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SYNAPTIC TRANSMISSION AT *N*-METHYL-D-ASPARTATE RECEPTORS IN THE PROXIMAL RETINA OF THE MUDPUPPY

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SUMMARY

1. The effects of excitatory amino acid analogues and antagonists on retinal ganglion cells were studied using intracellular recording in the superfused mudpuppy eyecup preparation.

2. Aspartate, glutamate, quisqualate (QA), kainate (KA) and *N*-methylaspartate (NMA) caused depolarization and decreased input resistance in all classes of ganglion cells. The order of sensitivity was QA $\geqslant$ KA > NMA > aspartate $\geqslant$ glutamate. All of these agonists were effective when transmitter release was blocked with 4 mM-Co^{2+} or Mn^{2+}, indicating that they acted at receptor sites on the ganglion cells.

3. At a concentration of 250 μM, 2-amino-5-phosphonovalerate (APV) blocked the responses of all ganglion cells to NMA, but not to QA or KA, indicating that NMA acts at different receptor sites from QA or KA. Responses to bath-applied aspartate and glutamate were reduced slightly or not at all in the presence of APV, indicating that they were acting mainly at non-NMDA (*N*-methyl-D-aspartate) receptors.

4. In all ganglion cells 250 μM-APV strongly suppressed the sustained responses driven by the 'on'-pathway but not those driven by the 'off'-pathway.

5. In most on–off ganglion cells the transient excitatory responses at 'light on' and 'light off' were not reduced by 500 μM-APV. APV-resistant transient excitatory responses were also present in some on-centre ganglion cells. APV did not block the transient inhibitory responses in any class of ganglion cells.

6. At concentrations which blocked the sustained responses of ganglion cells, APV did not affect the sustained responses of bipolar cells, indicating that it acted at sites which were post-synaptic to bipolar cells.

7. The simplest interpretation of these results is that the transmitter released by depolarizing bipolar cells acts at NMDA receptors on sustained depolarizing amacrine and ganglion cells. It may act at non-NMDA receptors at synapses which produce transient excitatory responses, but this could not be proved. The transmitter released by hyperpolarizing bipolar cells does not appear to act at NMDA receptors on any post-synaptic cells.

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INTRODUCTION

Although there is considerable evidence that γ-aminobutyric acid (GABA) and glycine may be mediators for specific types of inhibition in the proximal retina in the mudpuppy (Frumkes, Miller, Slaughter & Dacheux, 1981; Belgum, Dvorak & McReynolds, 1984) and many other species, relatively little is known about the transmitters which are mediators for specific excitatory connexions in the proximal retina. Recent studies have suggested that the transmitter released by bipolar cells may be an excitatory amino acid. It has been reported that this may be glutamate in the mudpuppy (Slaughter & Miller, 1983*a*, *b*) and aspartate in the cat (Ikeda & Sheardown, 1982*b*).

Aspartate and glutamate may act at several different receptor sites; the three main types are usually classified as quisqualate (QA), kainate (KA) and *N*-methyl-D-aspartate (NMDA) receptors based on the relative potency of these aspartate or glutamate analogues and the actions of pharmacological antagonists (Watkins, Davies, Evans, Francis & Jones, 1981; Watkins & Evans, 1981; McLennan, 1983). What appears to be a fourth type of excitatory amino acid receptor, at which 2-amino-4-phosphonobutyrate (APB) is an agonist, is present on depolarizing bipolar cells (Slaughter & Miller, 1981), and still other types may also exist. The present study attempts to characterize different excitatory pathways in the proximal retina by using the specific NMDA receptor antagonist 2-amino-5-phosphonovalerate (APV) (Davies, Francis, Jones & Watkins, 1981). The results suggest that the transmitters released by depolarizing and hyperpolarizing bipolar cells may act at different types of post-synaptic receptor sites.

METHODS

The systems for electrical recording and optical stimulation have been described in detail (Belgum, Dvorak & McReynolds, 1982, 1983) and will only be explained briefly here. Intracellular recordings were made from ganglion cells in the eyecup preparation of the mudpuppy (*Necturus maculosus*) using micropipettes of 300–800 MΩ resistance filled with 4 M-potassium acetate. Current–voltage (*I–V*) relations were made by passing constant current steps or pulses through the recording electrode and measuring the resulting steady-state voltage displacement. In some experiments light stimuli were presented during the current step and the voltage displacement was measured at a specific time during the light response. The amplifier (Colburn & Schwartz, 1972) contained a bridge circuit to balance out the voltage drop across the electrode resistance. *I–V* relations measured when the electrode was outside the cells were used to correct for electrode rectification, which often occurred with current intensities greater than $\pm 0{\cdot}05$ nA. Responses were stored on magnetic tape and displayed on a chart recorder (Gould 2200S) from which the illustrated responses were photographed.

The eyecup was superfused with a continuous flow of Ringer solution whose composition was (mM): NaCl, 110; KCl, 2·5; $CaCl_2$, 1·8; glucose, 11; HEPES buffer, 5·0. The solution flowing over the preparation could be rapidly switched to any one of several other solutions containing Ringer solution plus known concentrations of $CoCl_2$, QA, KA, NMA, APV, etc., either singly or in various combinations. All solutions were adjusted to pH 7·8. The time required for the new solution to reach the preparation was 1–2 min, and exchange of the fluid at the preparation was 90% complete within another 30–90 s. 2-amino-5-phosphonovaleric acid and 2,3-*cis*-piperidinedicarboxylic acid (PDA) were obtained from Cambridge Research Biochemicals, Inc., and 2-amino-4-phosphonobutyric acid from Calbiochem-Behring. All other drugs were obtained from Sigma Chemical Co.

The light stimulus was a 250 μm diameter spot of white light which was centred in the cell's receptive field. Unless otherwise indicated the intensity of the light stimulus at the plane of the

retina was equivalent to $5 \cdot 2 \times 10^{10}$ photons cm^{-2} s^{-1} at 572 nm, the λ_{max} for mudpuppy cones (Fain, 1975). Only those stimulus intensities different from this value are indicated in the Figure legends, where they are expressed as $\log_{10}$ units of additional attenuation by neutral density filters.

Ganglion cells were classified as on-centre, off-centre or on–off based on their characteristic responses to stationary light stimuli. They could usually be distinguished from amacrine cells by their response characteristics and receptive field properties as described previously (Belgum *et al.* 1982, 1983, 1984; Werblin, 1977).

RESULTS

Effects of QA, KA and NMA on ganglion cells

Responses to aspartate, glutamate and the specific agonists for the three principal types of excitatory amino acid receptor types (QA, KA and NMDA) were recorded in all three classes of ganglion cells (ten on-centre, six off-centre and eighteen on–off). Instead of NMDA the more easily obtainable racemic mixture of *N*-methylaspartate (NMA), which has a similar action (Dingledine, 1983; Slaughter & Miller, 1983*b*), was used. Unless otherwise stated, the results for all the agonists reported in this section were the same for all classes of ganglion cells. Aspartate, glutamate, QA, KA, and NMA each caused a depolarization and a reduction in the amplitude of the light-evoked responses (Fig. 1). The depolarization produced by each of the agonists was associated with a decrease in input resistance, as will be shown below. The reduction of the light response could thus have been due to competition between the applied agonist and the endogenous transmitter if the two substances were acting at the same receptor sites, or by shunting if they were acting at different types of receptors. Dose–response properties of the agonists were not studied in any detail, but the concentrations which produced about half-maximal responses were found to be approximately 10–20 μM-QA and KA, 50–100 μM-NMA, and 1–2 mM-aspartate and glutamate.

To verify that the agonists were acting directly on ganglion cells rather than on a presynaptic neurone the agonists were also applied when synaptic transmission was blocked with 4 mM-Co^{2+}. Co^{2+} did not reduce the responses of any ganglion cells to QA ($n = 18$), KA ($n = 22$), aspartate ($n = 4$) or glutamate ($n = 5$), indicating that all of these substances were acting at receptors located on the ganglion cells. An example is illustrated in Fig. 2, which compares the response of an on-centre ganglion cell to 20 μM-QA in normal Ringer solution and in the presence of 4 mM-Co^{2+}. The responses to these agonists were usually larger in the presence of Co^{2+}; this was probably due to an increased input resistance since, as described elsewhere (Belgum *et al.* 1982, 1983), the addition of Co^{2+} causes a significant increase in resistance by blocking tonic synaptic inputs which are active in darkness.

In the cell shown in Fig. 2 input resistance was monitored by passing pulses of hyperpolarizing current across the membrane (arrows); the smaller voltage drop during the response to QA indicates a decrease in resistance. When measured in this manner the responses to aspartate, glutamate, QA, and KA were associated with a decrease in resistance, both in normal Ringer solution and in the presence of Co^{2+}. In normal Ringer solution the responses to NMA were also associated with a decrease in resistance, but they were suppressed in the presence of Co^{2+}, as described below. In order to determine whether the resistance changes produced by QA, KA and NMA were due to voltage-dependent membrane properties, the I–V relations of some cells

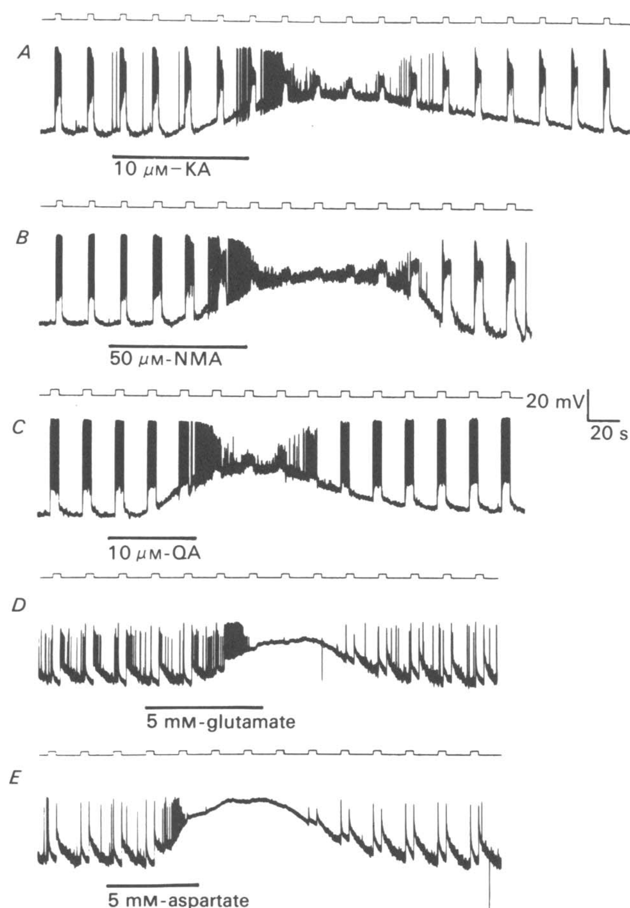

Fig. 1. Responses of ganglion cells to KA, NMA, QA, glutamate and aspartate. Each trace shows the effect of a subsaturating concentration of the applied agonist on the membrane potential and light-evoked response. *A*, *B* and *C* are from a single on-centre ganglion cell. *D* and *E* are from a single on–off ganglion cell. In this and all subsequent Figures the continuous line above the responses is the light stimulus monitor; an upward deflexion indicates light stimulus was on. The thick horizontal lines below the responses indicate when the superfusion fluid contained the indicated test substance. Relative light intensity was −0·6 in *B* and −1·2 in *C*. Resting potential was −64 mV in *A*, *B* and *C* and −57 mV in *D* and *E*.

were measured during the responses to KA ($n = 5$), QA ($n = 4$) and NMA ($n = 3$). The *I–V* relations for KA and QA were measured in the presence of Co^{2+}, whereas those for NMA were measured in normal Ringer solution. In every case the agonist-induced depolarization was associated with a decrease in resistance which was not voltage-dependent within the range of membrane potential measured (resting potential ± 20 mV). Fig. 3 shows an example from an off-centre ganglion cell; 25 μM-KA caused a depolarization of 15 mV which was associated with a decrease in resistance from 320 to 150 MΩ.

In contrast to the results with QA, KA, aspartate and glutamate, the responses

to NMA were reduced or eliminated in the presence of Co^{2+} (Fig. 4*A*). This does not necessarily indicate that the NMDA receptors were on a presynaptic cell, however, since Co^{2+} and certain other divalent cations may interfere post-synaptically with responses to NMA, but not to QA or KA (Ault, Evans, Francis, Oakes & Watkins, 1980). Since Mn^{2+} was reported to be much less effective than Co^{2+} in this respect

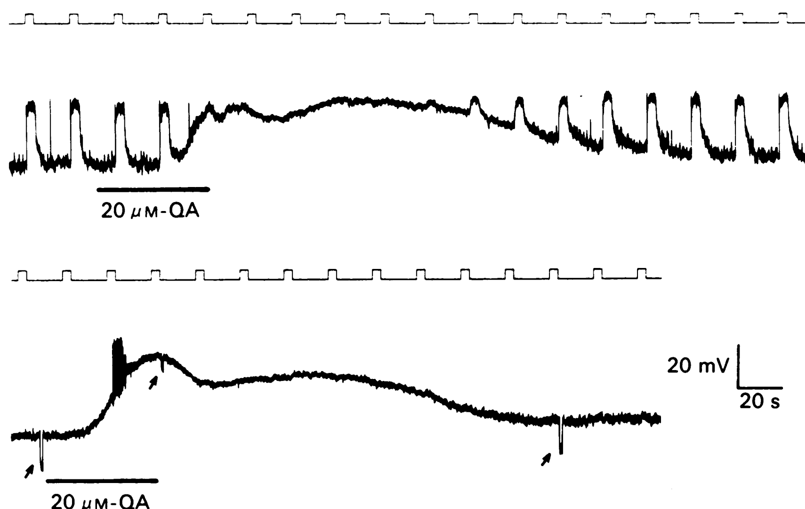

Fig. 2. Effect of Co^{2+} on responses to QA. Responses of an on-centre ganglion cell to 20 µM-QA in normal Ringer solution (upper trace) and after transmission was blocked with 4 mM-Co^{2+} (lower trace). The three downward deflexions in the lower trace (indicated by arrows) are voltage drops produced by a −0·05 nA current pulse. Light intensity −0·6, resting potential −58 mV.

(Ault *et al.* 1980), additional experiments were done using Mn^{2+} instead of Co^{2+} to block transmitter release. In the four cells tested, 4 mM-Mn^{2+} reduced transmitter release by more than 90%, as judged by the amplitude of the light responses, but reduced the NMA-evoked depolarization by less than 20%. The fact that NMA could produce nearly normal responses when transmitter release was strongly suppressed by Mn^{2+} suggests that NMA receptors were present on ganglion cells. Furthermore, the suppression of NMA responses by Co^{2+} could be overcome by using higher concentrations of NMA (0·5–2 mM). The responses obtained under these conditions often showed large fluctuations in potential and were associated with an apparent increase in resistance (Fig. 4*B*), although complete *I–V* relations were not measured. The apparent increase in resistance may have been due to a voltage dependence of the NMA-gated channels in the presence of Co^{2+} (see Discussion).

Since depolarizing bipolar cells in the mudpuppy have an unusual type of excitatory amino acid receptor at which APB acts as an agonist (Slaughter & Miller, 1981), experiments were also done to determine whether this type of receptor was present on ganglion cells. In the presence of 4 mM-Co^{2+}, which did not block its action on depolarizing bipolar cells (see also Slaughter & Miller, 1981), 250 µM-APB had no effect on membrane potential or input resistance of ganglion cells (two on-centre, one

off-centre and two on–off), indicating that such 'APB' receptors were not present on these cells.

APV selectively blocks NMDA receptors. APV has been shown to be a selective antagonist at NMDA receptors in central neurones (Davies *et al.* 1981; Watkins *et al.* 1981). The selectivity of APV in this preparation was confirmed in eighteen

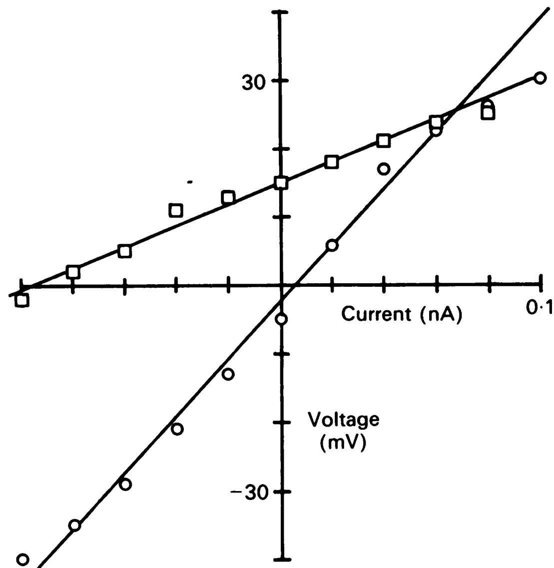

Fig. 3. The effect of KA on the *I–V* relation of an off-centre ganglion cell. Data points indicate the steady-state voltage displacement produced by steps of constant current. Measurements made in Co^{2+} alone (○) and during the response to 25 μM-KA in Co^{2+} (□). All potentials are plotted relative to the membrane potential in control Ringer solution. Note that the membrane potential in the presence of Co^{2+} was 5 mV more negative than in control Ringer solution. Least-square regression lines were fitted to all data points except +30 mV.

ganglion cells (six on-centre, four off-centre and eight on–off). Fig. 5 shows the effect of APV on ganglion cell responses to subsaturating concentrations of NMA, KA and QA. The response to 50 μM-NMA was completely blocked in the presence of 250 μM-APV, while the responses to 10 μM-KA and QA were not reduced by 500 μM-APV. These results indicate that specific NMDA receptors, as well as other receptors that respond to QA and/or KA, are present on all classes of ganglion cells. The effects of 500 μM-APV on responses to aspartate and glutamate (2–5 mM) were studied in four ganglion cells. In two cells APV had no effect on the responses to aspartate or glutamate, while in two others it reduced the responses to these agonists by about 20%.

Effects of APV on light-evoked responses of ganglion cells

In order to determine which components of the synaptically driven responses were mediated by NMDA receptors, the effects of APV on light-evoked responses were

studied in seventy ganglion cells (twenty-one on-centre, ten off-centre and thirty-nine on–off). All effects of APV were reversible when the drug was washed away.

On-centre ganglion cells. APV strongly suppressed the light-evoked sustained depolarizing responses in all on-centre ganglion cells tested ($n = 21$). In 250 μM-APV

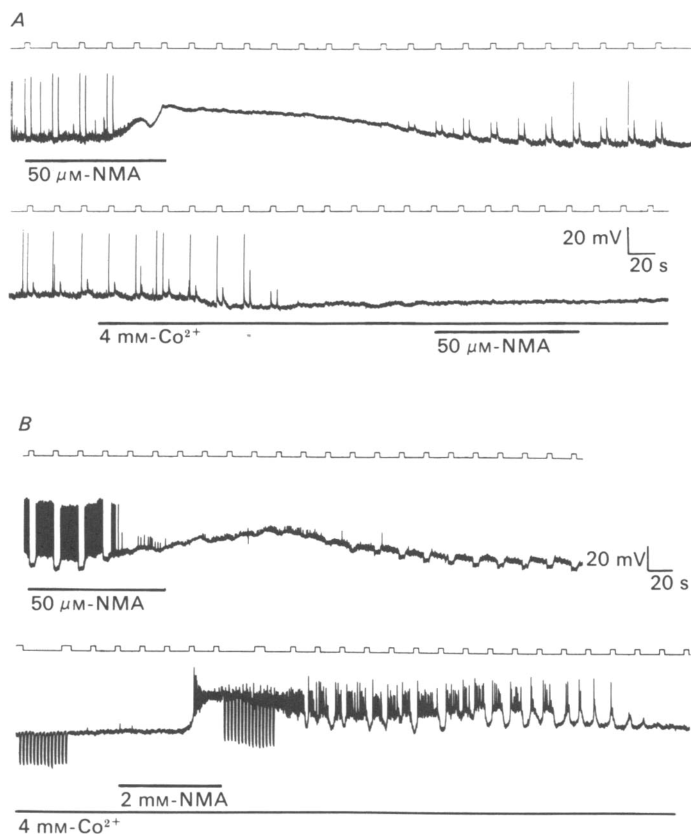

Fig. 4. Effect of Co^{2+} on responses to NMA. *A*, responses of an on–off ganglion cell to 50 μM-NMA in normal Ringer solution (upper trace) and in the presence of 4 mM-Co^{2+} (lower trace). Resting potential was −63 mV. *B*, responses of an off-centre ganglion cell to 50 μM-NMA in normal Ringer solution (upper trace) and to 2 mM-NMA in the presence of 4 mM-Co^{2+} (lower trace). Lower trace begins 5·5 min after addition of Co^{2+}. The two trains of downward deflexions are voltage drops produced by −0·03 nA current pulses. Resting potential was −55 mV.

the sustained responses were always reduced to less than 10% of their control amplitude. Fig. 6*A* shows the time course of the action of 250 μM-APV on an on-centre ganglion cell. Fig. 6*B* shows, on a faster time scale, individual responses from the same cell recorded in normal Ringer solution (left) and in 250 μM-APV (right) when the membrane potential was displaced to different levels with steps of extrinsic current. *I–V* relations (see below) were measured from these and other records. Transient inhibitory responses (Belgum *et al.* 1984) could be seen at the onset and termination of the light stimulus when the cell was depolarized with extrinsic current

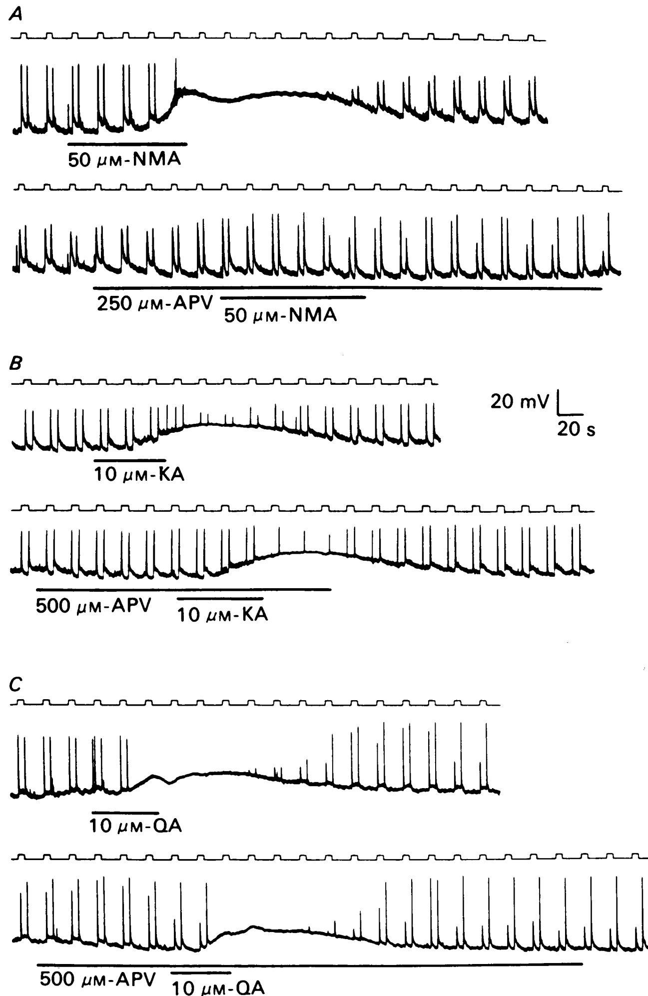

Fig. 5. Effect of APV on responses of ganglion cells to NMA, KA and QA. In each section the upper trace shows the response of a ganglion cell to a subsaturating concentration of the agonist and the lower trace shows the response to the same concentration of agonist in the presence of APV. *A*, *B* and *C* are each from a different ganglion cell.

(upper traces); they became more prominent when the sustained excitatory response was blocked by APV.

I–V relations for this cell are shown in Fig. 7. Measurements made in normal Ringer solution are indicated by open symbols and those made in the presence of APV by filled symbols. In normal Ringer solution the input resistance in darkness was 340 MΩ; this was not affected by APV (● *vs.* ○), indicating that the reduction of the light response in APV was not due to a shunting effect. *I–V* relations were also measured during illumination, just before the end of the light stimulus (squares). In normal Ringer solution the sustained depolarizing response (□) was associated with

a decrease in resistance to 150 MΩ, but in the presence of APV the resistance at this time (■) was not measurably different from that in darkness.

In addition to blocking the sustained response to light, APV often had two additional effects on on-centre ganglion cells. In five of the twenty-one cells APV caused a hyperpolarization and resistance increase in darkness, as illustrated in Fig. 8. The hyperpolarization in darkness was probably due to blocking residual

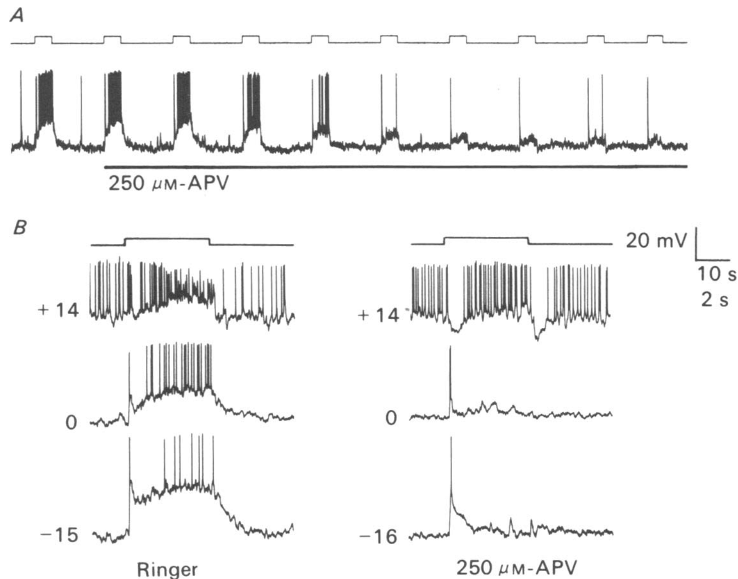

Fig. 6. Effect of APV on an on-centre ganglion cell. *A*, APV suppressed the sustained light-evoked depolarizing response, but did not affect the dark potential. *B*, responses recorded in normal Ringer solution (left) and in 250 µM-APV (right) when membrane potential was displaced by the indicated amounts (in mV) by extrinsic current. Relative light intensity −0·6, resting potential −68 mV.

excitatory input from the 'on'-pathway, since 250 µM-APB, which selectively blocks the 'on'-pathway by an action on depolarizing bipolar cells (Slaughter & Miller, 1981), caused a similar hyperpolarization and resistance increase. APB hyperpolarized only those cells which were hyperpolarized by APV. Also, in seven on-centre ganglion cells large, transient, depolarizing responses remained at the onset and termination of the light stimulus when the sustained excitatory post-synaptic potential (e.p.s.p.) was blocked with APV. This result can also be seen in the cell of Fig. 8. The fact that transient e.p.s.p.s were not always seen in the presence of APV could indicate that these responses were sometimes blocked by APV, but it is also possible that the transient excitatory input was not present in all on-centre ganglion cells. The latter possibility is suggested by the observation that the responses of those on-centre ganglion cells with APV-resistant transient e.p.s.p.s usually had the fastest rise times in normal Ringer solution.

Off-centre ganglion cells. The sustained hyperpolarization of off-centre ganglion cells during centre illumination is primarily due to activation of a sustained inhibitory

input (Belgum *et al.* 1982), although a reduction in the tonic release of excitatory transmitter may also contribute to the hyperpolarization (Dacheux, Frumkes & Miller, 1979). APV strongly suppressed the light-evoked sustained hyperpolarizing response, but not the transient inhibitory response at 'light on' in all off-centre ganglion cells studied ($n = 10$). Fig. 9 shows the effect of APV on two off-centre ganglion cells.

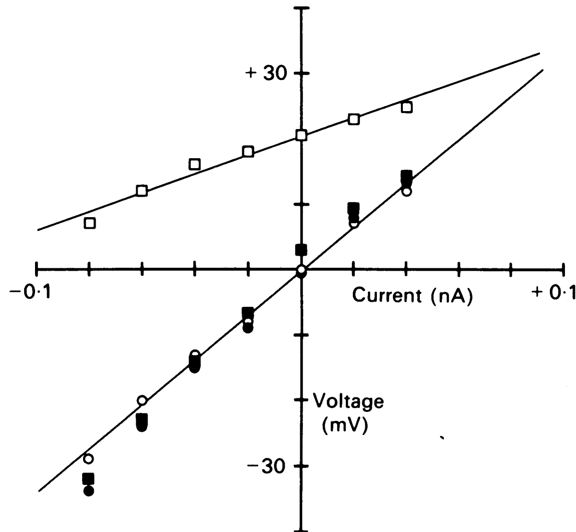

Fig. 7. *I–V* relations from an on-centre ganglion cell in the presence and absence of APV. Data are from the cell shown in Fig. 6. Measurements were made in normal Ringer solution in darkness (○) and during the steady response to light (□), and then in the presence of APV in darkness (●) and in steady light (■). The straight lines were fitted by least-squares regression to the data points measured in normal Ringer solution (○ and □).

APV did not cause a hyperpolarization of the dark potential in any off-centre ganglion cells, indicating that it did not block the tonic excitatory input which these cells receive in darkness. The presence of this excitatory input could be revealed by blocking transmitter release with 4 mM-Co^{2+}, which caused a hyperpolarization and increase in resistance (Fig. 9*B*, see also Belgum *et al.* 1982). Since the tonic excitation in darkness is resistant to APV, any component of the response due to a reduction of tonic excitatory input should be detectable when the sustained inhibition is blocked with APV. The amplitude of the sustained hyperpolarization remaining in the presence of 250 μM-APV was, however, never more than 10 % of its control value. If the remaining sustained light-evoked hyperpolarization was due to a reduction in tonic excitatory input rather than an incomplete block of the inhibitory input, then it should have been associated with a resistance decrease, but unfortunately the resistance changes under such conditions were too small to measure accurately. In two cells, however, increasing the concentration of APV to 500 μM did not cause any further reduction in the sustained hyperpolarization, suggesting that the residual sustained response may have indeed been due to modulation of excitatory input.

On–off ganglion cells. The characteristic response of on–off ganglion cells consists

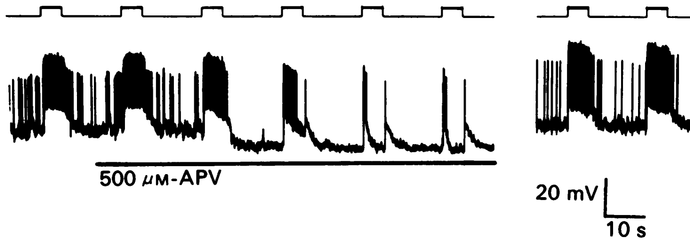

Fig. 8. APV-resistant transient e.p.s.p.s in an on-centre ganglion cell. 500 μM-APV caused a hyperpolarization in darkness and blocked the sustained light-evoked e.p.s.p., leaving transient depolarizing responses at 'light on' and 'light off'. The transient e.p.s.p.s persisted undiminished in amplitude for the entire 3 min exposure to APV. *I–V* measurements (not shown) indicated that the 8 mV hyperpolarization in darkness was accompanied by a 46 MΩ decrease in resistance. Responses at extreme right of trace were recorded 4 min after switching back to control Ringer solution. Relative light intensity −0·6, resting potential −62 mV.

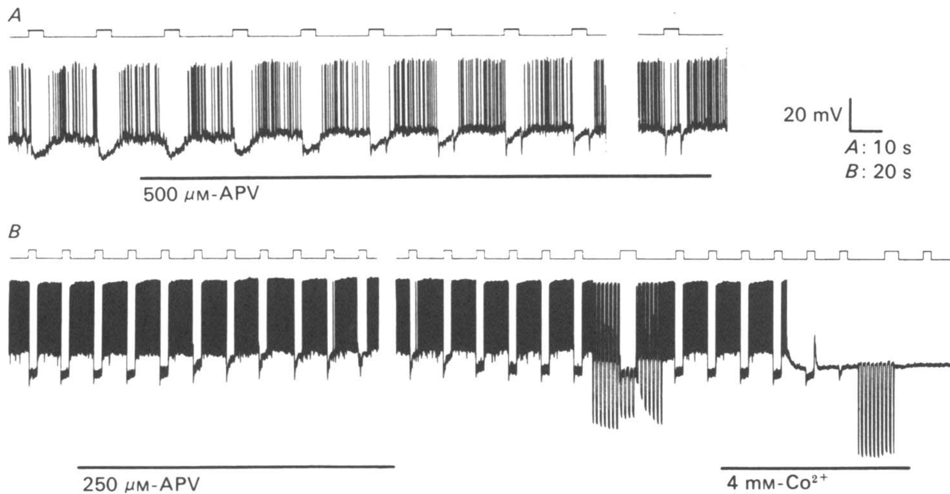

Fig. 9. Effects of APV on off-centre ganglion cells. *A*, 500 μM-APV blocked the sustained light-evoked hyperpolarization and revealed APV-resistant transient i.p.s.p.s at 'light on' and 'light off'. APV did not cause a hyperpolarization of the dark potential. Gap in trace marks a 40 s period when *I–V* relations (not shown) were measured. Resting potential was −55 mV. *B*, comparison of the effects of APV and Co^{2+} on another off-centre ganglion cell. 250 μM-APV suppressed the sustained hyperpolarizing light response, leaving a transient i.p.s.p. at 'light on'. No transient hyperpolarization was seen at 'light off', but it may have been masked by transient excitatory input. A transient excitatory response at 'light off' can be seen during the onset of the block by Co^{2+}. The two groups of downward deflexions are voltage drops produced by trains of −0·08 nA current pulses. The first train of pulses shows that resistance was decreased during the sustained hyperpolarizing light response, with an even further transient decrease at 'light off'. 4 mM-Co^{2+} caused a hyperpolarization and increase in resistance. The chart speed was increased by a factor of 2 during the periods when the resistance measurements were made. The gap in the record represents a 32 s break. Light intensity −0·6, resting potential −54 mV.

of transient e.p.s.p.s at 'light on' and 'light off'. During steady illumination the membrane potential usually returns to the same level as in darkness, although in some cases there may be small, sustained depolarizing or hyperpolarizing components to the response. In most (thirty of thirty-nine) on–off ganglion cells APV did not reduce the transient responses, but any sustained light-evoked components that were present were suppressed. Several examples can be seen in Fig. 5. In the remaining nine cells 250 or 500 μM-APV reduced the amplitude of the transient e.p.s.p.s by up to 50% at 'light on' and up to 24% at 'light off'. In eleven on–off ganglion cells 250 μM-APV caused a small hyperpolarization of the dark potential, suggesting that there was some sustained excitatory input in darkness, but 250 μM-APB caused a similar hyperpolarization in these cells ($n = 5$), indicating that this input was from the 'on'-pathway.

Evidence that APV acts at sites post-synaptic to bipolar cells

Since excitatory amino acids have been implicated as transmitters released by photoreceptors (Cervetto & MacNichol, 1972; Murakami, Ohtsu & Shimazaki, 1975; Wu & Dowling, 1978; Shiells, Falk & Naghshineh, 1981; Slaughter & Miller, 1981, 1983*b*, *c*; Lasater & Dowling, 1982; Lasater, Dowling & Ripps, 1984) it is possible that the observed effects of APV were due to an action at the synapses from photoreceptors to depolarizing bipolar cells. However, 250 μM-APV did not cause any reduction in the responses of depolarizing bipolar cells ($n = 4$). 500 μM-APV caused a 12–22% decrease in the initial peak response in five of seven depolarizing bipolar cells, but did not affect the sustained responses. Thus the effects of APV on sustained responses of ganglion cells cannot be accounted for by an action on depolarizing bipolar cells and must have occurred at sites which were post-synaptic to the bipolar cells. 500 μM-APV had no effect on the responses of horizontal cells ($n = 8$) or hyperpolarizing bipolar cells ($n = 5$).

Other antagonists

Attempts to selectively block responses to QA and KA were not successful. Streptomycin is reported to selectively block responses of cortical neurones to QA (Stone & Perkins, 1983), but at concentrations of 1–10 mM it had no effect on ganglion cell responses to applied QA or KA and it did not reduce any light-evoked responses. The non-specific excitatory amino acid receptor antagonist PDA reportedly blocks QA, KA and NMDA receptors (Watkins *et al.* 1981; Watkins & Evans, 1981). Slaughter & Miller (1983*a*) found that 1–5 mM-PDA reduced the responses of all types of mudpuppy ganglion cells to applied KA and NMA, and reduced the light-evoked responses at 'off' to a greater extent than those at 'on'. In the present study PDA was also tested on a few ganglion cells (eight on-centre, ten on–off and two off-centre). The results were generally similar to those reported by Slaughter & Miller (1983*a*) except that in six on–off ganglion cells the transient e.p.s.p.s at 'on' were not affected, even by 5 mM-PDA. In the remaining four on–off cells the transient e.p.s.p. at 'on' was reduced by up to one-third, but it is not known whether these responses were resistant to APV, since these cells could not be held long enough to test both drugs.

DISCUSSION

Although receptors which respond to QA, KA and NMA appear to be present on all types of ganglion cells, the fact that APV selectively blocked the depolarization evoked by NMA indicates that the receptors for NMA are distinct from those at which QA and KA act. The responses to NMA were affected by the presence of Co^{2+} in a way which is similar to the behaviour of some central neurones, where NMA may appear to cause a decrease in conductance due to voltage-dependent properties of the NMA-gated channels (Dingledine, 1983; MacDonald, Porietis & Wojtowicz, 1982; Mayer & Westbrook, 1984). In cultured mouse neurones, the voltage dependence of the NMA response, which prevents channel opening at normal resting potential, is induced by the presence of Mg^{2+} in the extracellular medium (Nowak, Bregestovski, Ascher, Herbert & Prochiantz, 1984). A similar mechanism might account for the suppression of responses to NMA in the presence of other divalent cations such as Co^{2+}.

The ability of APV to selectively block responses at NMDA receptors allows the demonstration of pharmacologically distinct excitatory synaptic pathways in the inner retina. For example, in all classes of ganglion cells APV consistently blocked the sustained responses which were produced by illumination of the receptive field centre. These include the sustained 'on'-depolarization in on-centre ganglion cells, the sustained 'on'-hyperpolarization in off-centre ganglion cells and the sustained 'on'-components (both depolarizing and hyperpolarizing) of the responses in on–off ganglion cells. These responses are all thought to depend on the activity of depolarizing ('on') bipolar cells, either directly or through amacrine interneurones. The suppression of the sustained hyperpolarizing responses of off-centre and on–off ganglion cells was probably due to blocking the excitatory input from depolarizing bipolar cells to depolarizing sustained amacrine cells, which in turn provide the sustained inhibitory input to the ganglion cells. It is unlikely that APV directly blocked the sustained inhibitory input to ganglion cells since direct application of NMA caused a depolarization in these cells. The concentrations of APV which produced these effects did not reduce the sustained responses of depolarizing bipolar cells, indicating that its site of action must have been post-synaptic to the depolarizing bipolar cells.

In contrast, APV did not reduce any of the sustained ganglion cell responses which are thought to depend on the activity of hyperpolarizing ('off') bipolar cells. Such responses include the tonic excitation of off-centre ganglion cells in darkness and the tonic inhibition of on-centre ganglion cells in darkness (Belgum *et al.* 1982). In the presence of APV the tonic inputs from the 'off'-pathway were active in darkness, but they did not appear to be strongly modulated by light. It is possible that under other conditions these inputs play a more significant role in the generation of sustained 'on' responses in ganglion cells.

Thus, considering only the sustained responses of ganglion cells, the results suggest that the transmitter released by depolarizing bipolar cells acts at NMDA receptors while that released by hyperpolarizing bipolar cells does not. These results do not necessarily imply that the transmitters are different. This interpretation assumes that depolarizing bipolar cells make excitatory synapses directly onto on-centre ganglion

cells and the inhibitory sustained amacrine cells. It is possible, however, that these connexions are via excitatory amacrine interneurones, in which case the transmitter acting at NMDA receptors could be released by either the depolarizing bipolar cell or the amacrine interneurones.

Ikeda & Sheardown (1982*b*) studied action potential discharges of cat ganglion cells and concluded that both types of bipolar cells released aspartate. Their conclusions were based on the finding that aspartate was more effective than glutamate in causing firing of on-centre and off-centre sustained ganglion cells and that the light-evoked discharges of these cells were reduced by APV. However, it seems unlikely that APV blocked any tonic excitatory input to off-centre ganglion cells in darkness since it did not decrease their rate of spontaneous firing in the dark.

Slaughter & Miller (1983*a*, *b*) reported that light-evoked depolarizing responses of mudpuppy amacrine and ganglion cells were not blocked by the relatively weak NMDA antagonists D-α-aminoadipate and D-α-aminosuberate but were reduced by PDA at concentrations which did not reduce the responses of bipolar cells. They concluded that the transmitter released by both types of bipolar cells acted at KA or QA receptors and was therefore probably glutamate. Another report, however, claims that the effect of PDA on off-centre ganglion cells may be entirely accounted for by its action in the outer plexiform layer (Dvorak, 1984). Whether or not the transmitter released by 'off'-bipolar cells is an excitatory amino acid remains to be determined; the results of the present study indicate only that it does not appear to act at NMDA receptors.

The effects of APV on the transient responses of ganglion cells are more difficult to interpret. There are several possible explanations for the finding that APV did not usually reduce the transient responses at 'on', even though it blocked the sustained responses driven by the 'on'-pathway. The apparent APV resistance of the transients at 'on' could be due to an increased amount of transmitter release, sufficient to overcome the antagonism of APV, at the onset of the light response. Unfortunately, it was not possible to test adequately whether the receptors in question could be blocked by increased concentrations of APV, since this also caused a reduction in the responses of depolarizing bipolar cells. Thus it is possible that the transient and sustained e.p.s.p.s at 'on' are mediated by the same receptors.

On the other hand, the transient responses at 'on' may not involve NMDA receptors. If it is assumed that the sustained and transient e.p.s.p.s of ganglion cells at 'on' are both due to monosynaptic excitatory input from bipolar cells, then a given depolarizing bipolar cell could make both sustained and transient synapses at which the post-synaptic receptor types were different, or separate populations of depolarizing bipolar cells could mediate for sustained and transient excitation. In the latter case the transmitters used could also be different.

It is also possible that the transient e.p.s.p.s of on–off ganglion cells are driven by transient (on–off) amacrine cells (Werblin, 1977). If an amacrine interneurone were present, then the above discussion regarding the nature of the receptor sites would also apply to the synapses between bipolar and amacrine cells. The transient excitatory responses seen in some on-centre ganglion cells may also be driven by on–off amacrine cells. The transient i.p.s.p.s of ganglion cells were also resistant to APV, and since they are also thought to be mediated by transient amacrine cells (Werblin,

1977; Frumkes *et al.* 1981; Belgum *et al.* 1984), it is likely that both excitatory and inhibitory transient amacrine cells receive input from depolarizing bipolar cells via the same type of receptor.

A similar pharmacological difference between the transient and sustained excitatory pathways has been reported in the cat retina, where APV reduced the light-evoked firing of sustained ganglion cells but had no effect on the light-evoked firing of transient ganglion cells (Ikeda & Sheardown, 1982*b*).

The identity of the transmitter which mediates excitatory input from transient amacrine to ganglion cells in the mudpuppy is not known. In contrast to the evidence that acetylcholine may mediate this input in the cat (Ikeda & Sheardown, 1982*a*) and rabbit (Masland & Ames, 1976; Ariel & Daw, 1982; Masland, Mills & Cassidy, 1984), it is reported not to do so in the mudpuppy (Frumkes *et al.* 1981). It is possible that there is more than one type of excitatory transient amacrine cell in the mudpuppy since in the relatively few on–off ganglion cells in which APV reduced the transient responses at 'light on' it also reduced the response at 'light off'. The reason for this is not known, but one possibility is that part of the transient excitatory input to these ganglion cells may be from a population of on–off amacrine cells whose transmitter acts at NMDA receptors.

Although the use of pharmacological antagonists can help to characterize the type of excitatory amino acid receptor sites at a given synapse, the results of such studies do not necessarily indicate whether the transmitter is aspartate, glutamate or some other related substance, since both aspartate and glutamate can act at multiple receptor types. Thus, although it is often stated that sensitivity of a synaptic response to NMDA antagonists implicates aspartate as the natural transmitter, recent evidence suggests that such inferences may not be generally valid. For example, at rat brain synapses glutamate appears to have a much higher affinity than aspartate for NMDA receptors (Olverman, Jones & Watkins, 1984). In the present study the finding that APV had little effect on the responses to aspartate and glutamate suggests that, at least when applied in the bathing medium, both of these agonists acted primarily at non-NMDA receptors. Thus, the relative effectiveness of different exogenously applied agonists may not reflect the situation at a given synapse if the substances also have access to other receptors on that cell, either at other synapses or at extrasynaptic locations. For these reasons the present results do not favour aspartate over glutamate or some other closely related substance as the endogenous transmitter whose action is antagonized by APV.

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