Journal of Physiology (1992), **445**, *pp.* 741–757
With 5 figures
Printed in Great Britain

EFFECTS OF 2-AMINO-4-PHOSPHONOBUTYRIC ACID ON CELLS IN THE DISTAL LAYERS OF THE TIGER SALAMANDER'S RETINA

BY WILLIAM A. HARE AND W. GEOFFREY OWEN

From the Division of Neurobiology, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA

(*Received 29 April 1991*)

SUMMARY

1. We studied the effects of 2-amino-4-phosphonobutyric acid (APB) on the response properties of rods, horizontal cells and bipolar cells in the isolated, perfused retina of the tiger salamander, *Ambystoma tigrinum*. A concentration of 100 μM was found to be sufficient to elicit maximal effects.

2. Rods hyperpolarized slightly upon exposure to 100 μM-APB and their response amplitudes were slightly reduced. The amplitude of the cone-generated component of the rod's response to 700 nm light was not significantly affected by APB.

3. Horizontal cells hyperpolarized by 2–5 mV upon exposure to 100 μM-APB. The rod-driven component of the horizontal cell response increased in amplitude while the cone-driven component decreased in amplitude. APB thus causes an increase in voltage gain between rods and horizontal cells and a decrease in cone/horizontal cell gain. These findings can be explained in terms of an APB-induced reduction in transmitter release from the cones.

4. APB at a concentration of 100 μM caused an increase in the length constant of the horizontal cell syncytium. Our analysis shows this to be due primarily to a 50% reduction in the coupling impedance between the cells of the syncytium.

5. The effects of APB on off-centre bipolar cells were qualitatively similar to those on horizontal cells. APB increased the amplitudes of rod-driven responses and reduced those of cone-driven responses. The length constants, both of the receptive field centre and of the surround, were increased and the strength of the surround relative to the centre was reduced by about 20%.

6. APB abolished the depolarizing light responses of the receptive field centres of on-centre bipolar cells. A hyperpolarizing response remained whose spatial properties were similar to those of the receptive field surround. We believe this response to reflect a direct (feedforward) input to on-centre bipolar cells from horizontal cells.

INTRODUCTION

The drug 2-amino-4-phosphonobutyric acid (APB) is a glutamate agonist which, in mudpuppy and dogfish, was found to eliminate the light responses of on-centre bipolar cells while leaving those of off-centre cells and horizontal cells intact (Slaughter & Miller, 1981; Sheills, Falk & Naghshineh, 1981). These findings led to the notion that APB could be used to isolate the 'off' pathway in the retina though

subsequent studies revealed a variety of more subtle effects on horizontal cells and off-centre bipolar cells that must be considered if APB is to be used for that purpose (Slaughter, 1986; Nawy, Sie & Copenhagen, 1989; Dong & McReynolds, 1989; Nawy & Copenhagen, 1990).

Our interest in APB grew out of our attempts to establish the pathway by which light responses contribute to the receptive field surround of the bipolar cell. There is longstanding evidence that horizontal cells feed back onto cones (Baylor, Fuortes & O'Bryan, 1971; O'Bryan, 1973; Burkhardt, 1977; Gerschenfeld & Piccolino, 1980; Lasansky, 1981), and this feedback signal should be relayed, in turn, from the cones to the bipolar cells, thereby contributing to the bipolar cell's receptive field surround. The synapse between the cone and bipolar cell should thus mediate input to both regions of the bipolar cell's receptive field. On the other hand, conventional chemical synapses between horizontal cells and bipolar cells have been identified in the salamander (Lasansky, 1978) suggesting that a direct feedforward pathway may contribute to the surround response of the bipolar cell. If the surround response were due exclusively to a feedback from horizontal cells onto the photoreceptors driving the bipolar cell, elimination of the receptive field centre should always be accompanied by a loss of surround responses. This cannot easily be tested in off-centre cells because agents such as *cis*-2,3-piperidinedicarboxylic acid (PDA), which block photoreceptor input to those cells, also block the light responses of horizontal cells and, in consequence, the receptive field surrounds of bipolar cells receive no input (Hare & Owen, 1990*a*). If, however, in tiger salamander as in mudpuppy, APB does not block the light responses of horizontal cells the existence of a feedforward pathway from horizontal cells to on-bipolar cells should be revealed.

In this paper we present evidence that a significant component of the receptive field surround of the on-centre bipolar cell is mediated by a feedforward pathway. We show also that application of APB causes an increase in the voltage gain of synaptic transmission from rods to horizontal cell and off-centre bipolar cells and leads to a decrease in the coupling resistance between horizontal cells. Possible mechanisms underlying these effects are discussed.

Brief summaries of some of our results were presented in 1989 at the ARVO meeting in Sarasota, Florida, and at the Eleventh Taniguchi Symposium on Visual Science, in Katata, Japan (Hare & Owen, 1989; Owen & Hare, 1989).

METHODS

Experimental procedures

All procedures involving animals conformed to the recommendations of the NIH Guide for the Use of Laboratory Animals and the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. Following overnight dark adaptation during which they were cooled to induce torpor, larval tiger salamanders, *Ambystoma tigrinum* (supplied by Carl Lowrance, Tulsa, OK, USA) were killed by decapitation and pithing under dim red light. Dissection of the eye and isolation and mounting of the retina, receptor-side up, in a perfusion chamber were carried out under infra-red illumination with the aid of image converters to preserve dark adaptation.

Retinae were superfused with a control Ringer solution containing (in mM): NaCl, 111; KCl, 2·5; $CaCl_2$, 1·5; $MgCl_2$, 1·5; glucose, 9; and $NaHCO_3$, 22; buffered to pH 7·8 by bubbling with a mixture of 95% O_2/5% CO_2. Test solutions were identical except for the addition of an appropriate concentration of APB. APB, in a racemic mixture of the two optical isomers, was obtained from Sigma Chemical Company, St Louis, MO, USA).

Standard intracellular recording methods were used to measure the responses of rods, horizontal cells and bipolar cells to stimuli derived from a two-beam photostimulator and focused in the plane of the photoreceptors. These stimuli were flashes, 20 ms in duration, of either 500 or 700 nm light, the intensities of which were typically adjusted to elicit weak, near-linear range responses. Previous work in our laboratory showed that weak 500 nm illumination stimulates primarily rods while weak 700 nm illumination stimulates both rods and cones (Capovilla, Hare & Owen, 1987). Circular or annular stimuli were used to excite primarily the centre or surround, respectively, of the bipolar cell's receptive field (Hare & Owen, 1990*a*).

Cells were identified by criteria which had earlier been verified by injection of the enzyme horseradish peroxidase and subsequent optical microscopy (Hare, Lowe & Owen, 1986).

Input resistance measurements were made using double-barrelled electrodes pulled from theta tubing. The fabrication and use of these electrodes was essentially as described by Torre & Owen (1983). The coupling resistance between the current-passing barrel and the voltage-recording electrode was measured in Ringer solution both before and after recording from a cell. Coupling resistances were typically of the order of a few megaohms.

A complete set of data, including careful measurement of receptive field properties and, in some cases, input resistances, under control and test conditions, required that stable recordings be maintained for periods of 2 h. Except for the rods, whose receptive field properties we did not examine, all of the data described in this paper were drawn from cells in which complete data sets were obtained.

A detailed description of our procedures is given in an earlier publication (Capovilla *et al.* 1987).

Analysis of receptive fields

Receptive fields were analysed using circular stimuli of low, fixed intensity and variable radius as described earlier (Hare & Owen, 1990*a*). If horizontal cells formed a continuous two-dimensional network, the relation between the peak response amplitude (V_r) of a horizontal cell and stimulus radius (r) should be exactly defined by an expression that includes a Bessel function (Lamb & Simon, 1976). In our earlier study, however, we noted that, because of the discrete size of the horizontal cells in the syncytium, that function does not accurately describe the amplitudes of responses to stimuli whose radii are comparable with, or less than, the syncytial length constant. We found that for the range of stimuli used in our experiments, our data were better described by the equation:

$$V_r = V_\infty\left[1-\left(1+\frac{r}{\bar{\lambda}}\right)e^{-r/\bar{\lambda}}\right], \tag{1}$$

where $\bar{\lambda}$ is the apparent length constant and is equal to 0·81 of the true length constant, λ. V_∞ is the peak amplitude of the response to a diffuse stimulus.

The receptive fields of bipolar cells were analysed in a similar fashion though it was necessary to include two terms to describe the two functionally antagonistic regions of the field. Provided, again, weak responses are elicited we can write:

$$\frac{V_r}{V_\infty} = \left[1-\left(1+\frac{r}{\bar{\lambda}_c}\right)e^{-r/\bar{\lambda}_c}\right] - A\left[1-\left(1+\frac{r}{\bar{\lambda}_s}\right)e^{-r/\bar{\lambda}_s}\right], \tag{2}$$

where $\bar{\lambda}_c$ and $\bar{\lambda}_s$ are the apparent length constants of the receptive field centre and surround respectively, V_∞ is the amplitude the response to a diffuse stimulus would have if the surround were eliminated and A is a scaling factor that defines the strength of the surround mechanism relative to that of the centre. The derivation of these equations is given in Hare & Owen (1990*a*).

Throughout this paper we shall give values of the true length constant, λ ($= 1{\cdot}24\ \bar{\lambda}$).

RESULTS

Effects of APB on rods

When rods are stimulated with weak 500 nm light, their responses consist of a single component which peaks some 500 ms after stimulus presentation. When weak 700 nm stimuli are presented, on the other hand, rod responses exhibit two components, a rapid component, due to an input from red-sensitive cones, which is

superimposed upon a slower component generated by the rods themselves. In contrast to the finding of Wu & Yang (1988) that in salamanders, cone–rod coupling is detectable in only a fraction of rods, we find it to be a general phenomenon in the dark-adapted retina, though the magnitude of the cone-driven component varies

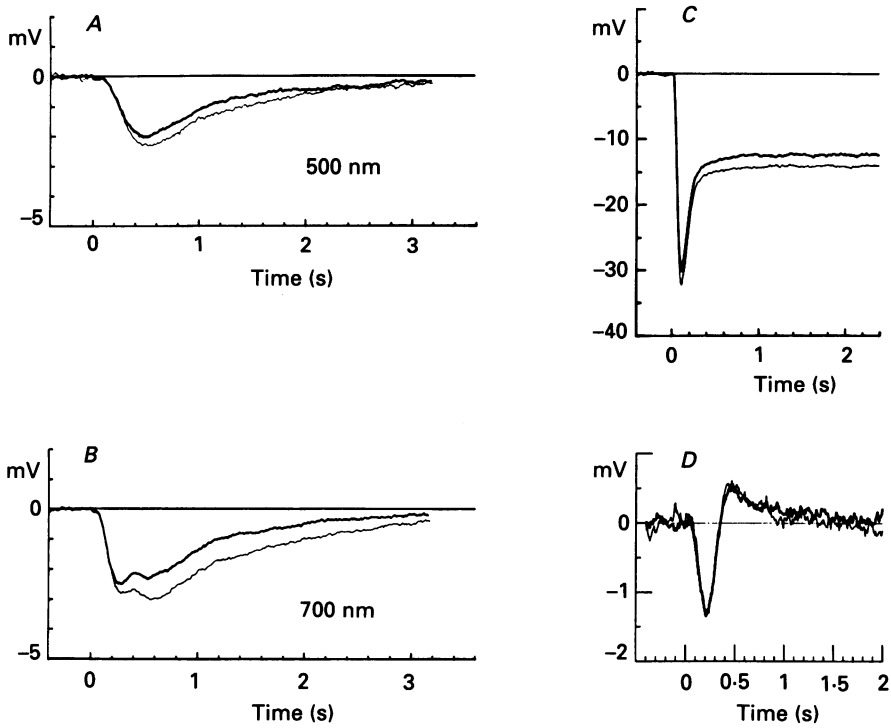

Fig. 1. Effects of 100 μM-APB on rods. *A*, responses elicited by dim 500 nm stimuli, 20 ms duration, 600 μm diameter, of intensity 0·447 photons μm^{-2} per flash (i.e. 8·9 Rh* per rod). Each trace is the average of ten responses. In this and all subsequent figures, responses elicited in the presence of APB are plotted as thick traces, control responses as thin traces. *B*, Responses elicited by dim 700 nm stimuli, 20 ms duration, 600 μm diameter, of intensity 763 photons μm^{-2} per flash (i.e. 9·6 Rh* per rod). Each trace is the average of ten responses. *C*, saturated responses elicited by 20 ms flashes of 500 nm light of intensity 2240 photons μm^{-2} per flash ($4{\cdot}48 \times 10^4$ Rh* per rod). *D*, the cone-generated components of the rod responses elicited in control Ringer solution (thin trace) and APB Ringer solution (thick trace), isolated as described in the text.

considerably from rod to rod and is sometimes barely discernible. Examples of these two types of response are shown in Fig. 1*A* and *B*.

We recorded from five rods while applying 100 μM-APB. Stimuli were 600 μm in diameter, more than sufficient to illuminate the entire receptive field of the rod (Hare & Owen, 1990*a*), and were adjusted in intensity to elicit responses of about 3 mV in amplitude in control Ringer solution. In each case, APB caused the rod to hyperpolarize slightly over a period of 20 min. Return to the control value of the dark potential after switching back to control Ringer solution was so slow that we could not reliably determine when recovery was complete. Our best estimate is that more than 1 h was required for recovery. Thus the effect of APB application on the rod's dark potential develops slowly and is slow to wear off.

The small APB-induced hyperpolarization was accompanied by a 10–20% decrease in the amplitudes of responses elicited by weak 500 nm stimuli (Fig. 1*A*). As can be seen, the initial hyperpolarizing phase of the response was little affected but the peak of the response was attenuated and the time-to-peak was reduced by almost 100 ms. When a weak 700 nm stimulus was used the early peak of the response, which reflects input from cones, was also reduced in amplitude (Fig. 1*B*), and both peaks occurred slightly earlier. The saturated response of this same rod was reduced in amplitude by about 7% in the presence of APB, as shown in Fig. 1*C*. Reductions in the saturated response amplitude of up to 20% were seen in other rods.

To determine whether or not the cone-generated component of the rod's response to a 700 nm stimulus was affected by APB we made use of the fact that the responses shown in Fig. 1*A* and *B* lay within the rod's linear range and that cone responses to such stimuli are relatively brief. We scaled the 500 nm response elicited in control Ringer solution so that its time course matched that of the 700 nm control response at times greater than 1 s following stimulus presentation. We then subtracted the scaled 500 nm response from the 700 nm response to obtain the cone-driven component. This procedure was repeated using the responses elicited in the presence of APB. The cone-generated components of the rod responses, thus isolated, are shown in Fig. 1*D*. It can be seen that APB had no effect upon either the amplitude or the time course of the cone-generated component. The reduction in amplitude of the early peak of the 700 nm response was entirely due to the effect of APB on the slow rod-generated response, therefore.

These conclusions are broadly consistent with the observations of Yang & Wu (1989), that APB had little effect on cone response amplitudes but caused some attenuation of rod responses. On the other hand, they found APB to have no significant effect upon the dark potential of either cell.

Our findings are not easily explained. If the effect of APB was to induce a shunt of the rod's plasma membrane, one would expect the rod's response to 500 nm light to be reduced throughout its time course, yet we find the initial hyperpolarizing phase to be unaffected. Moreover, we would have expected the cone-generated component to be attenuated in the same way as the rod-generated response. A time-varying, voltage-dependent conductance is known to shape the rod's response to weak stimuli (Owen & Torre, 1983), and one might suppose that APB affects its action. This should also change the time course of the cone-generated component, however, whereas it clearly remained unchanged. An interesting possibility, though an unexpected one, is that the action of APB exerts some, perhaps indirect, effect upon the rod's phototransduction mechanism leading to a change in the kinetics of the later phase of the response. We are currently exploring this possibility in other experiments.

Effects of APB on horizontal cells

We measured the effects of APB, in concentrations between 100 μM and 1 mM, upon the receptive field properties of fourteen large-field horizontal cells. Since similar results were obtained at each of the concentrations tested, we conclude that the effects produced by 100 μM-APB were maximal. Typical records are shown in Fig. 2. Upon exposure to APB, horizontal cells hyperpolarized by 2–5 mV in darkness (Fig. 2*A*). This hyperpolarization persisted until the control solution was

restored at which point the cells repolarized to their pre-drug level and response amplitudes returned to their control values. Following brief exposures to APB this recovery took only a few minutes while after long exposure, more than half an hour was required.

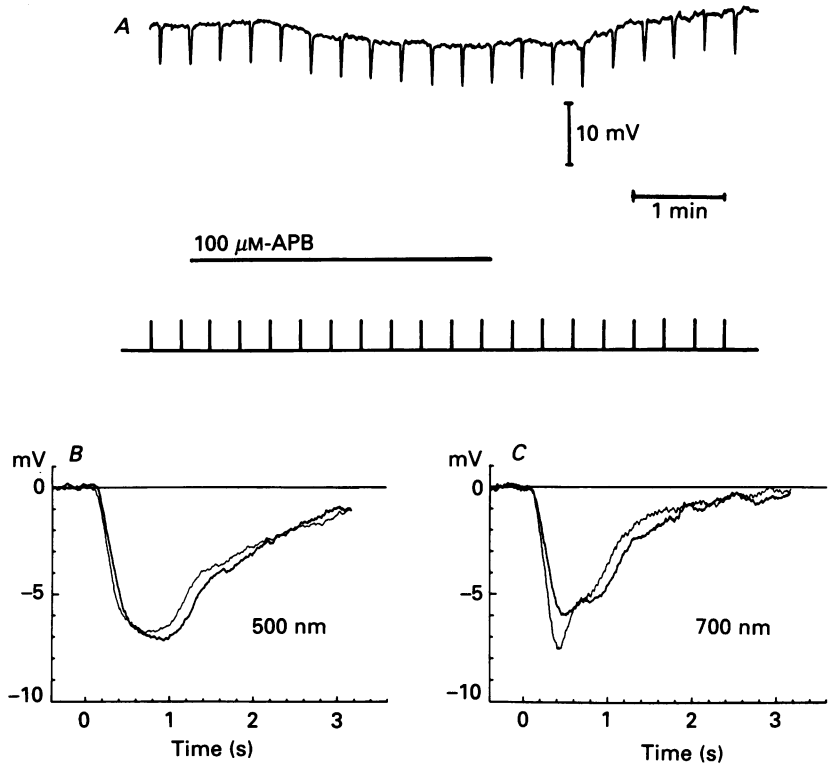

Fig. 2. Effects of 100 μM-APB on the responses of horizontal cells. *A*, effect of a brief application of APB on the dark potential. Stimuli were 1500 μm in diameter, 700 nm wavelength and intensity 200 photons μm^{-2} per 20 ms flash (nearly half-saturating). *B*, averaged responses ($n = 10$) to 500 nm stimuli of 2000 μm diameter and intensity 1·23 photons μm^{-2} per 20 ms flash. (Thin trace plots control response.) *C*, averaged responses ($n = 10$) elicited by 700 nm stimuli, 2000 μm in diameter, of intensity 430 photons μm^{-2} per 20 ms flash. (Thin trace plots control response.) Note that responses in *B* and *C* were not within the linear intensity–response range.

In their study of salamander horizontal cells, Yang & Wu (1989) found 500 μM-APB to cause a hyperpolarization of about 45 mV. This difference probably reflects the lower dark potential of horizontal cells in that preparation, about −25 mV (Wu, 1988), compared with −55 mV in the present study. When we switch from our bicarbonate-buffered Ringer solution to Wu's HEPES-buffered Ringer solution, horizontal cells depolarize to about −25 mV and their saturated light responses grow in amplitude by the same amount (W. A. Hare & W. G. Owens, unpublished observations). We are currently studying this effect in order to establish its basis.

APB caused a slight increase in the times-to-peak of responses to 20 ms flashes of 500 nm light of moderate intensity (diameter 2000 μm), and enhanced their amplitudes, in this case by 6% (Fig. 2*B*), though increases of up to 20% were seen in other cells. The response to diffuse illumination increased in amplitude by 13%

(see Discussion). A small APB-induced enhancement of the horizontal cell's response amplitude was also observed in the mudpuppy by Slaughter (1986). Responses to 20 ms, 700 nm stimuli of diameter 2000 μm and moderate intensity, though they also peaked more slowly, were significantly attenuated in amplitude, in this case by 21% (Fig. 2*C*), with attenuations ranging between about 12 and 25% in other cells. When near-saturating intensities were used, APB attenuated the responses elicited by both 500 and 700 nm wavelengths (not shown). A marked attenuation of horizontal cell responses to bright 700 nm light in the presence of APB was also reported by Yang & Wu (1989).

APB also increased the tightness of coupling of horizontal cells as found in mudpuppy by Dong & McReynolds (1989). This is illustrated in Fig. 3*A*. In this particular cell (whose responses are shown in Fig. 2*B* and *C*) the length constant of the receptive field measured with weak 500 nm stimuli increased from 217 to 304 μm. The magnitude of the increase in length constant produced by APB was larger in cells that were initially less tightly coupled.

In order to determine whether the APB-induced increase in length constant is due to a decrease in the coupling resistance between neighbouring cells of the syncytium, to an increase in the membrane impedance of each cell, or both, we measured the steady-state input impedance of the horizontal cell network both under control conditions and in the presence of APB. We did this in five of the fourteen horizontal cells whose receptive field properties were measured. The curves shown in Fig. 3*B* were measured in the cell from which the data of Figs 2*B*, *C* and 3*A* were obtained. The results from the other cells were essentially similar. Note that in the presence of APB, the input impedance of this cell was reduced by about 46% from its control value, i.e. from 23·7 to 12·8 MΩ. A roughly 50% reduction was seen in each of the horizontal cells analysed in this way.

Effects of APB on off-centre bipolar cells

Complete data sets were obtained from six off-centre bipolar cells. The typical effects of applying 100 μM-APB are illustrated in Fig. 4. The drug caused a rapid, sustained hyperpolarization of approximately 2 mV (Fig. 4*A*). Upon restoring control Ringer solution, the potential returned to its original dark value within about 8 min. APB caused a significant increase in the amplitudes of responses elicited by 600 μm diameter, 20 ms flashes of dim 500 nm light, in one case by almost 100%. In the cell whose responses are shown in Fig. 4*B*, responses to 800 μm diameter stimuli increased in amplitude by a more typical 23% and the decay phase of the response became more rapid. Note, too, that the depolarizing responses elicited by large annuli were also enhanced, again by 23% in this case, and their times-to-peak increased.

As in horizontal cells, APB attenuated responses elicited by dim 700 nm stimuli (Fig. 4*C*). In this particular cell, the same one from which the responses in Fig. 4*B* were elicited, responses to dim, 800 μm diameter spots were attenuated by about 25% while those elicited by dim annuli of this wavelength were also reduced, by a similar amount, with a marked slowing in time course.

Figure 4*D* illustrates the effect of APB on the receptive field of the off-centre cell as measured with dim 500 nm spots of increasing diameter. Applying 100 μM-APB

increased the length constant of the receptive field centre, in this case from 85 to 110 μm, and also that of the surround, here from 225 to 320 μm. This increase in the length constant of the surround was consistent with the increase in the length constant of the horizontal cell syncytium measured under the same conditions. The

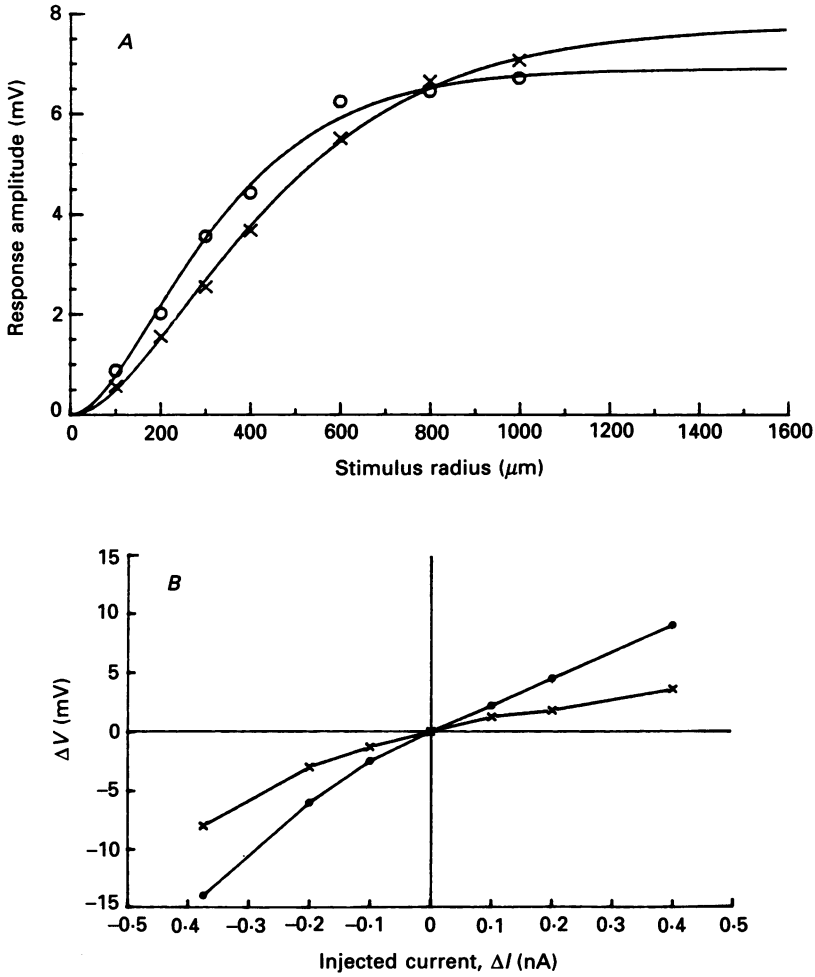

Fig. 3. *A*, effect of APB on the spatial profile of the horizontal cell's receptive field. Stimuli were circular spots of different radii, 500 nm wavelength and fixed intensity, which elicited maximum response amplitudes of 6·9 mV (control, diffuse light) and 7·8 mV (APB, diffuse light). The smooth curves were generated by eqn (1) with length constants, λ, of 271 μm (control, ○) and 304 μm (APB, ×). *B*, steady-state input resistance of the same horizonal cell in control Ringer solution (●) and 100 μM-APB (×). The slopes at the origin are 23·7 MΩ (control) and 12·8 MΩ (APB).

strength of the surround relative to that of the centre, defined by the value of A in eqn (2), was also decreased by almost 20%. This is consistent with our observation that the gain between rods and off-centre bipolar cells was enhanced by APB to a greater degree than the gain between rods and horizontal cells.

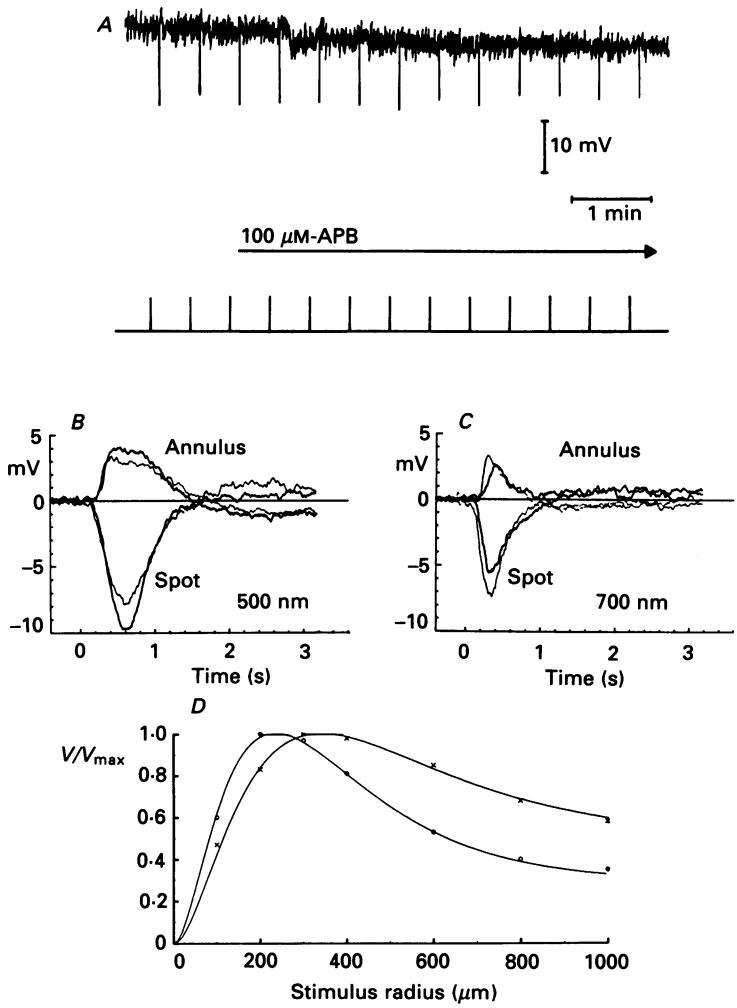

Fig. 4. Effects of 100 μM-APB on off-centre bipolar cells. *A*, effect upon the dark potential. Stimuli were 20 ms flashes of 700 nm light, 600 μm in diameter, each of which delivered 22 photons μm^{-2}. *B*, averaged responses ($n = 10$) to centred, 500 nm spots (800 μm diameter, 0·16 photons μm^{-2} per 20 ms flash) and annuli (1000 μm i.d., 1700 μm o.d., 0·47 photons μm^{-2} per 20 ms flash). (Thin traces plot control responses.) *C*, averaged responses ($n = 10$) to centred 700 nm spots and annuli of the same dimensions which delivered 48 and 68 photons μm^{-2} per 20 ms flash, respectively. (Thin traces plot control responses.) *D*, effect of APB upon the spatial profile of the off-centre cell's receptive field. Stimuli were circular spots of different radii, 500 nm wavelength and fixed intensity that elicited maximum amplitudes of 4·8 mV (control, ○) and 9·95 mV (APB, ×). The smooth curves were generated by eqn (2) with length constants $\lambda_c = 85$ μm, $\lambda_s = 225$ μm (control) and $\lambda_c = 110$ μm, $\lambda_s = 320$ μm (APB). The values of A were 0·85 (control) and 0·7 (APB).

Effects of APB on on-centre bipolar cells

Complete data sets were obtained from four on-centre bipolar cells with consistent results in each case. Application of 100 μM-APB caused on-centre bipolar cells to hyperpolarize rapidly by 4–5 mV as shown in Fig. 5*A*. This hyperpolarization was

accompanied by an apparent loss of light responses elicited by near-saturating 600 μm diameter spots of light. On closer examination, however, we noticed that small responses persisted during drug application but they were hyperpolarizing responses, opposite in sign to the control responses. These are shown in Fig. 5*B*.

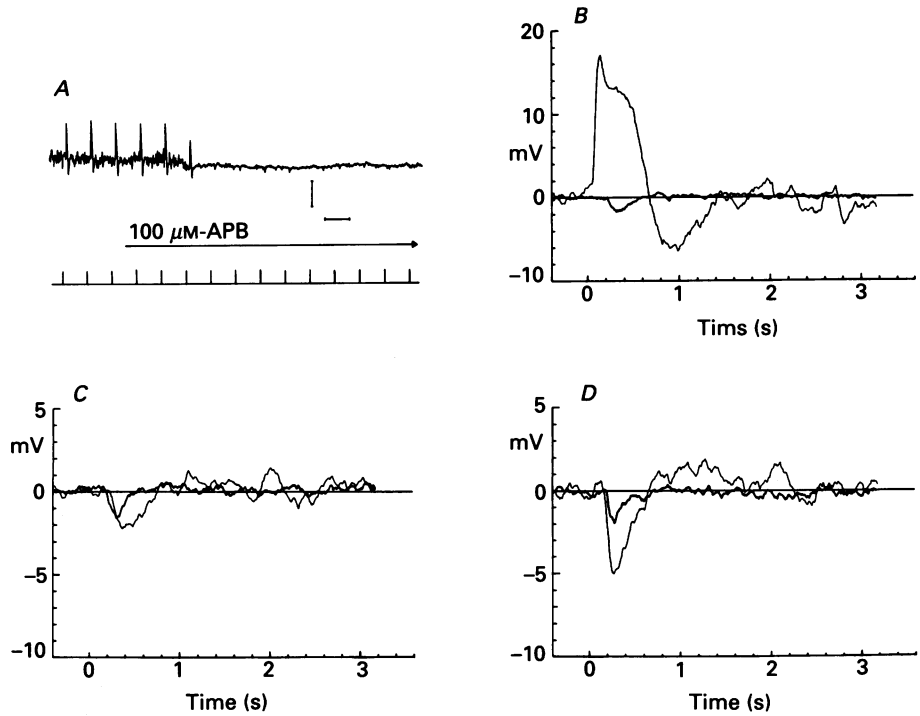

Fig. 5. Effects of 100 μM-APB on on-centre bipolar cells. *A*, effect upon the dark potential. Stimuli were 20 ms flashes of 700 nm light, 600 μm in diameter, each of which delivered 23700 photons μm^{-2}. *B*, responses averaged from *A*, shown on an expanded time scale. (Thin trace plots control response.) *C*, averaged responses ($n = 10$) to 20 ms flashed annuli of 500 nm wavelength and 1000 μm i.d., 1300 μm o.d., each of which delivered 0·532 photons μm^{-2}. (Thin trace plots control response.) *D*, averaged responses ($n = 10$) to 20 ms flashed annuli of 700 nm wavelength and the same dimensions as in *C*, each of which delivered 687 photons μm^{-2}. (Thin trace plots control response.)

When we presented annular flashes of similar intensity, clear hyperpolarizing responses were elicited. With 500 nm light, the amplitudes of these responses were about 72% of those elicited by the same stimuli in control Ringer solution (Fig. 5*C*). With 700 nm light, response amplitudes were only 40% of control values (Fig. 5*D*). Given that APB enhances the rod-driven component of the horizontal cell response by up to 20% and decreases the cone-driven component by about 20%, this suggest that the net gain of the pathway(s) between horizontal cell and on-centre bipolar cell was reduced by about 50% (see Discussion).

Presentation of a spot 2000 μm in diameter, sufficient to illuminate the entire receptive field of the bipolar cell, elicited a hyperpolarizing response which was larger in amplitude than responses to small spots, or annuli, of the same intensity (not shown). Thus, responses recorded in the presence of APB summed over an area

similar to that of the bipolar cell's receptive field surround. Moreover, they were entirely monophasic and hence showed no evidence of any contribution from the receptive field centre of the cell.

To be certain that this hyperpolarizing response was, indeed, insensitive to APB, we repeated these measurements using concentrations of 500 μM and 1 mM. The hyperpolarization produced in darkness developed more rapidly at these higher concentrations, peaking at about 8–10 mV but repolarizing to a steady level 5 mV more negative than the control potential. Otherwise, the effects were generally identical to those observed with 100 μM-APB.

Recovery of on-centre bipolar cells from exposure to APB was considerably slower than that of the off-centre bipolar cells. Following exposure to 100 μM-APB, it took about 30 min for the response of the receptive field centre to recover fully. After 1 mM-APB, more than an hour was required for full recovery to occur. This was much slower than recovery from exposure of these cells to other transmitters such as GABA but similar to the recovery we see in horizontal cells following exposure to dopamine whose action is known to involve a second messenger (Teranishi, Negishi & Kato, 1983, 1985; Piccolino, Neyton & Gerschenfeld, 1984; Lasater & Dowling, 1985; Lasater, 1987; DeVries & Schwartz, 1989; Maguire & Werblin, 1990). APB-mediated responses of on-centre bipolar cells in the tiger salamander (Nawy & Jahr 1990) and the dogfish (Sheills & Falk, 1990) were recently shown to involve the modulation of a G-protein/cyclic guanosine monophosphate system similar to that underlying phototransduction in rods.

DISCUSSION

The most detailed available picture of the actions of APB in the distal retina emerges from the work of Nawy & Copenhagen (1987, 1990) and Nawy *et al.* (1989) on the goldfish. As in other species, APB was found to act as an agonist at a unique class of receptors on the subsynaptic membrane of the on-centre bipolar cell. The channels controlled by those receptors have a reversal potential which is positive with respect to the dark potential and are normally closed by transmitter released from the rods. The transmitter released by cones, on the other hand, appears to open channels whose reversal potential is negative with respect to the dark potential and which are unaffected by APB. This is interpreted not as the effects of two different transmitters but as the effects of transmitter upon two different postsynaptic receptor types, one type being postsynaptic to cones, the other being postsynaptic to rods.

A second site of APB action appears to be the cone terminal where the rate of release of cone transmitter was markedly reduced by low concentrations of APB. The argument for this is that cone-driven horizontal cells, in the presence of APB, hyperpolarized by more than 15 mV and their light responses disappeared. The magnitude and time course of these effects were virtually identical to those produced by 1 mM-Co^{2+} which eliminated transmitter release from the cones. By contrast, in studies of enzymatically dissociated cones *in vitro* (Tachibana & Kaneko, 1988; Sarantis, Everett & Attwell, 1988), L-glutamate applied to the synaptic terminals was found to evoke an inward current at physiological potentials which should

depolarize the cones and thereby augment transmitter release. In the salamander, however, it was noted that kainate was similarly effective in evoking that current (Sarantis *et al.* 1988), which suggests that this mechanism is unlikely to be the one activated by APB.

Many of the results of our experiments on the salamander retina can be explained in terms of the two actions of APB identified in the goldfish. The APB-induced hyperpolarization of off-centre bipolar cells and horizontal cells, for example, and the decrease in the amplitudes of responses elicited in those cells by dim, full-field stimuli of 700 nm wavelength are what would be expected if APB acts to reduce transmitter release from the cones, though it should be noted that that action must be rather weaker in salamander than was observed in the goldfish since cone-generated responses in second-order cells were not eliminated. It is easily shown that an APB-induced reduction in transmitter release from the synaptic terminals of cones should cause an increase in the impedance of the postsynaptic membrane with a concomitant increase in the gain between rods and both horizontal and off-centre bipolar cells, but a net reduction in the voltage gain between cones and these classes of second-order cells (see Capovilla, Hare & Owen, 1987, eqn (6)). Thus, it is significant that, though responses of rods to dim, full-field stimuli of 500 nm wavelength were slightly reduced in amplitude by APB application, responses of off-centre bipolar cells and horizontal cells to the same stimuli were *increased* in amplitude. In all horizontal cells whose input impedance was measured, this APB-induced increase in rod/horizontal cell gain could be entirely accounted for by the calculated increase in resistance of the horizontal cell membrane (see below).

An alternative to this interpretation is suggested by the work of Knapp & Dowling (1987) which showed that, in teleost retinae, dopamine enhances the sensitivity of horizontal cells to glutamate. Maguire & Werblin (1990) reported similar effects of dopamine on both horizontal cells and off-centre bipolar cells in the tiger salamander. The observed APB-induced hyperpolarization of these cells might be explained in terms of this mechanism if we were to assume that APB inhibits an on-going release of dopamine in the retina. Two observations mitigate against this explanation, however. First, while a reduction in the efficacy of glutamate would result in an increase in the impedance of the postsynaptic membrane, it is easily shown that it would cause a net *decrease* in the voltage gain between *both* classes of photoreceptors and these second-order cells. As noted above, though we observed an APB-induced reduction in gain between cones and second-order cells, the gain between rods and both horizontal cells and off-centre bipolar cells was significantly *increased* by APB. Second, dopamine application in this retina causes rods to hyperpolarize and their response amplitudes to be diminished (Hare & Owen, 1990*b*). If the primary effect of APB were to inhibit on-going dopamine release we might expect, as a secondary effect of APB application, a depolarization of the rods and an enhancement of their responses. Again, this is the opposite of what we observed. For these reasons we doubt that the effects discussed above merely reflect an inhibition of dopamine release by APB though the involvement of such a mechanism cannot be ruled out.

An increase in the impedance of the postsynaptic cell should lead to an increase in the length constant of the syncytium of which that cell is a part and, indeed, APB-

induced increases in the length constants of the receptive fields of horizontal cells and off-centre bipolar cells were observed. If we analyse these increases quantitatively, however, we find that they are larger than can be explained by this single action of APB and that the drug must also affect the coupling pathway between neighbouring cells of the syncytium. To illustrate this we will consider the effects of APB on horizontal cells.

The horizontal cell syncytium can be modelled as a two-dimensional square lattice of cells, each having a membrane impedance R_m coupled to its four nearest neighbours by coupling resistances R_c (Lamb & Simon, 1976). The input resistance of such a lattice is given by:

$$R_{in} = \frac{2R_m}{\pi}\left(\frac{\gamma}{\gamma+4}\right)K\left[\left(\frac{4}{\gamma+4}\right)^2\right], \tag{3}$$

where $\gamma = R_c/R_m$ and K is a complete elliptic integral of the first kind (Lamb & Simon, 1976, their eqn (15)). Provided that the syncytial length constant, λ, is significantly larger than the mean distance, D, between adjacent cells, we can write, to a good approximation:

$$\frac{R_m}{R_c} = \left(\frac{\lambda}{D}\right)^2, \tag{4}$$

and

$$R_{in} = \frac{R_c}{2\pi}\ln\left(5{\cdot}66\frac{\lambda}{D}\right). \tag{5}$$

This is very similar to the function describing the input resistance to a continuous network given by Jack, Noble & Tsien (1975, their eqn (5.18)), which is based upon circular rather than square geometry (see also Minor & Maksimov, 1969). As they indicate, when $\lambda \gg D$, changes in the input resistance reflect primarily changes in R_c. The value of the R_c can be calculated from eqn (5) using the measured values of R_{in} and λ provided we know the lattice constant, D. We shall assume a value of 45 μm, consistent with the mean spacing of horizontal cells in other lower vertebrates (Witkovsky, Owen & Woodworth, 1983; Kamermans, Van Dijk & Spekreijse, 1990). It should be recognized, however, that estimates of *fractional* changes in R_c (using eqn (5)) and R_m (using eqns (4) and (5)) are relatively insensitive to any value of D chosen within reasonable limits.

The horizontal cell whose responses are shown in Fig. 2*B*, *C* and 3 will be used as an example though closely similar results were obtained in all of the five cells analysed in this way. In that particular case, APB caused the syncytial length constant to increase from 217 to 304 μm. This increase was accompanied by a decrease in input resistance from 23·7 to 12·8 MΩ. Substituting these values in eqn (5), we calculate that R_c fell from 45 to 22·1 MΩ, a fractional decrease of 51%. Substituting these values in turn in eqn (4) we find the resistance of the postsynaptic membrane to have remained essentially unchanged.

A second estimate can be obtained on the basis of the fractional changes in response amplitudes elicited by diffuse illumination and by illumination of a single horizontal cell. Under full-field illumination the syncytium responds isopotentially

and hence no net current flows laterally through the coupling pathways. The light-induced synaptic current (Δi) generated in a particular horizontal cell will thus flow to ground across the plasma membrane of that cell. The resulting potential change (V_∞) will be equal to $R_m \Delta i$. A stimulus of the same intensity but which stimulates only a single horizontal cell will elicit the same synaptic current, Δi, but this will flow to ground across the input resistance of the syncytium. The response (V_0) will thus be equal to $R_{in} \Delta i$. Denoting values measured in the presence of APB by an asterisk, we can write:

$$\frac{R_m^*}{R_m} = \frac{V_\infty^* V_0 R_{in}^*}{V_\infty V_0^* R_{in}}. \tag{6}$$

Given a mean cell spacing of about 45 μm, we take 22 μm as the radius of a spot that illuminates a single horizontal cell. Extrapolating from the functions fitted to the data in Fig. 3*A* we estimate V_0/V_0^* to have been 1·8 (the measured ratio using spots of radius 100 μm was 1·5), and V_∞^*/V_∞ to have been 1·13 (the measured ratio using spots of radius 1000 μm was 1·06). The measured value of R_{in}^*/R_{in} was 0·54. This yields a value of 1·1 for the ratio of R_m^*/R_m. From eqn (4) we calculate that APB induced a fall of 44% in the value of the coupling resistance.

In all horizontal cells thus analysed, the APB-induced reduction in R_c was found, by both estimates, to be within a few per cent of 50% while increases in R_m of up to 15% were observed. Thus, a second action of APB is to cause a decrease in the coupling resistance between horizontal cells. This effect is opposite to that of exogenously applied dopamine on salamander horizontal cells (Hare & Owen, 1990*b*), which raises the possibility that it might be mediated by an APB-induced inhibition of on-going dopamine release. Our reservations about such a mechanism are discussed above.

A simpler explanation is suggested by recent evidence that horizontal cells and off-centre bipolar cells may also possess APB receptors (Slaughter, 1986). The APB-induced reduction in coupling resistance between horizontal cells could thus be due to a direct action of APB, perhaps modulating the same second-messenger system that is modulated by exogenous dopamine (DeVries & Schwartz, 1989), but in the opposite manner. This leaves open the possibility that glutamate released from the photoreceptors might normally, in this way, regulate the coupling resistance, keeping it low.

The third action of APB is the well-established blockade of the direct input from photoreceptors to on-centre bipolar cells. In the goldfish, APB is thought to bind only to those receptors that are postsynaptic to rods. One must therefore consider why, in salamander, the cone input to on-centre cells also disappears. Our results clearly show that APB does not block transmitter release from salamander cones so the mechanism must be a postsynaptic one. Since the rod transmitter (and APB) *closes* channels in on-centre bipolar cells, one cannot argue in terms of a shunting of cone input. One possibility is that, in salamander unlike goldfish, APB also exerts a direct effect upon the cone-modulated channels. An alternative explanation is suggested by an observation of Ashmore & Falk (1980) that in on-centre cells of the dogfish, the input impedance was determined almost entirely by the properties of the dendrites. If this were true in salamander and the non-synaptic conductance were

very small, blockade of the rod-driven channels would polarize the cell to the reversal potential of the cone-driven channels and cone-generated responses would disappear. For this explanation to hold, the reversal potential of those channels must be only a few millivolts more negative than the dark potential, i.e. near −45 mV. This is not inconsistent with the findings of Nawy & Copenhagen (1987) in goldfish. The reversal potential of the cone-driven channels inferred from their Fig. 3*A*, for example, is about −45 mV.

Our observation that, in the presence of APB, when the direct input to on-centre bipolar cells was blocked, we could still record significant responses from their receptive field surrounds is particularly interesting. If the surround were mediated entirely by feedback from horizontal cells to photoreceptors, blockade of the receptive field centre should have eliminated any input from the surround. This was not the case and a feedforward pathway, insensitive to APB, was thus revealed. The approximately 50% reduction in net gain between the horizontal cells and the on-centre bipolar cell that results from APB application could be explained, if, under normal conditions, this feedforward pathway carries 50% of the input to the receptive field surround, the remainder arriving via feedback though photoreceptors.

One could imagine a 'feedforward' pathway in which signals pass first, by feedback or feedforward, from the horizontal cells to the off-centre bipolar cells and thence, either directly or via a sustained-type amacrine cell (Maguire, Lukasiewicz & Werblin, 1989), to the on-centre cell. The responses one would then observe, however, should reflect the spatial properties of the off-centre cells whose centre–surround organization remains intact in the presence of APB. Since we see no evidence of any centre–surround antagonism in the residual responses in on-centre cells, this possibility seems unlikely. The simpler explanation, that in the presence of APB signals pass between the horizontal cells and the on-centre bipolar cells via direct synapses similar to those described by Lasansky (1978), is entirely consistent with our findings, however.

We wish to acknowledge helpful discussions with Drs David Copenhagen and Greg Maguire. We are especially grateful to Dr Peter McNaughton for pointing out the approximations that led to eqn (5). This work was supported by research grant EY03785 from the National Eye Institute (to W.G.O.) and an institutional Biomedical Research Support Grant (NIH)

REFERENCES

ASHMORE, J. F. & FALK, G. (1980). Responses of rod bipolar cells in the dark-adapted retina of the dogfish, *Scyliorhinus canicula*. *Journal of Physiology* **300**, 115–150.

BAYLOR, D. A., FUORTES, M. G. F. & O'BRYAN, P. M. (1971). Receptive fields of cones in the retina of the turtle. *Journal of Physiology* **214**, 265–294.

BURKHARDT, D. A. (1977). Responses and receptive-field organization of cones in Perch retinas. *Journal of Neurophysiology* **40**, 53–62.

CAPOVILLA, M., HARE, W. A. & OWEN, W. G. (1987). Voltage gain of signal transfer from retinal rods to bipolar cells in the tiger salamander. *Journal of Physiology* **391**, 125–140.

DEVRIES, S. H. & SCHWARTZ, E. A. (1989). Modulation of an electrical synapse between solitary pairs of catfish horizontal cells by dopamine and second messengers. *Journal of Physiology* **414**, 351–375.

DONG, C.-J. & MCREYNOLDS, J. S. (1989). APB increases apparent coupling between horizontal cells in mudpuppy retina. *Vision Research* **29**, 541–544.

GERSCHENFELD, H. C. M. & PICCOLINO, M. (1980). Sustained feed-back effects of L-horizontal cells on turtle cones. *Proceedings of the Royal Society* B **206**, 465–480.

HARE, W. A., LOWE, J. S. & OWEN, W. G. (1986). Morphology of physiologically identified bipolar cells in the retina of the tiger salamander, *Ambystoma tigrinum*. *Journal of Comparative Neurology* **252**, 130–138.

HARE, W. A. & OWEN, W. G. (1989). Pharmacology of the bipolar cell's receptive field in the tiger salamander's retina. *Investigative Ophthalmology* **30**, suppl. 17.

HARE, W. A. & OWEN, W. G. (1990*a*). Spatial organization of the bipolar cell's receptive field in the retina of the tiger salamander. *Journal of Physiology* **421**, 223–245.

HARE, W. A. & OWEN, W. G. (1990*b*). Effects of dopamine on rods and horizontal cells in the tiger salamander retina. *Investigative Ophthalmology* **31**, suppl. 334.

JACK, J. J. B., NOBLE, D. & TSIEN, R. W. (1975). *Electric Current Flow in Excitable Cells*. Clarendon, Oxford.

KAMERMANS, M., VAN DIJK, B. W. & SPEKREIJSE, H. (1990). Interaction between the soma and the axon terminal of horizontal cells in carp retina. *Vision Research* **30**, 1011–1016.

KNAPP, A. G. & DOWLING, J. E. (1987). Dopamine enhances excitatory amino acid-gated conductances in cultured retinal horizontal cells. *Nature* **325**, 437–439.

LAMB, T. D. & SIMON, E. J. (1976). The relation between intracellular coupling and electrical noise in turtle photoreceptors. *Journal of Physiology* **263**, 257–286.

LASANSKY, A. (1978). Contacts between receptors and electrophysiologically identified neurons in the retina of the larval tiger salamander. *Journal of Physiology* **285**, 531–542.

LASANSKY, A. (1981). Synaptic action mediating cone response to annular illumination in the retina of the larval tiger salamander. *Journal of Physiology* **310**, 205–214.

LASATER, E. M. (1987). Retinal horizontal cell gap junctional conductance is modulated by dopamine through a cyclic AMP-dependent protein kinase. *Proceedings of the National Academy of Sciences of the USA* **84**, 7319–7323.

LASATER, E. M. & DOWLING, J. E. (1985). Dopamine decreases conductance of the electrical junctions between cultured retinal horizontal cells. *Proceedings of the National Academy of Sciences of the USA* **82**, 3025–3029.

MAGUIRE, G. W., LUKASIEWICZ, P. & WERBLIN, F. S. (1989). Amacrine cell interactions underlying the response to change in the tiger salamander retina. *Journal of Neuroscience* **9**, 726–735.

MAGUIRE, G. W. & WERBLIN, F. S. (1990). D1 dopamine receptors modulate excitatory amino acid gated currents in bipolar, horizontal and amacrine cells via a G-protein, cAMP-PK pathway in the tiger salamander retina. *Investigative Ophthalmology* **31**, suppl. 535.

MINOR, A. V. & MAKSIMOV, V. V. (1969). Passive electrical properties of the model of a flat cell. *Biofizika* **14**, 328–335 (in Russian).

NAWY, S. & COPENHAGEN, D. R. (1987). Multiple classes of glutamate receptor on depolarizing bipolar cells in retina. *Nature* **325**, 56–58.

NAWY, S. & COPENHAGEN, D. R. (1990). Intracellular cesium separates two glutamate conductances in retinal bipolar cells of goldfish. *Vision Research* **30**, 967–972.

NAWY, S. & JAHR, C. E. (1990). Suppression by glutamate of cGMP-activated conductance in retinal bipolar cells. *Nature* **346**, 269–271.

NAWY, S., SIE, A. & COPENHAGEN, D. R. (1989). The glutamate analog 2-amino-4-phosphonobutyrate antagonizes synaptic transmission from cones to horizontal cells in the goldfish retina. *Proceedings of the National Academy of Sciences of the USA* **86**, 1726–1730.

O'BRYAN, P. M. (1973). Properties of the depolarizing synaptic potential evoked by peripheral illumination in cones of the turtle retina. *Journal of Physiology* **235**, 207–223.

OWEN, W. G. & HARE, W. A. (1989). Signal transfer from photoreceptors to bipolar cells in the retina of the tiger salamander. *Neuroscience Research Supplement* **10**, S77–88.

OWEN, W. G. & TORRE, V. (1983). High-pass filtering of small signals by retinal rods: Ionic studies. *Biophysical Journal* **41**, 325–339.

PICCOLINO, M., NEYTON, J. & GERSCHENFELD, H. C. M. (1984). Decrease of gap junction permeability induced by dopamine and cyclic adenosine 3′:5′-monophosphate in horizontal cells of turtle retina. *Journal of Neuroscience* **4**, 2477–2488.

SARANTIS, M., EVERETT, K. & ATTWELL, D. (1988). A presynaptic action of glutamate at the cone output synapse. *Nature* **332**, 451–453.

SHEILLS, R. A. & FALK, G. (1990). Glutamate receptors of rod bipolar cells are linked to a cyclic GMP cascade via a G-protein. *Proceedings of the Royal Society* B **242**, 91–94.

SHEILLS, R. A., FALK, G. & NAGHSHINEH, S. (1981). Action of glutamate and aspartate analogues on rod horizontal and bipolar cells. *Nature* **294**, 592–594.

SLAUGHTER, M. M. (1986). APB receptors on Off bipolar cells and horizontal cells. *Investigative Ophthalmology and Visual Science* **27**, suppl. 224.

SLAUGHTER, M. & MILLER, R. F. (1981). 2-Amino-4-phosphonobutyric acid: A new tool for retina research. *Science* **211**, 182–185.

TACHIBANA, M. & KANEKO, A. (1988). L-Glutamate-induced depolarization in solitary photoreceptors: A process that may contribute to the interaction between photoreceptors *in situ*. *Proceedings of the National Academy of Sciences of the USA* **85**, 5315–5319.

TERANISHI, T., NEGISHI, K. & KATO, S. (1983). Dopamine modulates S-potential amplitude and dye-coupling between external horizontal cells in carp retina. *Nature* **301**, 243–246.

TERANISHI, T., NEGISHI, K. & KATO, S. (1985). Regulatory effect of dopamine on spatial properties of horizontal cells in carp retina. *Journal of Neuroscience* **4**, 1271–1280.

TORRE, V. & OWEN, W. G. (1983). High-pass filtering of small signals by the rod network in the retina of the toad, *Bufo marinus*. *Biophysical Journal* **41**, 305–324.

WITKOVSKY, P., OWEN, W. G. & WOODWORTH, M. (1983). Gap junctions among the perikarya and axon terminals of the luminosity-type horizontal cells of the turtle retina. *Journal of Comparative Neurology* **216**, 359–368.

WU, S. M. (1988). Synaptic transmission from rods to horizontal cells in dark-adapted tiger salamander retina. *Vision Research* **28**, 1–8.

WU, S. M. & YANG, X.-L. (1988). Electrical coupling between rods in the tiger salamander retina. *Proceedings of the National Academy of Sciences of the USA* **85**, 275–278.

YANG, X.-L. & WU, S. M. (1989). Effects of CNQX, APB, PDA and kynurenate on horizontal cells of the tiger salamander. *Visual Neuroscience* **3**, 207–212.