Pharmacology of the Skate Electroretinogram Indicates Independent ON and OFF Bipolar Cell Pathways

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ABSTRACT Organization of afferent information into parallel ON and OFF pathways is a critical feature of the vertebrate visual system. All afferent visual information in the vertebrate retina reaches the inner plexiform layer (IPL) via bipolar cells. It is at the bipolar cell level that separation of ON and OFF information first appears for afferent information from cones. This may also hold true for the rod pathway of cold-blooded vertebrates, but not for mammals. The all-rod retina of the skate presents an opportunity to examine such pathways in a retina having but a single class of photoreceptor. Immunocytochemical evidence suggests that both ON and OFF bipolar cells are present in the skate retina. We examined the pharmacology of the skate electroretinogram (ERG) to test the hypothesis that independent ON and OFF bipolar cell pathways are functional as rod afferent pathways from outer to inner plexiform layer in the skate. 100 μM 2-amino-4-phosphonobutyric acid (APB) reversibly blocked the skate ERG b-wave. A small d-wave–like OFF component of the ERG revealed by DC recording of response to a prolonged (10 s) flash of light was reduced or blocked by 5 mM kynurenic acid (KYN). We found that addition of 200 μM picrotoxin to the Ringer's solution revealed prominent ON and OFF components of the skate ERG while reducing the c-wave. These ON and OFF components were reversibly blocked by 100 μM APB and 5 mM KYN, respectively. Reversible block of the OFF component by KYN was also accomplished in the presence of 500 μM *N*-methyl-DL-aspartate. From these findings, we conclude that ON and OFF bipolar cells are likely to be functional as parallel afferent interplexiform pathways in the all-rod retina of the skate.

INTRODUCTION

Bipolar cells are the neurons by which afferent visual information passes from the outer (OPL) to the inner (IPL) plexiform layer of the vertebrate retina. There is now abundant evidence that this information is separated into ON and OFF pathways via corresponding ON (depolarizing in response to central light stimulation) and OFF (center hyperpolarizing) bipolar cells. In duplex retinas (i.e., retinas with the duplicity of having both rod and cone photoreceptors; Schultze, 1866; Pirenne, 1962), understanding the role of these parallel interplexiform pathways is usually complicated by the presence of inputs from four classes of photoreceptors, including one class of rods and three classes of cones having different peak spectral sensitivities that may influence the interpretation of pharmacological results (Famiglietti et al., 1977; Toyoda and Tonosaki, 1978; DeMarco and Powers, 1994; for review see Massey and Redburn, 1987). As a further complication, evidence from mammalian retinas has suggested a model in which the ON and OFF pathways differ in the photopic and scotopic ranges. In the photopic range, the ON and OFF information may be separated at the level of the OPL, while in the scotopic range information is carried via rod ON bipolar cells, and may be separated into ON and OFF components at the level of the IPL (Kolb and Famiglietti, 1974; Famiglietti and Kolb, 1975; McGuire et al., 1984; Dacheux and Raviola, 1986; Daw et al., 1990; Vaney et al., 1991). Accordingly, the distal processes of OFF bipolar cells may not be a part of the rod circuit in mammals (Dolan and Schiller, 1989; Yamashita and Wässle, 1991). In cold-blooded vertebrates, however, evidence has been found for mixed rod/cone input to both ON and OFF bipolar cells

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(Stell, 1967; Scholes, 1975; Stell et al., 1977; Kaneko and Tachibana, 1978). Therefore, a functional interplexiform OFF pathway for rod-generated information must generally be considered but cannot simply be assumed. The question of whether an OFF bipolar cell pathway is both present and functional in an all-rod retina holds special interest, since the preparation could provide the opportunity to examine the role of bipolar cells in ON and OFF pathways without the complication of considering possible chromaticity effects.

It was apparent some years ago that the skate (*Raja erinacea* or *R. oscellata*) may have an all-rod retina (Dowling and Ripps, 1970). Anatomically, this hypothesis was supported by examination of the fine structure of cone-like photoreceptors occasionally encountered in sections from retinas of younger animals and by autoradiography of their [^{3}H]fucose incorporation, both of which were typical of rods (Szamier and Ripps, 1983). Electrophysiologically, concerns regarding the retina's ability to respond to levels of illumination extending into the photopic range (Dowling and Ripps, 1970) and having double-branched flicker-fusion curves (Green and Siegel, 1975) were resolved by studies showing the ability of individual photoreceptors isolated from the skate retina to respond to incremental stimuli over a comparable range (Cornwall et al., 1989). One would like to know, therefore, whether ON and OFF bipolar cells are both present and functional as interplexiform pathways in this all-rod retina.

Fortunately, in examining questions concerning their presence, immunocytochemical markers have become available that relate well to general morphological distinctions between certain bipolar cells of the ON and OFF types, and these relationships have been confirmed by electrophysiological recording in some of them. For example, evidence for serotonin-like immunoreactivity (5-HT-IR) in bipolar cells of the vertebrate retina was first reported from studies in skate (Bruun et al., 1984). Subsequent investigations in the duplex retina of the turtle associated 5-HT-IR with OFF bipolar cells, which hyperpolarize in response to central illumination (Weiler and Schütte, 1985). PKC-like immunoreactivity (PKC-IR), on the other hand, was associated with ON bipolar cells, which depolarize in response to central illumination and have been identified as the rod (ON-depolarizing) bipolar cells of duplex retinas on the basis of their morphology (Negishi et al., 1988; Wood et al., 1988) and electrophysiology (Dacheux and Raviola, 1986; Karschin and Wässle, 1990).

Since the skate has an all-rod retina, it was not surprising when PKC-immunoreactive bipolar cells having a morphology typical of the rod bipolar cells in duplex retinas were reported from studies of its retina (Schlemermeyer and Chappell, 1991); but what of the 5-HT-IR reported by Bruun et al. (1984)? Interestingly, 5-HT-IR bipolar cells in the skate retina were found to have a morphology more typical of OFF bipolar cells of duplex retinas (Schlemermeyer and Chappell, 1991). This suggests the possibility that there might be two functional classes of bipolar cells in the all-rod retina of the skate serving the ON and OFF pathways, respectively.

Although the presence of both ON and OFF ganglion cells in the skate retina is well known (Dowling and Ripps, 1970; Brunken, 1983; Chappell and Glynn, 1993), electrophysiological evidence for independent ON and OFF bipolar cell pathways in skate is lacking. Results of recent pharmacological studies in the salamander retina (Stockton and Slaughter, 1989) indicated an approach that we have used to examine this question in the skate.

In their study of the local electroretinogram (ERG) and potassium-electrode responses in the salamander eyecup preparation, Stockton and Slaughter (1989) associated the salamander b-wave (the vitreal positive ON component) of the ERG with ON-bipolar cell activity and the d-wave (OFF) component with OFF bipolar and/or horizontal cell activity. They did so by using a variety of drugs, including 2-amino-4-phosphonobutyric acid (APB), a glutamate agonist that has been shown to block the ON pathway of the retina and eliminate the b-wave of the ERG (Slaughter and Miller, 1981: Shiells et al., 1981) but not OFF bipolar cell responses (Yang and Wu, 1991), as well as kynurenic acid (KYN), which has been shown to block the OFF pathway and eliminate the d-wave of the ERG (Slaughter and Miller, 1983a) without diminishing ON bipolar cell responses (Coleman et al., 1986). They found that APB application resulted in a loss of the ERG b-wave and the ON potassium flux, whereas KYN application resulted in the selective loss of the ERG d-wave and the OFF potassium flux. *N*-methyl-DL-aspartate (NMA) and glycine, known to suppress responses of third order neurons at the level of the IPL in amphibians, reduced neither the b- or d-waves nor the ON or OFF potassium fluxes. From this evidence, they suggested that APB and KYN were acting distally at the level of the OPL in the salamander retina. Recent studies suggest it is even possible that the b-wave may be generated directly from the ON bipolar cell response (Xu and Karwoski, 1994; Tian and Slaughter, 1995). On the basis of the observations made by Stockton and Slaughter (1989) in salamander, we have used vitreal ERG recording of the superfused skate eyecup preparation to pharmacologically dissect components of its ERG and relate these components to pathways that may be involved.

In this report, we present evidence from pharmacological manipulation of the skate ERG supporting the notion that independent ON and OFF bipolar cell pathways from outer to inner plexiform layers are func-

tional in this all-rod retina. We also introduce the use of picrotoxin (PTX) as a means to reveal prominent ON and OFF components of the skate ERG. Preliminary results from this study have been presented (Chappell and Rosenstein, 1993).

METHODS

Eyecup preparations from the skate (*R. erinacea* or *R. oscellata*) were used for all experiments. The fish were dark-adapted for 1 h and anesthetized in 0.1% 3-aminobenzoic acid ethyl ester (MS 222) and/or pithed prior to excising the eyes. The eyes were removed under dim red light. One eye was sectioned to remove the cornea and lens, drained of vitreous, and used immediately for experimentation. The other eye was stored temporarily under refrigeration for use later the same day with comparable results.

The eyecup was positioned in a superfusion chamber above a Ag/AgCl pellet (WPInstruments, Inc., New Haven, CT) reference electrode inside an electrically shielded enclosure. The recording electrode, consisting of a Ringer's-filled glass capillary held by an electrode holder containing another Ag/AgCl pellet electrode, was lowered into the eyecup. Solutions were oxygenated and adjusted to pH 7.6. Moist oxygen flowed through a capillary tube positioned directly over the preparation. Solutions flowed at a rate of about 1 ml/min from a manifold system used for the delivery of Ringer's and drugs that had a dead time of about 30 s when solutions were switched.

Recording Procedures

The leads from the electrodes were connected to a DC low noise pre-amplifier (model PARC 118; EG&G Instruments, Princeton, NJ) and this in turn to a TM 506 amplifier (Tektronics, Inc., Beaverton, OR) used in the DC mode to provide suitable DC offset. The output was then sent to a chart recorder (model 2200S; Gould, Inc., Cleveland, OH) as well as to a reel-to-reel tape recorder (model 3964A; Hewlett-Packard Company, San Diego, CA), where the data were recorded for later analysis. Analysis was accomplished with pCLAMP software (Axon Instruments, Inc., Foster City, CA) to subtract baseline drift, except for the raw data from chart recorder records shown in Fig. 3.

The eyecup was stimulated by light from a halogen lamp controlled by a voltage-regulated DC power supply (model SP40-10; Deltron, Inc., North Wales, PA) set for 6 amps and having an unattenuated intensity (log I = 0) at the level of the eyecup of about 20 $\mu W/cm^2$. The shutter was controlled by a Master-8 programmable pulse generator (Armon Micro-Processor Instruments, Jerusalem, Israel) and set to flash once a minute for a 10 s duration, except for the APB experiment of Fig. 1, in which an 80 ms flash was presented at 7 s intervals.

Drugs

The control Ringer's solution used consisted of 250 mM NaCl, 6 mM KCl, 20 mM $NaHCO_3$, 1 mM $MgCl_2$, 4 mM $CaCl_2$, 0.23 mM NaH_2PO_4, 360 mM urea, 10 mM glucose, and 5 mM HEPES buffer, adjusted to pH 7.6 with NaOH. The drugs or combinations of drugs used were dissolved directly in this Ringer's at the following concentrations: APB, 100 μM; KYN, 5 mM; NMA, 500 μM; PTX, 200 μM. Chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

RESULTS

The effect of APB on the skate retina was consistent with previous findings in duplex retinas of other vertebrates (cf. Slaughter and Miller, 1981; Massey et al., 1983; Knapp and Schiller, 1984; Porciatti et al., 1987). As shown in Fig. 1, the b-wave of the ERG in response to a brief (80 ms) flash was reversibly blocked by application of Ringer's solution containing 100 μM APB. 15 min after APB application (*middle trace*), the b-wave (the large upward, corneal positive peak) of the ERG was virtually eliminated. Essentially full recovery was observed after washing for 45 min in normal Ringer's (*bottom trace*).

The ERG of the skate is not known to have the corneal positive OFF or d-wave component, which is generally associated with the cone-driven photopic response of duplex retinas and not rod retinas (Granit, 1962; Dowling, 1987; Daw et al., 1990). We found, however, that by using DC coupling to record the ERG combined with a prolonged (10 s) light flash, a small d-wave-like OFF component of the ERG could often be observed (Fig. 2, *arrow*). A similar "positive-going off effect" was observed by Miller and Dowling (1970) both in the skate ERG and in intracellular Müller cell recordings. This component will be referred to here as the OFF component of the skate ERG response. This OFF component was reduced or eliminated after 5 min in Ringer's containing 5 mM KYN (Fig. 2, *lower trace*).

These results are consistent with Stockton and Slaughter's (1989) conclusion that the OFF component of the ERG they recorded is an expression of OFF bipolar and/or horizontal cell depolarization at the termination of illumination, since it is reduced by KYN, which selectively blocks the OFF channel at the level of the

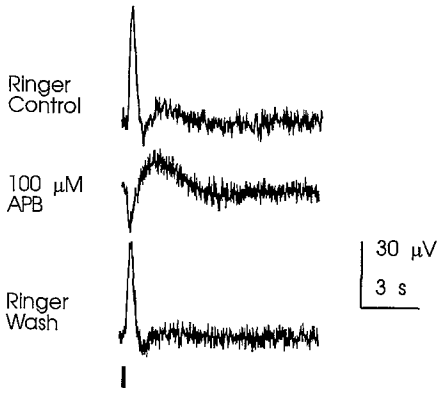

FIGURE 1. APB reversibly blocks the ERG b-wave. The b-wave of the ERG elicited by an 80 ms flash (*vertical bar, lower left*) presented at 7 s intervals is shown in the upper trace. After a 15 min superfusion of 100 μM APB, the b-wave was abolished (*middle trace*). The b-wave recovered almost completely 45 min after return to normal Ringer's, as shown in the lower trace. (Flash intensity: log I = −2.)

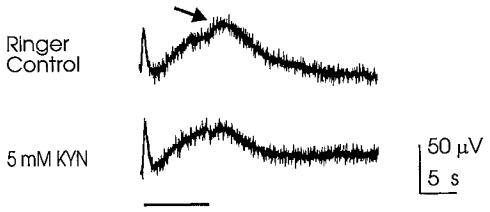

FIGURE 2. KYN reduces the OFF component observed in response to longer flashes. A small OFF-transient (*arrow, upper trace*) was revealed by DC recording of ERG responses to prolonged flashes. This component was selectively blocked after 5 min in 5 mM KYN (*lower trace*). Horizontal bar (*lower left*) indicates light ON (log I = −4).

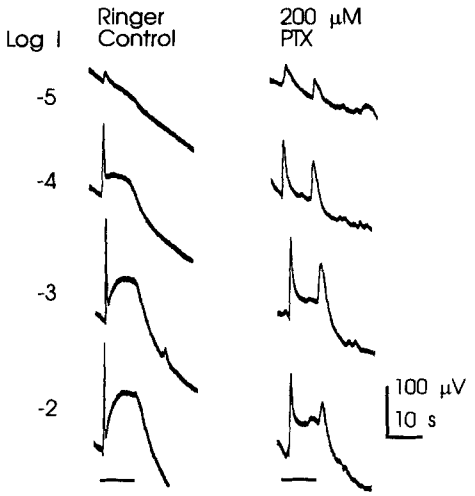

FIGURE 3. PTX application reveals a substantial OFF component of the skate ERG. Within 10 min of the application of 200 μM PTX, a substantial OFF component of the ERG was revealed over a range of 4 log units above threshold. The chart recorder records presented here have not been corrected for amplifier drift observed in the DC-recorded ERG. (*Bar*, light ON).

outer plexiform layer (Coleman et al., 1986). Since KYN is known to affect both OFF bipolar and horizontal cells (Coleman et al., 1986), however, they could not be certain which of these two cell types was the major contributor to the OFF component of the ERG in salamander.

Recent studies of γ-aminobutyric acid (GABA) sensitivity of isolated horizontal and bipolar cells by use of patch recording techniques have yielded two observations that are useful in considering this question in the skate. First, there is a difference between the GABA sensitivity of skate horizontal cells and that of its bipolar cells. The horizontal cells seem to respond via an electrogenic uptake mechanism that is not sensitive to GABA channel agonists or blockers (Malchow and Ripps, 1990; Malchow et al., 1990). Skate bipolar cells, on the other hand, respond via a ligand-gated conductance that is substantially blocked by PTX (Malchow et al., 1991). Second, the distal dendrites of the bipolar cells that extend into the outer plexiform layer region of the retina show substantial GABA sensitivity relative to the bipolar cell axon or its terminals that descend into the inner plexiform layer (Chappell et al., 1992). With this in mind, we decided to see whether the OFF component of the skate ERG and the action of KYN on this component might be affected by PTX application. If they were, it might suggest that bipolar cells played a predominant role in shaping that component of the response. If not, horizontal cells would be the more likely candidate. We found that both the amplitude of the OFF response and the amount by which it was reduced by KYN were greatly increased in the presence of PTX.

As shown in Fig. 3, the ERG recorded from the skate eyecup was changed dramatically by the application of 200 μM PTX. Under these conditions, a substantial OFF component comparable in amplitude and polarity to the ERG b-wave was revealed. A similar enhancement of the ON and OFF components of the intraretinally recorded L-ERG of the frog by 500 μM PTX has recently been reported by Xu and Karwoski (1994). In the skate, this effect was made even more obvious by a concomitant reduction in the corneal-positive ERG c-wave (PI component of Granit, 1933), a contribution to the ERG believed to arise primarily from glial cells that make up the pigment epithelium (Noell, 1954; Dowling and Ripps, 1970; Steinberg et al., 1970; Oakley et al., 1977). The two peaks of the ON (b-wave-like) and OFF (d-wave-like) components of the ERG in the PTX-treated eyecup preparation were especially pronounced over a range of intensities 1 or 2 log units above threshold (i.e., log I = −4 or −3 in Fig. 3). We therefore examined the pharmacology of these components of the ERG in the PTX-treated retina at these intensities.

Like the b-wave of the ERG in the untreated retina, the ON component of the ERG of the PTX-treated preparation was reversibly blocked by the application of 200 μM APB. Fig. 4 shows the ON and OFF components of the ERG control response obtained 15 min after application of Ringer's containing 200 μM PTX (Fig. 4, *upper trace*). The ON component of the response was blocked within 3 min of the addition of 100 μM APB to the control solution (Fig. 4, *middle trace*). After remaining over 30 min in APB, the ON component showed substantial recovery within 10 min of return to the control solution containing only the 200 μM PTX (Fig. 4, *lower trace*).

The OFF component that survived APB application (Fig. 4, *middle trace*), however, could be reversibly blocked by the application of 5 mM KYN. This is demonstrated in Fig. 5, where a response obtained 10 min

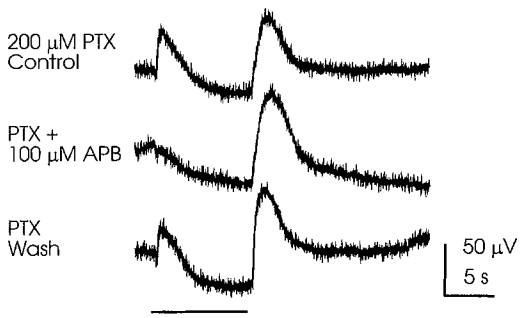

FIGURE 4. APB reversibly blocks the ON component of the PTX-treated skate retinal ERG. 15 min of superfusion with 200 μM PTX revealed pronounced peaks at light ON and light OFF in the ERG responses (*upper trace*). Addition of 100 μM APB for 3 min selectively blocked the ON component of this response while the OFF component was even slightly enhanced (*middle trace*). The ON component showed substantial recovery 10 min after APB had been removed from the superfusate (*lower trace*). (Log I = −3.)

after application of a control solution of Ringer's containing 200 μM PTX plus 100 μM APB to another retina is shown in the upper trace, where a prominent OFF component in the absence of the APB-blocked ON component is observed. Within 15 min of the addition of 5 mM KYN to this PTX + APB control solution, the OFF component was essentially eliminated (Fig. 5, *middle trace*). The OFF component showed substantial recovery 15 min after return to the PTX + APB control solution (Fig. 5, *lower trace*).

While KYN is a glutamate antagonist that has been shown to suppress synaptic transmission from photoreceptors to horizontal and OFF bipolar cells without diminishing the response of ON bipolar cells, it is also known to reduce transmission between bipolar cells and third order neurons (Slaughter and Miller, 1983b; Coleman et al., 1986). Consequently, the results obtained could have been due to blockage of a response of neurons postsynaptic to the OFF bipolar cell at the level of the IPL. For example, amacrine cells are thought to contribute to the skate proximal negative response (PNR), which shows distinct ON and OFF transients under light-adapted conditions (Dowling and Ripps, 1977). NMA, however, strongly suppresses the light responses of third-order neurons in amphibians through a depolarizing block (Slaughter and Miller, 1983c) and might be used as one test of this hypothesis. Miller and Slaughter (1986) caution, however, that this generalization developed in the mudpuppy retina may not be valid in the fish, where the action of NMA on third-order neurons has not been established, and studies in carp retina (Wu and Dowling, 1978) have shown block of the horizontal cell light response by the NMA antagonist α-aminoadipic acid. Indeed, it is known that during application of 50 mM α-aminoadipic acid, the b-wave of the skate ERG was greatly suppressed, and it was not possible to demonstrate electrical activity of its horizontal cells (Szamier et al., 1981). These effects in skate were concomitant with severe damage to its Müller cells, although full recovery of the b-wave was observed following return to Ringer's, even after 1 h in the acid. With this in mind, effects of NMA on the skate responses would be of considerable interest.

We found that the ON and OFF components of the ERG recorded after 15 min in 200 μM PTX (Fig. 6, *upper trace*) were not reduced after 15 min in 500 μM NMA (Fig. 6, *second trace*). Nevertheless, application of 5 mM KYN for 15 min selectively blocked the OFF component (Fig. 6, *third trace*), with recovery of the OFF component in the presence of NMA observed some 50

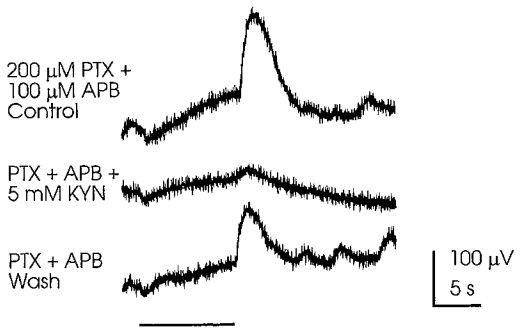

FIGURE 5. KYN reversibly blocks the PTX/APB-isolated OFF component of the skate ERG. After a 10 min application of 200 μM PTX plus 100 mM APB, a prominent OFF component of the ERG remained (*upper trace*). Addition of 5 mM KYN to the superfusate for 15 min blocked this OFF component (*middle trace*). Following a 15 min wash in the original PTX/APB solution, the OFF component showed substantial recovery. (Log I = −3.)

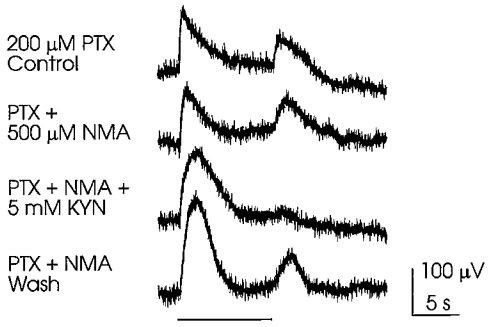

FIGURE 6. The PTX-isolated ON and OFF components of the ERG are not blocked by NMA even though reversible block or the OFF component by KYN can be demonstrated. The ON and OFF components of the skate ERG revealed after 15 min in 200 μM PTX (*upper trace*) are not blocked after an additional 15 min in 500 μM NMA (*second trace*). Even so, addition of 5 mM KYN for 15 min selectively blocked the OFF component (*third trace*), for which substantial recovery is shown after an additional 50 min in the PTX/NMA superfusate following KYN removal (*lower trace*). (Log I = −3.)

TABLE I

Action and Reversibility of Pharmacological Agents Tested

Drug applied	Concentration (mM)	Effect on ERG		Times observed	
		ON component	OFF component	Effect	Reversal
PTX	0.2 to 2	increase (small)	increase	27	7
APB	0.1 to 0.5	blocked	not blocked	22	9
KYN	1 to 5	not blocked	blocked	16	13
NMA	0.5	not blocked	not blocked	5	—

PTX, picrotoxin; *APB*, 2-amino-4-phosphonobutyric acid; *KYN*, kynurenic acid; *NMA*, *N*-methyl-DL-aspartate.

min after KYN removal (Fig. 6, *bottom trace*), thereby demonstrating that the OFF component could be selectively altered under these conditions even though NMA application showed no effect.

Similar results to those presented here were obtained by repeating the experiments on other preparations and/or as a part of experiments where other agents were being investigated. For example, the action of PTX to reveal ON and OFF components is discussed here with Fig. 3 but confirmed by the data presented in Figs. 4 and 6, where the response of the PTX-treated retina is shown as the original, control, response. Similarly, the ability to selectively block the ON component with APB is discussed with Fig. 4 and confirmed by the control response of Fig. 5. Using this approach, we have repeatedly been able to confirm the action and/or reversibility of all the pharmacological agents used as tabulated in Table I.

DISCUSSION

The pharmacology of the skate ERG observed in this series of experiments is consistent with that observed by Stockton and Slaughter (1989) in their studies of the salamander retina, from which they concluded that APB and KYN act at the level of the OPL to selectively block the ON and OFF pathways, respectively, from the outer to the inner retina. In addition to confirming the anticipated role for rod ON bipolar cells in the ON pathway of the skate's all-rod retina, our results provide electrophysiological evidence suggesting the presence of a functional afferent interplexiform OFF pathway. This OFF pathway presumably operates via the 5-HT-IR bipolar cells, which possess a morphology that is characteristic of OFF bipolar cells. Our interpretation of the experimental manipulations and results obtained is summarized in the schematic diagram presented as Fig. 7 and discussed below.

The block of the ON pathway by APB (Slaughter and Miller, 1981; Shiells et al., 1981) has become an important tool in retinal research and has been used to relate the ERG b-wave to ON bipolar cell activity in a variety of species (Massey et al., 1983; Porciatti et al., 1987; Müller et al., 1988; Smith et al., 1989; Dolan and Schiller, 1989; Stockton and Slaughter, 1989). Therefore, we were not surprised to observe (Fig. 1) that APB blocked the b-wave of the ERG of the all-rod skate retina, since many of the bipolar cells have PKC-IR and a morphology typical of rod ON bipolar cells in duplex retinas (Schlemermeyer and Chappell, 1991). These results are consistent with the findings of Kline et al. (1978, 1985), showing an increase in external potas-

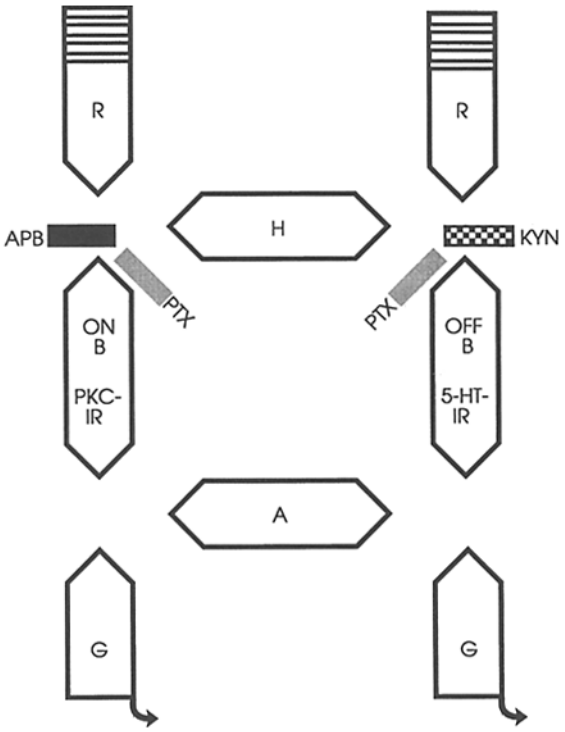

FIGURE 7. Summary diagram of possible relationships suggested from results of pharmacological manipulations of the skate ERG. A presumed ON bipolar cell (*ON B*) as identified by PKC-IR and a presumed OFF bipolar cell (*OFF B*) as identified by 5-HT-IR provide the respective ON and OFF afferent pathways from the outer (*OPL*) to the inner (*IPL*) plexiform layer. PTX (*stippled bars*) blocks the distal response to GABA at both ON and OFF bipolar cells, whereas APB (*dark bar*) selectively blocks the ON bipolar cells, and KYN (*checkered bar*) selectively blocks the OFF bipolar pathway at the level of the OPL. *R*, receptor; *H*, horizontal cell; *B*, bipolar cell; *A*, amacrine cell; *G*, ganglion cell. See text for details.

sium in the distal retina of the skate at light ON, and support their suggestion that this distal increase plays a prominent role in generation of the transient component of the b-wave, which is likely to represent bipolar cell activity.

Although 5-HT-IR bipolar cells having morphological characteristics of duplex retinal OFF bipolar cells had been reported in skate (Schlemermeyer and Chappell, 1991), the identification of an OFF component of the skate ERG was not at first obvious. Even going to the extremes of a DC-recorded ERG obtained from the skate retina in response to a prolonged flash, only a small positive OFF transient could be observed (Fig. 2, *upper trace*). The selective reduction of this component of the response by KYN (Fig. 2, *lower trace*), while consistent with the observations of Stockton and Slaughter (1989), was too subtle to justify extensive pharmacological studies of the effect on the Ringer's control ERG. Therefore, we pursued a possibility suggested from observations of the differential effects of PTX on skate isolated horizontal and bipolar cells. While the GABA response of skate bipolar cells is substantially reduced by PTX (Malchow et al., 1991), that of its horizontal cells is not affected by PTX, since their GABA response is due to an electrogenic uptake mechanism (Malchow and Ripps, 1990; Malchow et al., 1991). On this basis, we reasoned that it was worth looking to see whether PTX had an effect on the OFF component of the ERG, as this would provide evidence suggesting the involvement of bipolar cells.

The results of the application of 200 μM PTX were quite dramatic (Fig. 3). The OFF component of the ERG was significantly enhanced, possibly by PTX blockage of GABA action on OFF bipolar cells. Similarly, the b-wave ON component was more prolonged, and, unexpectedly, the corneal-positive c-wave appeared to be reduced. In addition to suggesting a role for bipolar cells in generating the OFF component of the skate ERG, since the horizontal cell GABA response is not affected by PTX, it provided us with conditions under which the relationships of ON and OFF components of the ERG response could be clearly examined. This is represented as a PTX block of GABA action on both ON and OFF bipolar cells (*stippled bars*) in the diagram of Fig. 7.

We should point out that although it not a subject of this investigation, the reduction of the corneal-positive c-wave response could be due simply to an effect on the pigment epithelial response by a distal K^+ increase of the type reported by Dick and Miller (1978) when ethanol and GABA were applied simultaneously to the frog retina. The possibility that Müller cells may contribute a corneal-positive component to the c-wave (Zeumer et al., 1994) should also be considered for skate, since its Müller cells have been shown to have a PTX-sensitive GABA response (Malchow et al., 1990; Qian et al., 1994).

Investigation of the ON and OFF components of the skate ERG became more straightforward once the PTX-isolated ON and OFF components of the ERG were available. The ON component of the ERG was selectively, and reversibly, blocked by APB (Fig. 4). KYN reversibly blocked the remaining ERG OFF component (Fig. 5). These findings are represented schematically in Fig. 7 by the APB and KYN block of the ON and OFF bipolar responses (*dark bar* and *checkered bar*, respectively).

Whereas these results are consistent with the findings of Stockton and Slaughter (1989) in the salamander, it should be noted that Katz et al. (1991) reported enhanced ON and OFF components of the PTX-treated ERG of the toad, *Bufo marinus*, which they related directly to an enhanced M-wave (Karwoski and Proenza, 1977) component of the ERG. They postulated that PTX acted by abolishing inhibition mediated by GABAergic amacrine cells, as noted by an enhanced magnitude of the proximal (IPL) K^+ increase. They tested this hypothesis by showing that 500 μM NMA reversed the PTX effects entirely. Assuming that NMA was acting at the level of the IPL in toad as it did in the salamander (Slaughter and Miller, 1983c), Katz et al. (1991) concluded that the PTX effects were generated at the level of the IPL. Furthermore, although Kline et al. (1985) noticed a transient increase in external potassium distally as well as proximally in the skate retina at light OFF, they suggested that the distal change was likely to be mediated by potassium effluxes of the proximal retina that diffuse to the distal recording site, rather than representing activity at the level of the distal retina.

To examine this possibility, we performed a test similar to that from which Katz et al. (1991) concluded that the PTX-enhanced OFF component might be generated proximally in the toad retina: we applied NMA to the PTX-treated skate retina to see how the OFF response was affected. Similar results would suggest that a proximal process might well be involved, although the effect of NMA on third-order neurons, established in salamander (Slaughter and Miller, 1983c), has not been examined in toad or skate. We found, however, that not only did the OFF component survive application of 500 μM NMA, but also, this OFF component could still be reversibly blocked by application of 5 mM KYN in the presence of the PTX and NMA (Fig. 6). This suggests that the mechanism involved is different from that associated with a proximally generated M-wave in the toad ERG (Katz et al., 1991) and supports the view that the KYN blockage of the skate OFF component may reflect its action on the OFF pathway via the OFF bipolar cell at the level of the OPL. This

raises the possibility that the distal increase in extracellular potassium at light OFF reported by Kline et al. (1985) may result from OFF bipolar cell activity at that level.

These observations in skate are consistent with the findings of Stockton and Slaughter (1989) from their observations in the salamander in which NMA did not reduce the OFF component of the ERG, as well as the recent findings by Seiving et al. (1994) from similar studies of the primate ERG in which they revealed a robust KYN-sensitive ERG component. Both of these studies associated the KYN-sensitive component with the OFF pathway at the level of the OPL. Like Seiving et al. (1994), we noticed a tendency for either the OFF (Fig. 4) or the ON (Fig. 6) component of the ERG to be enhanced when the other was blocked, consistent with their push–pull model relating ON and OFF bipolar contributions to the ERG. The enhancement we observed, however, was subtle by comparison with their observations and not sustained for the entire 10 s duration of the prolonged flash we used.

Our data support the notion that there are independent ON and OFF pathways from outer to inner retina via bipolar cells in the skate retina. The selective block of the ON and OFF components of the ERG by APB and KYN, respectively, suggests that there is indeed a functional role for a PKC-IR ON bipolar cell as well as a 5-HT-IR OFF bipolar cell, as presented schematically in the diagram of Fig. 7.

In summary, from these observations we conclude that parallel ON and OFF pathways via bipolar cells are functional as interplexiform afferent pathways in the all-rod retina of the skate. It is therefore reasonable to presume that the ON bipolar pathway in skate is via an identifiable class of PKC-IR bipolar cell similar to those identified in the duplex retinas of other vertebrates. The OFF pathway appears to be via an identifiable class of 5-HT-IR OFF bipolar cell in the skate retina, and it is the OFF bipolar cells, rather than horizontal cells, that are the most likely source of the OFF (d-wave-like) component of the skate ERG.

We note also that the amplitude of the ON and OFF components of the ERG were both increased by PTX application. We suggest that this may be the result of a release from inhibition at the distal dendrites of ON and OFF bipolar cells, respectively. Since both of these components are increased, it suggests further that GABA may act to open channels that tend to "voltage clamp" both ON and OFF bipolar cells nearer the chloride Nernst potential, and by blocking this inhibitory action with PTX, these components are enhanced.

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