respects to those of the 5-HT₂ antagonists, that is, they reduce the ON-responses of both ON- and OFF-center brisk cells. In many cases the spontaneous activity of ganglion cells is also reduced by these drugs. One exception to this may be the ON-center cells, which show in some cases an increase in maintained firing rates with 5-HT_{1A} agonists (Table 1). These data differ somewhat from those obtained with iontophoresis of 5-HT and 5-MeODT in cat retina (Thier and Wässle, 1984); in that study, 5-MeODT increased the activity of ON-center cat retinal ganglion cells. We have discussed this discrepancy elsewhere (Brunken and Daw, 1986). Briefly, this difference may reflect a real species difference, a difference in techniques, or a difference in the relative rod-versus-cone contribution to the light-evoked response of ganglion cells in the 2 experimental situations.

Functional interpretation of the role of indoleamine-accumulating neurons

These physiological results can be understood in terms of the anatomy of the putative indoleaminergic neurons of the rabbit

retina, several classes of which have recently been identified (Sandell and Masland, 1986; Vaney, 1986). These classes are distinguished from one another on the basis of their dendritic morphology, the size of their dendritic arbors, and the location of their perikarya. S1 and S2 cells are typical amacrine cells whose perikarya lie in the inner nuclear layer (INL). Their dendrites form a dense plexus in Cajal's layer 5 of the inner plexiform layer (IPL), with a sparse branching in layer 1 of the IPL. Each of these cells has a displaced analog (ds1 and ds2) with a dendritic morphology and arborizations similar to the ordinary amacrine. One unusual cell (S3), found only in the Sandell and Masland (1986) study, has its perikaryon in the outer regions of the INL among the horizontal and bipolar perikarya, and branches extensively in the outer plexiform layer (OPL), as well as having an extensive arbor in layer 5 of the IPL.

Electron-microscopic observation of the connections of the indoleamine-accumulating neurons demonstrates that these neurons are reciprocally connected to rod bipolar cells ending in layer 5 of the IPL (Ehinger and Holmgren, 1979). Connections to other amacrine cells are sparse, and no direct contacts were

Figure 8. Direct comparisons of the effects of 5-HT_{1A} agonists and 5-HT₂ antagonists were made on this ON-transient ganglion cell recorded in the eyecup. Both 8-OH-DPAT and LY 53857 reduced the ON-response of this cell. Some differences in the action of these drugs could be noted, in that the spontaneous activity was somewhat increased by the 5-HT_{1A} agonist, whereas LY 53857 decreased this activity. The cell was lost before recovery from the effects of LY 53857 was obtained.

found onto ganglion cells. The question of which of the 3 classes of indoleamine-accumulating amacrine cells contribute to the connectivity pattern is not yet fully resolved. However, since all 3 classes of indoleamine-accumulating cells contribute to the dense plexus of fibers in layer 5, and the EM studies have resulted in the conclusion that the main output of the indoleaminergic cells is in this layer, it seems reasonable to assume that all 3 classes of cell participate in this reciprocal synapse. Thus it appears that each class of indoleaminergic cell represents a separate channel carrying input from divergent sources, and that all converge onto a single site, the rod bipolar cell.

Therefore, we hypothesize that the putative indoleaminergic cells serve to increase the output of the rod bipolar cell and thereby facilitate signal transmission from this cell. Rod bipolar cells do not synapse directly onto ganglion cells in the mammalian retina, but rather exert their influence via the narrow-field bistratified amacrine cells (Dacheux and Raviola, 1986) called AII in the cat (Famiglietti and Kolb, 1975) (Fig. 10). The synapses between the rod bipolar cell and both the indoleaminergic cell and the narrow-field bistratified amacrine cell are sign-conserving (Dacheux and Raviola, 1986). Consequently, the output of the narrow-field bistratified amacrine cell should also be facilitated by the indoleaminergic neuron. It is presumed that the narrow-field bistratified amacrine cell excites ON-center ganglion cells via gap junctions between themselves and cone bipolar cells, followed by sign-conserving synapses between the cone bipolars and ON-center ganglion cells, as has been shown

in the cat retina (Famiglietti and Kolb, 1975). This would lead us to predict that the facilitation of the output of the rod bipolar by the endogenous indoleamine transmitter would lead to an increase in the ON-responses in ON-center cells. This is consistent with our data, which demonstrate that antagonists of the transmitter produce a decrease in ON-center responses.

The AII amacrine cell, the cat homolog of the narrow-field bistratified cell, is thought to be glycinergic and to convey center-type responses to OFF-center ganglion cells via sign-inverting synapses in sublamina a of the IPL. In contrast to the substantial reduction of ON-responses of ON-center cells, 5-HT₂ antagonists had little effect on the OFF-responses of OFF-center ganglion cells, suggesting that alternative pathways may exist for conveying rod-dominated responses to these cells. One possible pathway is provided by cone bipolar cell input to the narrow-field bistratified amacrine cell (Dacheux and Raviola, 1986); moreover, cones and cone bipolar cells are known to have rod input at mesopic levels in the cat (Nelson, 1977; Smith et al., 1986).

The profound reduction of the ON-response in the OFF-center cells is somewhat surprising and suggests that a considerable amount of this surround may be elaborated in the IPL. This pathway would require that an amacrine cell be interposed between the narrow-field bistratified amacrine cell and the OFF-center ganglion cell. It is conceivable that the surround input to ganglion cells could be influenced in the OPL by the S3 indoleamine cell, which arborizes in both the IPL and OPL. Current experiments are investigating these alternative pathways by recording simultaneously from horizontal and ganglion cells (Mangel and Brunken, 1987).

Function of multiple classes of serotonin receptors in the rabbit retina

The identification of functional correlates of 5-HT₁ receptors has been hampered by the lack of selective antagonists at this site; however, 2 forms of 5-HT autoreceptor have been defined functionally and appear to belong to the 5-HT₁ receptor group. They have distinct pharmacologies as well as different cellular locations, namely, one on the axonal terminals of serotonergic neurons and one on the perikarya of these neurons. The first, a presynaptic terminal autoreceptor, decreases, when activated, the release of serotonin from terminals (Cerrito and Raiteri, 1979; Gothert, 1980; Engel et al., 1986). The ability of RU 24969 and the inability of 8-OH-DPAT to produce this decrease in K⁺-evoked release of 5-HT from rat frontal cortex has been taken by some to indicate that this receptor is of the 5-HT_{1B} type (Middlemiss, 1984, 1985; Engel et al., 1986). The second 5-HT autoreceptor is located on the perikarya and dendrites of raphe neurons and, when activated, decreases the firing rate of these neurons. The ability of 8-OH-DPAT to produce this effect suggests that these are receptors of the 5-HT_{1A} variety.

The somewhat surprising finding that both 5-HT_{1A} agonists and 5-HT₂ antagonists have the same effect on receptive-field properties of rabbit retinal ganglion cells can be understood in terms of the multiple types of receptors for serotonin. Given that both 5-HT₁ and 5-HT₂ receptors are present in the rabbit retina (Mitchell and Redburn, 1985), 2 possible models of the distribution of these receptors are consistent with our observations (Fig. 11). These 2 models differ only in the proposed location of the 5-HT_{1A} receptors. In either case, we hypothesize that the activation of 5-HT₂ receptors located on the rod bipolar terminal facilitates signal transmission from the rod bipolar cell

Figure 9. Direct comparison of the effects of LY 53857 and the D_1 antagonist SCH 23390 reveals several differences in the actions of these drugs (see text for details). Records from 2 different ON-sustained cells are presented (*columns*); the height of the *stimulus marker* corresponds to a firing rate of 50 spikes/sec in each case.

to follower cells; thus, the blockade of these receptors by specific 5-HT_2 antagonists produces a depression of ON-responses. On the other hand, the models differ in the placement and action of the 5-HT_{1A} receptors. In the first model, activation of pre-synaptic autoreceptor decreases the activity of the indoleaminergic neuron. In the second model, the 5-HT_{1A} receptors are placed postsynaptically on the same bipolar terminal as the 5-HT_2 receptors. In this case, the 5-HT_{1A} receptors are linked to an inhibitory process in the bipolar cell and thereby reduce the transmission of ON-signals from the bipolar cell to follower cells. Both models assume that 8-OH-DPAT acts selectively as an agonist at the 5-HT_{1A} receptor. The second model seems to be the more plausible explanation at the moment, since terminal autoreception appears to be linked to 5-HT_{1B} receptors, and 5-HT_{1A} receptors are generally found postsynaptically in the cerebral cortex. Further experiments are needed to support the validity of either of these 2 models (autoreception vs postsynaptic inhibition).

Comparison of the effects of serotonergic and dopaminergic drugs

Jensen and Daw (1984, 1986) have demonstrated that dopaminergic drugs have many effects on the responses of rabbit ganglion cells that are similar to those of the serotonergic drugs employed in this study. Especially noteworthy are the effects on OFF-transient cells, which seem identical. Two explanations for this similarity of action are that (1) it is a pharmacological artifact from the cross-reactivity of the D_1 receptor and the 5-HT_2 receptor, or (2) there is a common pathway by which both of the systems converge onto the ganglion cell. We favor the latter explanation for a variety of reasons. First, we have used several 5-HT_2 antagonists and D_1 antagonists that are selective in *in vitro* assays, both biochemically and pharmacologically. Second, and more important, there are several differences to be noted between the effects of these drugs on ON-center ganglion cells. The most striking of these is the effect of SCH

Figure 10. Connectivity of dopaminergic (DA) and indoleaminergic (IA) amacrine cells in the mammalian retina. Rod bipolar cells (*Rb*) synapse reciprocally with IA cells and onto the narrow-field bistratified amacrine cell (*NFB*). DA cells also synapse onto NFB, which in turn synapses with OFF-ganglion cells at chemical synapses and with other NFB and cone bipolar cells at gap junctions (*I*). Cone bipolar cells (*Cb*) in outer sublaminae of the IPL provide additional input to NFB.

Figure 11. Two possible receptor arrangements that are consistent with our physiological studies.

23390, which increases the spontaneous activity of some ON-center cells, while 5-HT₂ antagonists reduce it. In addition, D₁ drugs produce a period of sustained ON-inhibition in ON-sustained cells; this effect has not been seen with 5-HT₂ drugs. Thus, sufficient differences exist for us to conclude that these classes of drugs are acting at sites distinct from one another.

Anatomically there is an obvious candidate for a common pathway from indoleaminergic and dopaminergic neurons to

ganglion cells, namely, the narrow-field bistratified amacrine cell (Fig. 10). In cat, this cell receives heavy dopamine input in layer 1 of the IPL (Pourcho, 1982) and has input from indoleamine cells via rod bipolar cells in layer 5 of the IPL. It is tempting to speculate that the reason for the great similarity of the actions of dopaminergic and serotonergic drugs on OFF-cells is that both transmitters lie on the pathway to the narrow-field bistratified amacrine cell, which is responsible for convey-

ing some of the rod signal to the outer third of the IPL. The differences in the effects of these drugs on the response of ON-center cells could be due to some additional input to these cells that does not involve the narrow-field bistratified amacrine cell.

Autoinhibition may be a common factor in retinal processing

Two transmitter systems (dopamine and serotonin) have now been described in the rabbit retina; in both, agonists at one receptor type have the same physiological effect on ganglion cells as do antagonists at another receptor type. A similar result has been reported for serotonin in the cat retina (Thier and Wässle, 1984). In other parts of the CNS, this has been described as autoinhibition. One function of such a receptor arrangement might be to produce transient postsynaptic responses from more sustained presynaptic generator potentials, but this hypothesis remains to be fully tested. It seems possible that this arrangement is a general feature of processing in the retina.

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