

RESEARCH NOTE

APB INCREASES APPARENT COUPLING BETWEEN HORIZONTAL CELLS IN MUDPUPPY RETINA

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Abstract—2-Amino-4-phosphonobutyrate (APB), an agonist at a unique type of glutamate receptor on depolarizing bipolar cells, caused an apparent increase in coupling between horizontal cells as evidenced by a decrease in amplitude of responses to illumination of the receptive field center and an increase in responses to illumination of the peripheral part of the receptive field. APB also caused a hyperpolarization of horizontal cells in darkness and increased the amplitude of responses to full-field illumination, which cannot be explained by an increase in electrical coupling between horizontal cells. Possible mechanisms for these actions are discussed.

2-Amino-4-phosphonobutyric acid APB Retina Horizontal cells Electrical coupling

INTRODUCTION

In the retinas of many vertebrates, horizontal cells contribute to the center-surround receptive field organization of cones and bipolar cells, and thus indirectly to the receptive field organization of more proximal neurons such as ganglion cells (Werblin & Dowling, 1969; Baylor, Fuortes & O'Bryan, 1971; Naka & Witkovsky, 1972; Burkhardt, 1977; Marchiafava, 1978; Mangel & Miller, 1987). The large receptive fields of some types of horizontal cells are due to electrical coupling through gap junctions (Kaneko, 1971) and recent evidence suggests that this coupling may be physiologically regulated; dopamine causes a decrease in electrical coupling between certain types of horizontal cells in fish and turtle (Hedden & Dowling, 1978; Teranishi, Negishi & Kato, 1983; Mangel & Dowling, 1985; Lasater & Dowling, 1985; Piccolino, Neyton & Gerschenfeld, 1984). In the present study we report that apparent coupling between horizontal cells in the mudpuppy retina is markedly *increased* in the presence of 2-amino-4-phosphonobutyrate (APB) at concentrations as low as 5 μ M. This is of interest because APB is often used to block the "On"-pathway in the retina, due to its action as an agonist at a unique type of glutamate receptor thought to be restricted to depolarizing bipolar cells (Slaughter & Miller, 1981; Shiells, Falk & Naghshineh, 1981; Nawy & Copenhagen, 1987). APB may also have some

effects on horizontal cells (Slaughter, 1986; Nawy, Copenhagen & Lisberger, 1988), but none that could account for an apparent increase in coupling. The present results suggest that APB might be suppressing a tonic release of a transmitter whose action on horizontal cells is similar to that of dopamine in fish, although the possibility of a direct action of APB on horizontal cells cannot be completely ruled out. The results also suggest that some of the APB-induced changes in response properties of more proximal neurons may be due to alterations in lateral interactions in the outer plexiform layer rather than solely to elimination of input from depolarizing bipolar cells.

METHODS

Intracellular recordings were made from horizontal cells in the superfused eyecup preparation of the mudpuppy (*Necturus maculosus*). Details of the preparation, electrical recording and light stimulation are described in detail elsewhere (Miyachi, Lukasiewicz & McReynolds, 1987). The dissection was carried out in room light and the preparation was then dark-adapted for 10–20 min before attempting to penetrate a cell. A tungsten-halogen lamp provided two independently-shuttered beams of white light whose intensities were adjusted by calibrated neutral density filters to the values

indicated in the figure legends. The light stimuli were spots (dia. $240\ \mu\text{m}$) or annuli (i.d. $800\ \mu\text{m}$, o.d. greater than diameter of retina) centered on the recording site. Horizontal cells were identified by their large, hyperpolarizing responses, wide receptive fields and depth in the retina. During the experiments the retina was maintained in a mesopic condition. APB (Sigma Chemical Co.) was applied by switching the superfusate from normal Ringer to a solution containing normal Ringer plus the desired concentration of APB. The composition of the normal Ringer solution was (in mM): NaCl, 110; KCl, 2.5, CaCl_2 , 1.8; glucose 11; HEPES buffer 5. pH was adjusted to 7.8 with NaOH.

RESULTS AND DISCUSSION

APB had markedly different effects on the responses of horizontal cells to illumination of the central and peripheral portions of the receptive field. Figure 1 shows responses of a horizontal cell which was alternately illuminated with a small spot and an annulus which was concentric with the spot; the intensities of the two stimuli were adjusted with neutral density filters to give approximately half-saturating responses of equal amplitude. The addition of $5\ \mu\text{M}$ APB to the medium caused a hyperpolarization in darkness, a decrease in the amplitude of the response to the spot and an increase in the amplitude of the response to the annulus. APB caused similar changes in responses to spot and annular illumination in 15 of the 18 horizontal cells in

which this was tested, and in all but three of these cells the effect was rapidly reversed when the APB was washed out. The different effects of APB on the spot and annulus responses could not have been due to differences in light intensity of the two stimuli, since in other experiments similar results were observed regardless of whether the intensity of the spot was less than or greater than that of the annulus.

The above results could be explained if APB caused an increase in electrical coupling between horizontal cells. An increase in coupling (i.e. a decrease in coupling resistance) would allow more of the current generated in the recorded horizontal cell by a small spot to leak away into neighboring horizontal cells, thus decreasing the amplitude of the spot response, and it would allow more of the current generated by an annulus in peripheral horizontal cells to reach the recorded horizontal cell, thus increasing the amplitude of the response to the annulus.

APB also had two other effects on horizontal cells; it caused a hyperpolarization of the membrane in darkness, which can be seen in Figs 1 and 2, and it increased the amplitude of responses to full-field illumination (Fig. 2). Neither of these two effects can be explained by an increase in coupling, since in both darkness and diffuse illumination all of the coupled horizontal cells would presumably have been at the same membrane potential, with no lateral current flow between horizontal cells. This result is also opposite to the effect of dopamine in fish retina,

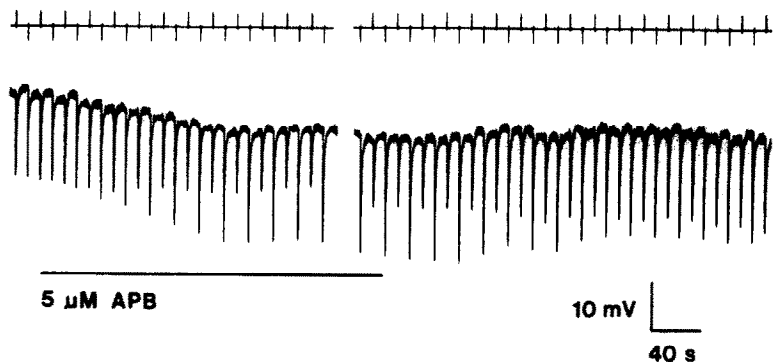


Fig. 1. Effect of APB on horizontal cell responses to spot and annulus illumination. The top trace indicates the timing of the light stimuli; upward deflections indicate presentation of a spot stimulus (dia. $240\ \mu\text{m}$) and downward deflections indicate presentation of a concentric annulus (i.d. $800\ \mu\text{m}$, o.d. greater than diameter of retina). The spot and annulus intensities were adjusted with neutral density filters to give horizontal cell responses of equal amplitude prior to exposure to APB. Under these conditions the intensity of the spot stimulus was equivalent, for mudpuppy cones, to 4×10^{10} photons $\text{cm}^{-2} \text{sec}^{-1}$ at 575 nm and that of the annulus was $0.6 \log_{10}$ units dimmer. During the time indicated by the horizontal line below the recording the superfusate was switched to the test solution, which contained $5\ \mu\text{M}$ APB.

The break in the response trace represents a period of 2 min. Resting potential was $-33\ \text{mV}$.

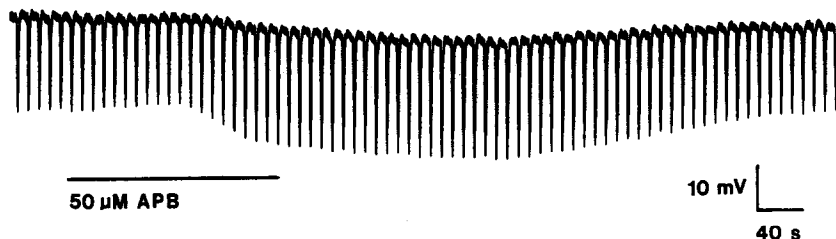


Fig. 2. Effect of APB on responses of a horizontal cell to full-field illumination. Downward deflections of the membrane potential are responses of the horizontal cell to a 100 msec flash of diffuse illumination presented every 10 sec. The intensity of the light stimulus was $0.6 \log_{10}$ units less than that of the spot stimulus in Fig. 1. During the time indicated by the horizontal line below the recording the superfusate was switched to the test solution, which contained $50 \mu\text{M}$ APB. Similar results were obtained in 6 other cells using $5\text{--}10 \mu\text{M}$ APB. Resting potential was -26 mV .

where it caused a decrease in the response to diffuse illumination and a depolarization in darkness. Those effects of dopamine also could not be explained by a change in coupling (Hedden & Dowling, 1978; Mangel & Dowling, 1985), but subsequent experiments on isolated horizontal cells showed that they were due to an increase in the sensitivity of glutamate receptor sites which in the intact retina presumably receive transmitter released by photoreceptors (Knapp & Dowling, 1987). All of the above-mentioned actions of dopamine on horizontal cells in fish and turtle appear to be mediated via an increase in intracellular cyclic AMP concentration (Van Buskirk & Dowling, 1981; Teranishi et al., 1983; Piccolino et al., 1984; Lasater & Dowling, 1985; Knapp & Dowling, 1987).

The effects of APB on bipolar cells were essentially as described previously (Slaughter & Miller, 1981). $5\text{--}100 \mu\text{M}$ APB eliminated the response to light and caused a $4\text{--}10 \text{ mV}$ hyperpolarization in darkness in all of the four depolarizing bipolar cells tested. In two presumed hyperpolarizing bipolar cells $5 \mu\text{M}$ APB had no noticeable effect on the dark potential or response to center illumination. The effect of APB on the surround response in hyperpolarizing bipolar cells could not be tested because in both of these cells the response to surround illumination disappeared soon after penetration. Because hyperpolarizing bipolar cells were rarely encountered in our experiments, it was not possible to study them further.

The fact that the effects of APB on horizontal cell coupling, membrane potential in darkness, and responses to diffuse illumination are all opposite to those of dopamine in fish suggests that APB might cause a reduction in the tonic

release of dopamine (or another transmitter with a similar action) onto horizontal cells. This could be explained by the known hyperpolarizing action of APB on depolarizing bipolar cells (Slaughter & Miller, 1981), if that in turn led to a reduction of tonic excitatory input to neurons which release a dopamine-like transmitter. It has been reported that relatively high concentrations of dopamine can cause uncoupling of horizontal cells in mudpuppy (Frumkes, Eysteinson & Denny, 1987), and we have made similar observations. However, dopamine was often without any effect in mudpuppy, even at concentrations as high as 2 mM . Since APB was consistently effective at very low concentrations, it is possible that in mudpuppy some other transmitter has an action similar to that of dopamine in fish retina.

The possibility that APB might act directly on horizontal cells must also be considered. The primary action of APB in the retina is on depolarizing bipolar cells, where it mimics the action of an endogenous photoreceptor transmitter by closing sodium channels at a unique type of glutamate receptor (Slaughter & Miller, 1981; Shiells et al., 1981; Nawy & Copenhagen, 1987). Hyperpolarization of horizontal cells by APB has also been reported; this was attributed to closing sodium channels at non-synaptic sites on horizontal cells in mudpuppy (Slaughter, 1986) or to a presynaptic action on cone terminals in goldfish (Nawy et al., 1988). However, neither of these suggested actions, nor an action at the horizontal cell receptor sites which normally receive photoreceptor transmitter, could account for the opposite effects of APB on horizontal cell responses to spot and annulus stimuli. Thus, it seems unlikely that the effects reported here are due to a direct effect of APB

on horizontal cells, unless they are mediated by some as yet unidentified type of receptor, for example one whose activation caused a decrease in cyclic AMP concentration. Unfortunately, it could not be determined whether APB affected coupling when transmitter release was blocked with cobalt, since cobalt itself hyperpolarized horizontal cells and eliminated their light responses, and we were not able to successfully measure changes in input resistance. Other techniques, such as measuring the effect of APB on coupling between pairs of isolated horizontal cells, may help to resolve this question. Nevertheless, the results suggest that interpretation of APB-induced changes in responses of neurons in the inner retina should take into account the fact that APB may alter lateral interactions in the outer retina.

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