# Identification and Functional Characterization of a Dual GABA/ Taurine Transporter in the Bullfrog Retinal Pigment Epithelium

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ABSTRACT  Intracellular microelectrodes, fluorescence imaging, and radiotracer flux techniques were used to investigate the physiological response of the retinal pigment epithelium (RPE) to the major retinal inhibitory neurotransmitter, $\gamma$-aminobutyric acid (GABA). GABA is released tonically in the dark by amphibian horizontal cells, but is not taken up by the nearby Müller cells. Addition of GABA to the apical bath produced voltage responses in the bullfrog RPE that were not blocked nor mimicked by any of the major GABA-receptor antagonists or agonists. Nipecotic acid, a substrate for GABA transport, inhibited the voltage effects of GABA. GABA and nipecotic acid also inhibited the voltage effects of taurine, suggesting that the previously characterized $\beta$-alanine sensitive taurine carrier also takes up GABA. The voltage responses of GABA, taurine, nipecotic acid, and $\beta$-alanine all showed first-order saturable kinetics with the following $K_m$'s: GABA ($K_m = 160$ $\mu$M), $\beta$-alanine ($K_m = 250$ $\mu$M), nipecotic acid ($K_m = 420$ $\mu$M), and taurine ($K_m = 850$ $\mu$M). This low affinity GABA transporter is dependent on external Na, partially dependent on external Cl, and is stimulated in low $[K]_o$, which approximates subretinal space $[K]_o$ during light onset. Apical GABA also produced a significant conductance increase at the basolateral membrane. These GABA-induced conductance changes were blocked by basal $Ba^{2+}$, suggesting that GABA decreased basolateral membrane K conductance. In addition, the apical membrane Na/K ATPase was stimulated in the presence of GABA. A model for the interaction between the GABA transporter, the Na/K ATPase, and the basolateral membrane K conductance accounts for the electrical effects of GABA. Net apical-to-basal flux of [$^3$H]-GABA was also observed in radioactive flux experiments. The present study shows that a high capacity GABA uptake mechanism with unique pharmacological properties is located at the RPE apical membrane and could play an important role in the removal of GABA from the subretinal space (SRS). This transporter could also coordinate the activities of GABA and taurine in the SRS after transitions between light and dark.

INTRODUCTION

In the posterior part of the vertebrate eye the retinal pigment epithelium (RPE) forms a polarized, multifunctional sheet of cells that separates the neural retina from the choriocapillaris. The apical (retinal facing) membrane of this epithelium contains a number of transport mechanisms that regulate the ionic, metabolic, and hydration state of the subretinal space (SRS), an extracellular volume defined by the RPE, the photoreceptors and the distal portion of the Müller cells (Oakley and Green, 1976; Bok, 1985; Steinberg, 1985; Linsenmeier, 1986; Borgula, Karwoski, and Steinberg, 1989; Adorante and Miller, 1990; Miller and Edelman, 1990; Uehara, Matthes, Yasumura, and LaVail, 1990; Lin and Miller, 1991). The RPE serves a number of important glial functions: (a) it has the ability to form an elaborate, protective ensheathment of the photoreceptor outer segments (Steinberg and Wood, 1974); (b) it buffers changes in $[K]_o$ during retinal activity (Immel and Steinberg, 1986; Bialek and Miller, 1994); (c) it secretes the Na necessary to maintain the photoreceptor dark current (Miller, Steinberg, and Oakley, 1978); (d) it transports lactate out of the SRS during retinal glycolysis and mediates the net transport of essential metabolites such as retinoids (vitamin A) and taurine between the SRS and the blood supply (Kenyon, Yu, la Cour, and Miller, 1994; Bauman, 1970; Miller and Steinberg, 1979).

In the present study we show that the RPE has another glial-like function, which is to control the subretinal space concentration of a major inhibitory neurotransmitter, γ-aminobutyric acid (GABA). Electrophysiological, radiotracer, and fluorescent imaging techniques were used to demonstrate the presence of an electrogenic GABA transporter at the apical membrane. GABA is taken up and released by horizontal and amacrine cells (Marshall and Voaden, 1974; Yang and Yazulla, 1988; O'Malley, Sandell, and Masland, 1992; Barnstable, 1993). Carriers for various neurotransmitters and amino acids include those of GABA, glutamate, glycine, serotonin, dopamine, taurine, and choline (Amara and Arriza, 1993; Lester, Mager, Quick, and Corey, 1994). In addition to terminating neurotransmission, these neuronal and glial uptake systems are proposed to control extracellular levels of the neurotransmitter, shape the kinetic response in the postsynaptic cell to the neurotransmitter, and release neurotransmitter by reversing the transporter from its uptake mode (Henn and Hamberger, 1971; Iversen, 1971; Schwartz, 1982; Szatkowski, Barbour, and Attwell, 1990; Isaacson, Solis, and Nicoll, 1993; Mennerick and Zorumski, 1994). In various systems, the GABA transporter is directly coupled to Na and Cl transport; it has a putative stoichiometry of Na:Cl:GABA of 2:1:1, and transfers net positive charge inward with the uptake of each GABA molecule (Kanner and Schuldiner, 1987; Mager, Naeve, Quick, Labarca, Davidson, and Lester, 1993).

Autoradiographic studies in the frog retina demonstrate a Na-dependent accumulation of GABA in horizontal and amacrine cells with an apparent $K_m$ of 25 μM (Voaden, Marshall, and Murani, 1974). Because amphibian Müller cells do not take up GABA (Neal, Cunningham, and Marshall, 1979), diffusion of this neurotransmitter from the horizontal cell layer could lead to an accumulation in the SRS. The present experiments identify a novel GABA transporter at the RPE apical membrane and suggest that the RPE plays a role in clearing excess GABA from the subretinal space.

METHODS

## Preparation

Medium-sized bullfrogs, *Rana Catesbeiana*, were obtained from Western Scientific (Sacramento, CA) and kept on a 12 h light/dark cycle for 1–6 wk before using. The dark-adapted RPE-choroid was separated from the retina and sclera, placed on a supporting mesh, and mounted apical side up between two halves of a modified Ussing chamber, which allowed for separate perfusion of apical and basolateral membranes. The exposed surface area of the apical membrane was 0.07 cm². The techniques for handling this tissue have been previously reported (Miller and Steinberg, 1977).

## Solutions

Control Ringer's contain the following (in millimolar): 82.5 NaCl, 2.0 KCl, 27.5 NaHCO₃, 1.0 MgCl₂, 1.8 CaCl₂, and 10.0 glucose. This solution was bubbled continuously with 95% O₂/5% CO₂, and had a pH of 7.4. Na-free solutions were made by replacing all NaCl with NMDG (*N*-methyl-D-glucamine) titrated with HCL, and replacing NaHCO₃ with NMDG bubbled to pH 7.4 with 100% CO₂, and then maintained at pH 7.4 with 95% O₂/5% CO₂. Cl-free solutions were made by replacing NaCl with either Na-cyclamate, Na-gluconate, or Na-methane sulfonate and all other Cl salts with gluconate salts. HCO₃-free and Cl-free solutions were made by replacing all NaCl and 20.0 mM NaHCO₃ with Na-cyclamate, and replacing the remaining 7.5 mM NaHCO₃ with 4.3 mM Na-HEPES and 5.7 mM HEPES (titrated to pH 7.4). These HEPES-containing solutions were bubbled with 100% O₂. Stock solutions of GABA, taurine, nipecotic acid and β-alanine were added to the apical perfusate to achieve the desired concentrations. Solution changes at the apical bath that involved an increase of more than 2 mosm were compensated with mannitol.

GABA, taurine, β-alanine, muscimol, phenobarbital, picrotoxin, bicuculline, BaCl₂, DIDS (4,4'-diisothiocyanostilbene-2,2'-disulfonic acid), bumetanide, ouabain, HEPES, and NMDG were purchased from Sigma Chemical Co. (St. Louis, MO). (+/−)-Nipecotic acid, (+/−)-baclofen and phaclofen were purchased from Research Biochemical International (Natick, MA).

## Electrophysiology

The recording set-up and perfusion system have been described previously (Miller and Steinberg, 1977; Joseph and Miller, 1991). Calomel electrodes in series with Ringer solution-agar bridges were used to measure the transepithelial potential (TEP) and the signals from intracellular microelectrodes were referenced to either the apical or basal bath to measure the membrane potentials, $V_A$ and $V_B$, where TEP $= V_B - V_A$. Conventional microelectrodes were made from fibre filled borosilicate glass tubing with 0.5 mm inner diameter and 1 mm outer diameter (Sutter Instrument Co., Novato, CA) and were back-filled with 150 mM KCl, and had resistances of 80–250 M$\Omega$.

The transepithelial (total) resistance, $R_t$, and the ratio of the apical to basolateral membrane resistance, $a$, were obtained by passing 2 μA current pulses across the tissue and measuring the resultant change in TEP and membrane potentials. Current pulses were bipolar, with a period of 3 s applied at various time intervals. $R_t$ is the resulting change in TEP divided by 2 μA, and $a$ is the absolute value of the ratio of voltage change in $V_A$ divided by the change in $V_B$ ($a = |\Delta V_A/\Delta V_B|$). The current-induced voltage deflections were digitally subtracted from the records for clarity.

## Equivalent Circuit

The electrical properties of the RPE can be modelled as an equivalent circuit shown in Fig. 1. The apical and basolateral membranes of the RPE are each represented as an equivalent electromotive force (EMF), $E_A$ or $E_B$, in series with a resistor, $R_A$ or $R_B$, respectively. The paracellular pathway is represented as a shunt resistor, $R_s$, which is the parallel combination of the paracellular resis-

tances between neighboring cells and the resistance of the seal around the tissue. Because of this shunt resistance and the differences between the membrane EMFs, a current loop, $I_s$, flows around the circuit. The observed membrane potentials, $V_A$ and $V_B$, are as follows:

$$V_A = E_A - I_S \cdot R_A \tag{1}$$

$$V_B = E_B + I_S \cdot R_B. \tag{2}$$

The effect of the loop current is to depolarize the apical membrane and hyperpolarize the basolateral membrane (Miller and Steinberg, 1977).

The transepithelial resistance, $R_t$ and $a$ are expressed in terms of the membrane and shunt resistances as follows:

$$R_t = \frac{R_s(R_A + R_B)}{R_A + R_B + R_s} \qquad a = \frac{R_A}{R_B} \tag{3}$$

The apical and basolateral membrane voltages are electrically coupled via $R_s$ so that any voltage change at one membrane will be shunted to the opposite membrane. For example, a membrane
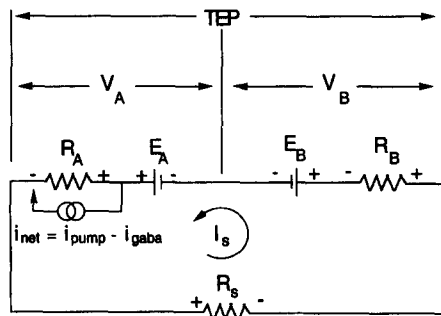


FIGURE 1.  Equivalent circuit for the retinal pigment epithelium. Apical and basolateral membranes are each represented by its resistance, $R_A$ or $R_B$, respectively, in series with its battery, $E_A$ or $E_B$. Shunt resistance, $R_s$, represents the parallel combination of the junctional complex resistance and the seal resistance around the tissue. A loop current, $I_s$, flows through the circuit due to the difference between $E_A$ and $E_B$. The net current, $I_{net}$, with the indicated polarity across the apical membrane is the difference between the current generated by the Na/K ATPase, $I_{pump}$, and the GABA transporter, $I_{gaba}$. The measured apical and basolateral membrane voltages are represented as $V_A$ and $V_B$, respectively. The potential across the tissue is called the transepithelial potential, TEP, and represents the difference between $V_A$ and $V_B$.

voltage change originating at the apical membrane, $\Delta V_A$, will produce a smaller voltage change during the same period at the basolateral membrane, $\Delta V_B$. If $\Delta V_A$ occurs without any changes in membrane or shunt resistances, then $\Delta V_B$ can be represented in terms of $\Delta V_A$ as (Oakley, Miller, and Steinberg, 1978; Hughes, Miller, Joseph, and Edelman, 1988):

$$\Delta V_B = \frac{R_B}{R_B + R_s} \cdot \Delta V_A \tag{4}$$

The measured parameters $R_t$, $a$, $\Delta V_A$, and $\Delta V_B$ in Eqs. 3 and 4 can be used to determine $R_A$, $R_B$, and $R_s$.

The addition of GABA to Ringer bathing the apical bath immediately depolarized $V_A$ and $V_B$ by a few millivolts during the first 10–15 s after GABA reached the apical membrane. No measurable changes in either $R_t$ or $a$ were observed during this period. We measured the GABA-induced changes in membrane potentials, $\Delta V_A$ and $\Delta V_B$, during these initial 10–15 s. We also determined $a$ and $R_t$ before the addition of GABA. These measurements then allowed us to determine the membrane and shunt resistances before the electrical effects of GABA (Eqs. 3 and 4). Since GABA is assumed to activate an electrogenic carrier that can be represented as a current generator, we can

calculate the GABA-elicited transporter current, $I_{gaba}$, from the following equation (Miller et al., 1978):

$$I_{gaba} = \Delta V_A \cdot \frac{R_A + R_B + R_s}{R_A (R_B + R_s)} .$$   (5)

### Dose Response

Because changes in TEP as low as 50 μV can be detected, the TEP is a very sensitive measure of the GABA-induced voltage responses (see Figs. 2 and 12). Because of its sensitivity the initial rate change of TEP, $(d\text{TEP}/dt)_{init}$, was used to characterize the dose dependence of the RPE to successively higher concentrations of GABA. Immediately after the GABA-induced changes in TEP, the initial TEP slopes were determined using a least squares linear regression fit to groups of 30 points that were sequentially sampled every 250 ms. $(d\text{TEP}/dt)_{init}$ represents the largest initial TEP slope and had $r^2$ values ranging from 0.92 to 0.998, demonstrating good linear fits. The maximum TEP slope generally occurred 5–10 s after the TEP began to change. This delay in the GABA-induced TEP response is predicted by the diffusion equation (Crank, 1956) for the movement of GABA molecules across the unstirred layer (200 μm), which is known to separate the bulk solution and the RPE apical membrane (Miller et al., 1978). Solution flow rates were kept constant throughout the course of the dose-response experiments to ensure that $(d\text{TEP}/dt)_{init}$ was not a function of different flow rates. We found $(d\text{TEP}/dt)_{init}$ to be a highly repeatable parameter. For example, addition of four or more consecutive GABA pulses to Ringer perfusing the apical membrane produced almost identical $(d\text{TEP}/dt)_{init}$ responses (no individual $(d\text{TEP}/dt)_{init}$ response deviated from the mean by more than 4%).

The apparent affinity, or $K_m$, of the GABA-mediated TEP responses showed first-order saturation kinetics and was determined by plotting $(d\text{TEP}/dt)_{init}$ as a function of GABA concentration and fitting the points to a simple Michaelis-Menten equation using nonlinear regression analysis (Marquardt-Levenberg algorithm, Sigmaplot). The same protocol and analysis were used for determining the apparent $K_m$'s for taurine, nipecotic acid and β-alanine.

### Radioactive Flux

Transepithelial unidirectional fluxes of GABA were made in paired tissues from the same eye mounted in Lucite chambers. The apical and basolateral membranes of each tissue were bathed in a 1.8 ml vol of Ringer whose composition could be separately controlled. Both sides of the tissue were exposed to equal concentrations of "cold" GABA, and a tracer amount (15 μCi) of [2,3-³H(N)]-GABA was added to the apical bath for one tissue and to the basal bath for the paired tissue. The apical-to-basal ($A \rightarrow B$) or basal-to-apical ($B \rightarrow A$) fluxes were measured by sampling the "cold" solution on the opposite side of the tissue every 30 min. The sample size was 100 μl and was replaced with an equal amount of Ringer. Bath Ringer was well stirred and maintained at a pH of 7.4 by bubbling with 5% $CO_2$/95% $O_2$. Tissues having a TEP of less than 6 mV, a total resistance <200 Ω·cm², or exhibiting a decrease in short-circuit current (SCC) of >30% during the course of the experiment were not used in the analysis. A detailed protocol for radiotracer flux measurements has been described previously (Miller and Steinberg, 1976).

Samples were counted using a Beckman LS 7500 liquid scintillation counter. Unidirectional fluxes were calculated from the rate of tracer appearance on the cold side, the specific activity of the hot side, and the exposed area of the tissue (0.07 cm²). [2,3-³H(N)]-GABA was purchased from Dupont (NEN) products.

### Intracellular Na Fluorescence

Approximate intracellular Na levels were monitored with the Na sensitive, single wavelength dye Sodium Green tetraacetate (Molecular Probes, Inc., Eugene, OR). The procedures of intracellu-

lar fluorescent imaging have been described elsewhere (Lin and Miller, 1991; Kenyon et al., 1994). The xenon excitation source was filtered through a 500-nm filter with a 10-nm bandwidth (Omega Optical, Brattleboro, VT), and the epifluorescent emission measured through a 40-nm bandwidth filter at 520–560 nm.

After the tissue was mounted in the chamber, 50 μg of a membrane-permeant form of the dye (sodium green tetraacetate) dissolved in 10 μl of a 10% Pluronic Acid-DMSO stock was added to 15 ml Ringer perfusing the apical membrane. Background fluorescence was determined prior to dye loading and subtracted from subsequent records. Maximal loading of dye generally took 30–60 min. During the time course of loading, the TEP decreased by 1–2 mV and $R_t$ decreased by 10–15 $\Omega \cdot cm^2$. TEP and $R_t$ partially returned to baseline values 15 min after loading. Because of significant dye loss, calibration of $a^i_{Na}$ levels at the end of each experiment was unsuccessful. Results are reported in terms of relative fluorescent intensities.

## RESULTS

### Effect of Apical GABA on Membrane Voltages and Resistances

Fig. 2, $A$ and $B$, shows typical voltage and resistance responses after 1 and 5 min additions of 500 μM GABA to the solution bathing the RPE apical membrane. Apical addition of GABA depolarized $V_A$ faster than $V_B$ (Fig. 2 $B$, continuous traces) and caused a decrease in TEP (Fig. 2 $A$, continuous trace). The maximal drop in TEP occurred in ~40 s and then quickly recovered after GABA removal. During longer GABA pulses (5 min), the TEP slowly increased and $V_A$ hyperpolarized (over ~3 min) to a new steady state. The mechanisms underlying the generation of this "sag" in the voltage traces probably result from subsequent intracellular events (see Discussion). The open squares in Fig. 2 $A$ show that $R_t$ decreased in the presence of apical GABA, and the open triangles in Fig. 2 $B$ show that the ratio of apical to basolateral membrane resistance, $a$, increased.

In 33 tissues, apical addition of 500 μM GABA maximally depolarized $V_A$ and $V_B$ by 4.0 ± 1.0 mV and 2.5 ± 0.8 mV, respectively (mean ± SD). These GABA-induced voltage responses are consistent with an electrical event that originated at the apical membrane. The apical membrane voltage change could have been caused by a change in membrane conductance or by the activation of a current generator. Apical GABA also produced significant resistance changes. In 21 tissues where $R_t$ and $a$ were measured, the mean values for $R_t$ and $a$ were 286 ± 72 $\Omega \cdot cm^2$ and 0.45 ± 0.12, respectively. Addition of 500 μM GABA decreased $R_t$ by 10.5 ± 4.2 $\Omega \cdot cm^2$ and increased $a$ by 0.14 ± 0.06. This drop in $R_t$ and increase in $a$ are consistent with a decrease in basolateral membrane resistance. These resistance changes were observed only after a delay of 10–15 s after the onset of the GABA-evoked voltage responses. These electrical measurements suggest that GABA produced a larger voltage response at the apical membrane and a significant conductance change at the basolateral membrane.

Removal of GABA led to an overshoot and recovery of the TEP toward baseline levels, especially for larger concentrations of GABA (>100 μM); this is evident in Fig. 2 $B$ for both durations of GABA application. Both $V_A$ and $V_B$ hyperpolarized upon removal of GABA. Examination of these responses shows that $V_A$ and $V_B$ hyperpolarized to a voltage slightly below baseline and then slowly recovered toward baseline levels. These changes determine the characteristic TEP response.
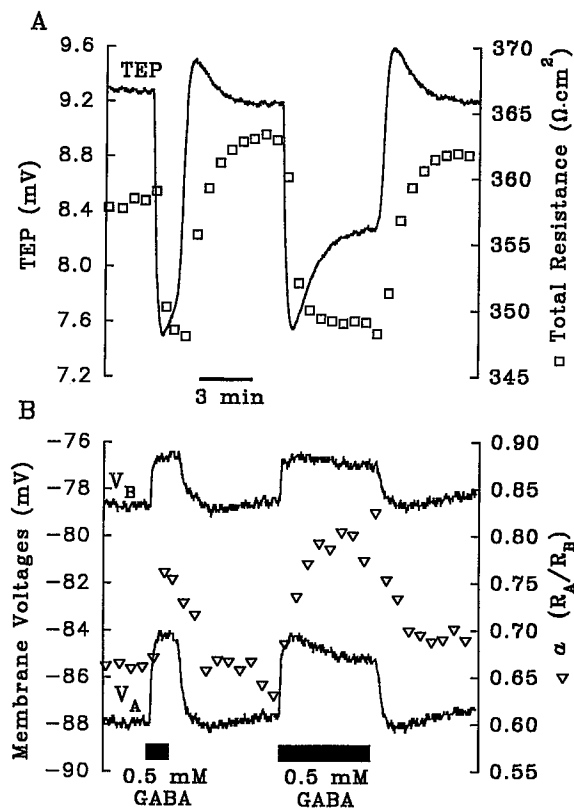
FIGURE 2. Effects of a one minute and a five minute addition of 500 μM GABA to Ringer perfusing the apical membrane. Upper trace shows TEP (*continuous line*) and $R_t$ (*open square*). Lower trace shows $V_A$, $V_B$ (*continuous lines*), and a (*open triangle*). The black bars indicate when GABA-containing Ringer was perfused into the apical chamber. (*A*) Apical addition of GABA caused a rapid drop in TEP and decrease in total resistance. (*B*) The continuous traces show that $V_A$ depolarized faster than $V_B$. a increased in the presence of GABA. Electrical measurements in *A* and *B* were reversible upon return to GABA-free Ringer.

## *The GABA-induced Electrical Responses Are Not Due to GABA Receptors*

Since RPE intracellular Cl activity is distributed above equilibrium (Wiederholt and Zadunaisky, 1985; Miller and Edelman, 1990; Bialek and Miller, 1994), the GABA-induced depolarization of $V_A$ may be due to an efflux of Cl ions associated with the opening of the ionotropic $GABA_A$ or $GABA_C$ receptor/channel (Takeuchi and Onodera, 1972; Shimada, Cutting, and Uhl, 1992). This notion was tested by adding various $GABA_A$ and $GABA_C$ agonists and antagonists to the apical bath. The $GABA_A$ agonists, muscimol (250 or 500 μM) or phenobarbital (250 μM), did not elicit substantial voltage responses in control Ringer nor did they potentiate the GABA responses ($n = 5$). Fig. 3 *A* shows a typical $V_A$ trace of a control GABA response followed by the addition of 500 μM muscimol. Both measurements were made in the same cell. In addition the $GABA_A$-receptor antagonist bicuculline (500 μM) and the $GABA_A$- and $GABA_C$-receptor blocker picrotoxin (500 μM), which were separately perfused over the apical membrane for at least 3 min before the addition of 500 μM GABA, failed to block the GABA response ($n = 4$). Fig. 3 *B* shows a GABA response in the absence (*left panel*) and presence (*right panel*) of picrotoxin. 5 min preexposure to picrotoxin did not block the GABA-induced change in $V_A$. The additional observation that the GABA-elicited responses do not exhibit desensitiza-

tion, even after continuous exposure to GABA for 20 min (not shown), further supports our conclusion that these responses are not mediated by ionotropic GABA receptors (Bormann, 1988).

The GABA-induced voltage effects may also be due to activation of the metabotropic $GABA_B$ receptor, whose effects can be mimicked by baclofen and inhibited by phaclofen (Dutar and Nicoll, 1988). Fig. 4 $A$ shows the voltage response to 250 $\mu$M baclofen (*right panel*) is significantly smaller than that of 250 $\mu$M apical GABA (*left panel*). In four tissues, 1 mM baclofen depolarized $V_A$ by 0.7 ± 0.3 mV and $V_B$ by 0.3 ± 0.1 mV. A small decrease in $R_t$ and increase in $a$ were also observed in two of these tissues. These baclofen-elicited responses are most likely *not* due to the activation of $GABA_B$ receptors (see Discussion). The response of 250 $\mu$M GABA in the
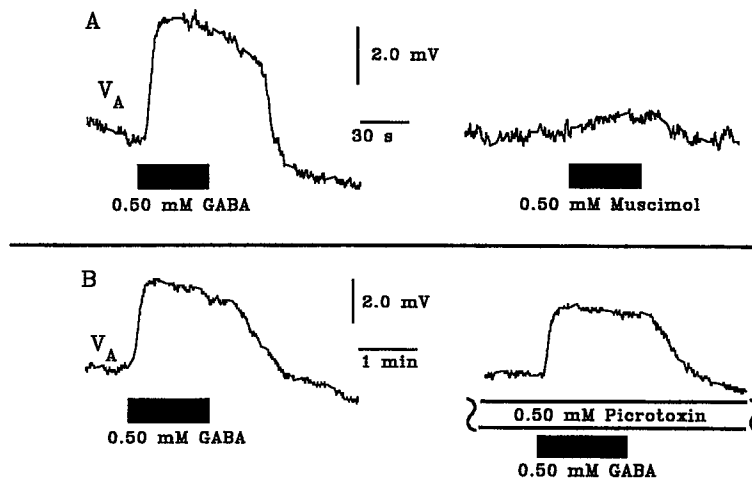


FIGURE 3.   Effects of $GABA_A$-receptor agonist muscimol and $GABA_A$/$GABA_C$-receptor antagonist picrotoxin in either mimicking or blocking the GABA-elicited depolarization of $V_A$. ($A$) Control depolarization of $V_A$ due to 500 $\mu$M GABA is not mimicked by 500 $\mu$M muscimol. Traces made in same cell. ($B$) Control response to 500 $\mu$M GABA is not inhibited by $GABA_A$/$GABA_C$-receptor blocker picrotoxin. Measurements made in the same cell in a different tissue from $A$.

absence and presence of the $GABA_B$-receptor antagonist phaclofen is shown in Fig. 4 $B$. In three tissues phaclofen failed to block the voltage effects of GABA, which suggests that $GABA_B$ receptors do not play a role in these responses.

*Nipecotic Acid Blocks the GABA Responses*

Many neuronal and glial cells contain electrogenic Na- and Cl-dependent GABA carriers that can be inhibited by nipecotic acid (Johnston, Krogsgaard-Larsen, and Stephanson, 1975). Fig. 5 $A$ and $B$ shows the response of apical GABA (200 $\mu$M) first in the absence and then in the presence of 1 mM nipecotic acid. Nipecotic acid completely and reversibly blocked the GABA-induced electrical responses.
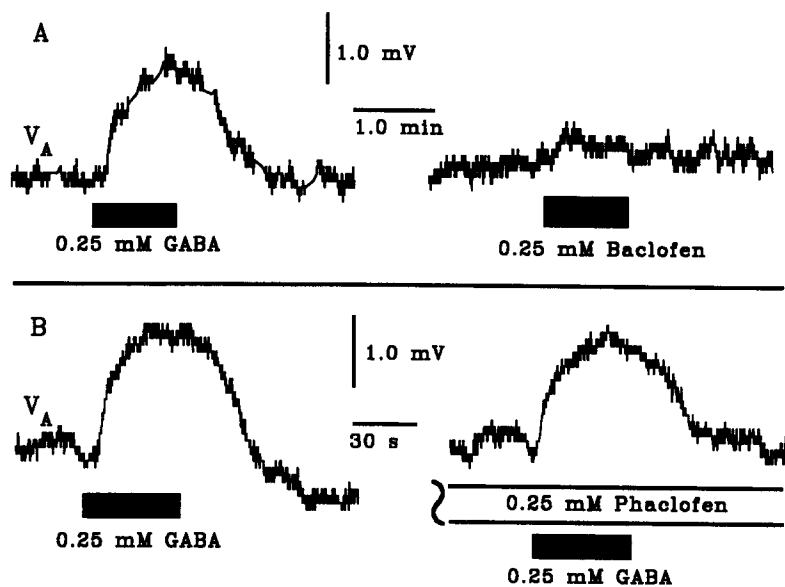
FIGURE 4.   Effect of GABA$_B$-receptor agonist, baclofen, and antagonist, phaclofen, on the GABA-mediated depolarization of $V_A$. ($A$) 250 μM apical baclofen failed to mimick the effects of GABA. Recordings were made from the same cell. ($B$) Preexposure to 250 μM apical phaclofen did not inhibit the voltage effect of GABA. Recordings were made from the same cell.

Similar observations were made in three other issues. The addition of nipecotic acid alone elicited voltage and resistance responses very similar to those of GABA. Inhibition of the GABA-mediated responses could have been caused in part by this nipecotic acid-induced depolarization. This possibility was eliminated, however, by first clamping $V_A$ back to its baseline value in the presence of nipecotic acid before the addition of GABA; the subsequent addition of GABA failed to elicit any electrical responses ($n = 2$; results not shown).

Nipecotic acid alone (1 mM) depolarized $V_A$ and $V_B$ by 3.8 ± 0.7 mV and 2.5 ± 0.5 mV, respectively ($n = 8$). The ability of nipecotic acid to block the GABA-induced voltage changes, presumably by competing as a substrate (Johnston et al., 1975), strongly suggests the presence of a GABA transporter at the RPE apical membrane.

### Ionic Dependence of the GABA Transporter

*Sodium.* GABA uptake is proposed to occur with the cotransport of 2–3 Na ions (Kanner and Schuldiner, 1987). To test if the GABA-induced change in $V_A$ is dependent on external Na, apical GABA was added to Na-free apical Ringer while monitoring $V_A$. Fig. 6 shows that removal of apical Na completely blocked the GABA-mediated depolarization of $V_A$, and that return to Na-containing Ringer restored the control GABA response. This observation was made in four other tissues. This result indicates that the voltage effects of apical GABA require external Na.
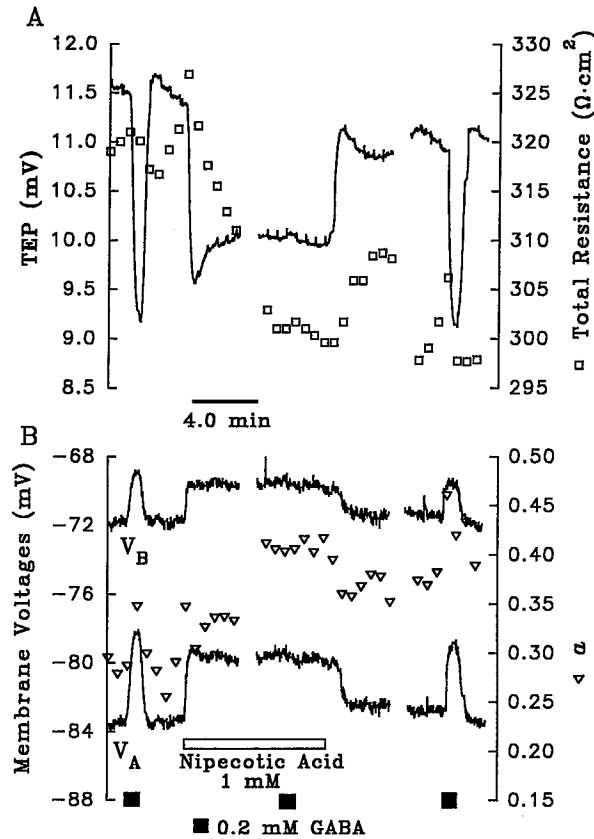
FIGURE 5. Effect of nipecotic acid alone and on the GABA-induced voltage and resistance changes. Solid black box shows when 200 μM GABA was added to apical Ringer, and open box shows when 1 mM nipecotic acid was added to apical Ringer. Nipecotic acid alone depolarized $V_A$ faster than $V_B$, decreased $R_t$, and increased $a$, and also reversibly blocked the GABA-induced voltage changes. Control and post-control GABA pulses show voltage responses similar to those of Fig. 1. Approximately 8 min elapsed during the break shown in the voltage traces while nipecotic acid was applied. 6 min elapsed between the second break shown in the voltage traces. All measurements were made from the same cell.

The apical membrane of the RPE contains an electrogenic ouabain-sensitive Na/K ATPase that maintains the Na gradient across the apical and basolateral membranes (Miller et al., 1978). This Na gradient could provide the driving force for GABA uptake. To test this hypothesis, we measured the GABA-induced voltage changes in the absence and presence of apical ouabin. Fig. 7 *A* shows that GABA reversibly depolarized $V_A$ by 2.8 mV in the absence of ouabain. Apical ouabain (200 μM) caused $V_A$ to slowly depolarized (see dashed line in Fig. 7 *B*), presumably because of a rundown of the Na gradient. In this case the addition of GABA to the apical bath only depolarized $V_A$ by 1.3 mV. In three tissues, the presence of 200 μM
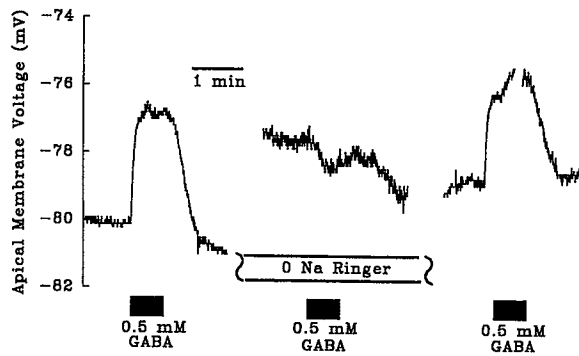
FIGURE 6. Effects of apical Na removal on the GABA-mediated depolarization of $V_A$. Initial control response shows the typical $V_A$ response to 500 μM apical GABA. Na-containing Ringer was then substituted with NMDG-containing Ringer for ~15 min before 500 μM GABA was added. In the absence of external Na the GABA-evoked depolarization of $V_A$ was blocked. A small hyperpolarization of $V_A$ was observed when GABA was added to Na-free Ringer. This effect was not investigated further. Return to Na-containing Ringer recovered most of the GABA-induced effects. Approximately 20 min elapsed between return to Na-containing Ringer and addition of third GABA pulse. Voltage traces were from the same cell.

ouabain reduced the GABA-elicited depolarization of $V_A$ by 41 ± 8%, indicating that the Na gradient is a crucial determinant of this response.

To directly demonstrate transporter-mediated influx of Na upon addition of GABA, the fluorescent dye sodium green was used to monitor intracellular levels of this ion. Fig. 8 shows that apical GABA produced a significant increase in Na inten-
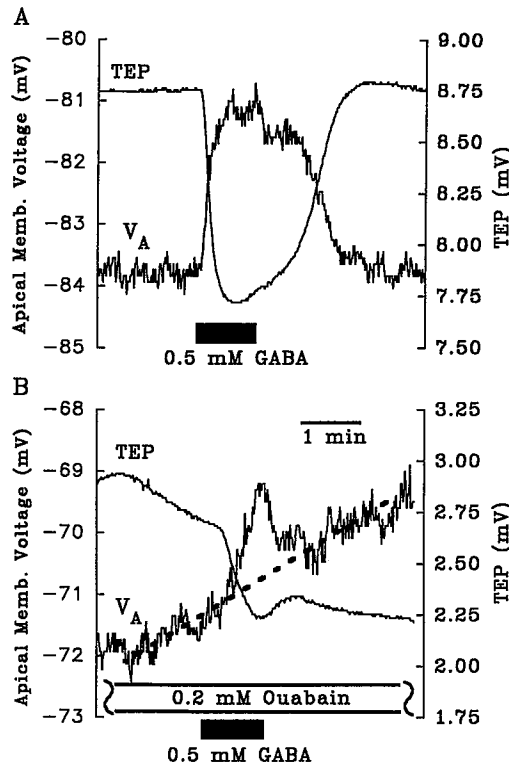


FIGURE 7. TEP and $V_A$ responses of a single cell to GABA in the absence and presence of 200 μM apical ouabain. (A) Control responses to a 1-min apical addition of 500 μM GABA. $V_A$ rapidly depolarized by 2.8 mV, and TEP decreased by 1.0 mV. Both voltage responses were fully reversible. After the removal of GABA, ouabain was added to the apical Ringer perfusate for ~15 min before the second addition of GABA. The initial voltage effects of ouabain are not shown. (B) Ouabain alone caused $V_A$ to depolarize linearly (represented by the dashed line). In the presence of ouabain, the GABA-induced depolarization of $V_A$ was only 1.3 mV, and the decrease in TEP response was <0.5 mV. TEP did not fully recover following GABA removal. Recordings in both figures were made in the same cell.

sity of approximately 8% above baseline. In five tissues, the addition of 500 μM GABA increased fluorescent intensities by 5 ± 3% above baseline. Because the magnitude of fluorescent intensity of this dye corresponds directly with the activity of intracellular Na, these results offer additional evidence for a GABA carrier that transports Na into the RPE.

*Chloride.* Many members of the neurotransmitter transporter family (including GABA) also transport Cl in addition to Na and substrate. Removal of external Cl almost completely abolishes transport (Cammack and Schwartz, 1993). Fig. 9 (*middle panel*) shows that Cl removal (gluconate substitution) from the apical bath had little effect on the GABA-induced apical membrane depolarization (pre- and post-controls in left and right panels, respectively). To eliminate the possibility that gluconate itself could replace Cl as a substrate for the putative GABA transporter, we also used methane sulfonate and cyclamate as Cl substitutes. The following results were obtained for the GABA-induced $\Delta V_A$ in control Ringer and in Cl-free Ringer
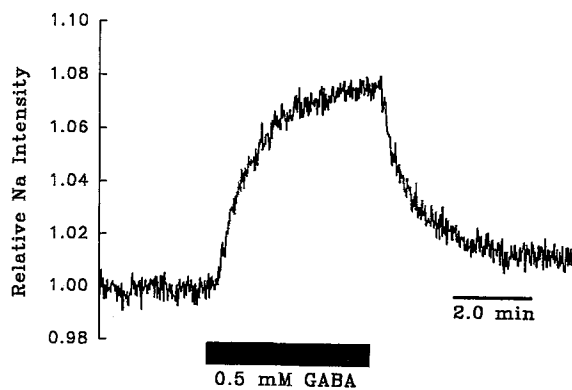


FIGURE 8. Effect of GABA on intracellular Na levels. Fluorescent intensities of the intracellular Na dye, sodium green, is shown normalized and monitored in the absence and presence of 500 μM GABA. The intensity of the fluorescent signal is monotonically related to $a_{Na}^i$. Addition of GABA reversibly increased fluorescence, indicating a significant increase in $a_{Na}^i$ in the presence of GABA and a recovery almost to baseline upon removal of GABA.

(paired results were obtained from the same tissue and reported as mean ± SEM): $\Delta V_A$ = 2.6 ± 0.6 mV (*control;* n = 4) and 2.7 ± 0.7 mV (gluconate); 3.6 ± 0.5 mV (*control;* n = 5) and 2.8 ± 0.6 mV (methane sulfonate); 4.1 ± 0.4 mV (*control;* n = 5) and 3.2 ± 0.5 mV (cyclamate). These results indicate that none of the anion substitutes significantly inhibited the voltage effects of GABA and strongly suggest that the carrier does not have an absolute requirement for external Cl.

The mean value for $R_A$ is 270 ± 20 Ω·cm² (n = 7; mean ± SEM) in Cl-containing Ringer and 380 ± 30 Ω · cm² in Cl-free Ringer (P < 0.01; paired t test). It is possible that the GABA-induced changes in voltage reported above were not different because the GABA-induced current ($I_{gaba}$) was smaller and $R_A$ was larger in Cl-free Ringer. To test this notion, we calculated $I_{gaba}$ in the presence and absence of apical Cl (see Methods). In Cl-containing Ringer, $I_{gaba}$ = 16.9 ± 2.3 μA/cm² (mean ± SEM; n = 7), and in Cl-free Ringer, $I_{gaba}$ = 10.3 ± 1.7 μA/cm². This 40% difference is significant and suggests a partial external requirement for Cl for transporter function (see Discussion).

It is possible that the GABA-induced responses were not completely blocked because Cl diffusion across the paracellular pathway was sufficient to keep apical Cl concentrations relatively high. This possibility is very unlikely because the GABA-evoked voltage changes were still present in the absence of Cl on both sides of the tissue. Cl was removed for at least 20 minutes before the addition of GABA. In three tissues, $I_{gaba}$ was 20.7 ± 2.8 mV in control Ringer (mean ± SEM) and 10.6 ± 1.0 mV in Cl-free Ringer in both baths.

Many Cl-dependent membrane transport mechanisms also utilize $HCO_3$. It is possible that apical Cl removal did not inhibit the GABA-mediated responses because the extracellular anionic requirement for GABA transport was met by the $[HCO_3]_0$. In Cl-free *and* $HCO_3$-free Ringer, 500 μM GABA depolarized $V_A$ by 3.5 ± 0.6 mV ($n = 2$; results not shown), which is not significantly different than the control. These results strongly suggest that $HCO_3$ cannot substitute for Cl to fulfill the anionic requirement for transport.
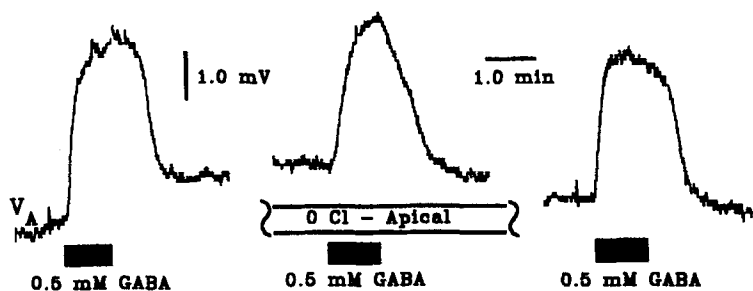


FIGURE 9.  Effects of apical Cl removal (gluconate substitution) on the $V_A$ response to 500 μM GABA. Control GABA response is shown in the first continuous trace. The tissue was then exposed to ∼15 min of Cl-free Ringer before the second addition of GABA. Removal of apical Cl did not abolish the GABA-mediated depolarization of $V_A$. Addition of Cl-free Ringer alone hyperpolarized $V_A$ and $V_B$, decreased TEP, increased $R_t$ and decreased $a$ (results not shown). The resistance changes produced by removal of apical Cl significantly reduced the current generated by the addition of GABA (see text). Following control is shown after return to Cl-containing Ringer. Approximately 15 min elapsed between return to Cl-containing Ringer and application of following control GABA pulse. Voltage traces were made from the same cell.

### Relationship between GABA and Taurine Transport

Previous work has demonstrated a β-alanine sensitive, Na-dependent taurine flux across the bullfrog RPE (Miller and Steinberg, 1976, 1979). In another study, evidence is presented that apical-enriched membrane vesicles from the bovine RPE contain a nipecotic acid-sensitive GABA transporter that also takes up taurine (Sivakami, Ganapathy, Leibach, and Miyamoto, 1992). We were interested to see if there exists a relationship between GABA and taurine transport in this tissue. Previous work in the bullfrog RPE (Scharschmidt, Griff, and Steinberg, 1988) showed that the addition of 10 mM taurine to the apical bath elicited a voltage response similar to that produced by 500 μM GABA (see Fig. 2).

To determine if the same transport mechanism utilizes GABA and taurine as substrates, we added apical taurine in the absence and presence of apical GABA. Fig. 10 shows the effects of 10 mM taurine on $V_A$ and TEP added to the apical bath in the absence (*top and bottom traces*) and presence (*middle traces*) of 500 μM GABA. The traces on the left side of Fig. 10 are of $V_A$ and the traces on the right side are of TEP. As the middle traces indicate, the taurine-induced changes in $V_A$ and TEP are significantly reduced in the presence of GABA and this effect is reversible (*lower trace*). In six tissues 500 μM GABA inhibited the taurine-elicited voltage responses by 79 ± 7%. In addition we found that 500 μM GABA inhibits the voltage effects of 1 mM taurine by 93 ± 3% ($n$ = 3; results not shown).
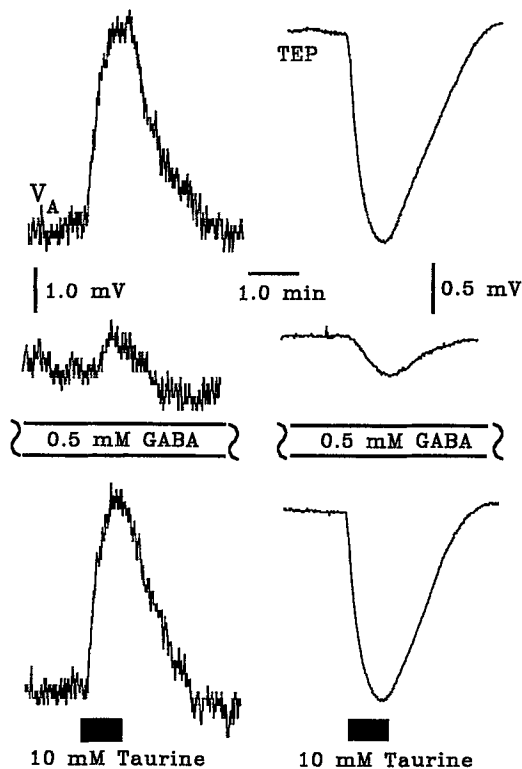


FIGURE 10. Voltage effects of apical addition of 10 mM taurine are significantly inhibited by 500 μM apical GABA. Left panel shows $V_A$ and right panel shows TEP. The magnitude of the $\Delta V_A$ responses correspond monotonically with the $\Delta$TEP responses. The middle traces in both panels show that the taurine-induced $\Delta V_A$ and $\Delta$TEP responses to taurine are reduced by approximately 80% in the presence of GABA. The addition of taurine following removal of GABA (*bottom traces*) shows almost a complete recovery. Electrical recordings were made in the same cell.

If taurine and GABA are substrates for the same transporter, then nipecotic acid should also block the taurine responses. Fig. 11 shows that nipecotic acid (1 mM) in the apical bath significantly inhibited a taurine-induced voltage change (*middle panel*; $V_A$ on left, TEP on right). In five tissues 1 mM nipecotic reduced the effects of 10 mM taurine by 74 ± 5% and the effects of 1 mM taurine by 90 ± 4% ($n$ = 3). The results summarized in Figs. 5, 10, and 11 strongly suggest that the GABA- and taurine-evoked electrical responses are generated by a single transport mechanism that utilizes GABA, taurine, and nipecotic acid as substrates.

*Dose Dependence of the GABA- and Taurine-induced Responses*

Since the TEP can provide a very sensitive measure of apical membrane potential changes, we used the initial rate change of TEP, $(d\text{TEP}/dt)_{\text{init}}$, to characterize the concentration dependence of the apical membrane voltage response to changes in $[\text{GABA}]_o$ (Methods). Fig. 12 A shows a typical TEP response to successively larger concentrations of GABA added to the apical bath. The initial slope representing $(d\text{TEP}/dt)_{\text{init}}$ is shown as a solid line and its value is given above each response. Fig.
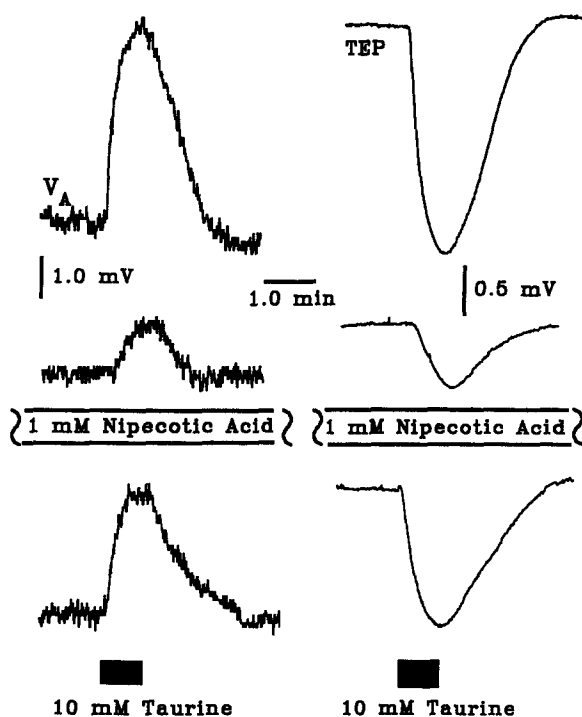
FIGURE 11. Voltage effects of apical addition of 10 mM taurine are significantly inhibited by 1 mM nipecotic acid. The traces were generated in the manner described in the legend for Fig. 10. The depolarization of $V_A$ and the drop in TEP due to taurine was inhibited by ~75% in the presence of nipecotic acid. Voltage responses were taken from the same cell.

12 A shows that successively higher concentrations of GABA (30 µM to 1 mM) produced larger TEP responses. The absolute value of $(d\text{TEP}/dt)_{\text{init}}$ is plotted against GABA concentration in Fig. 12 B and fitted with a continuous curve using nonlinear regression analysis based on first order Michaelis-Menten kinetics with an apparent $K_m$ of 210 µM and a $V_{\text{max}}$ of 3.88 mV/min.

The same experimental protocol was used to determine the concentration dependence of $(d\text{TEP}/dt)_{\text{init}}$ on taurine concentration, and Fig. 13, A and B, shows the typical TEP response from a tissue exposed to successively larger concentrations of taurine (250 µM to 8 mM). Approximately fivefold higher concentrations of taurine were required to elicit TEP responses comparable in magnitude to those obtained with GABA. Fig. 13 B shows that the taurine dose response could also be fitted with a first order Michaelis-Menten curve with an apparent $K_m$ of 0.96 mM and a $V_{\text{max}}$ of 4.31 mV/min.

The TEP responses to apical nipecotic acid and β-alanine are very similar to the GABA and taurine responses (Figs. 12 and 13), and also show first-order saturable kinetics. Fig. 14 shows a compilation of the average dose responses from a number of tissues for each of the four substrates. The average apparent $K_m$ for each substrate is 160 μM for GABA (*filled circles*), 250 μM for β-alanine (*filled triangles*), 420 μM for nipecotic acid (*filled squares*), and 850 μM for taurine (*open circles*). These $K_m$ values for GABA and taurine indicate that this putative transporter is of a low affinity type ($K_m > 50$ μM) for both substrates, but because its specificity is fivefold higher for GABA than for taurine, this carrier should be classified as a GABA transporter.
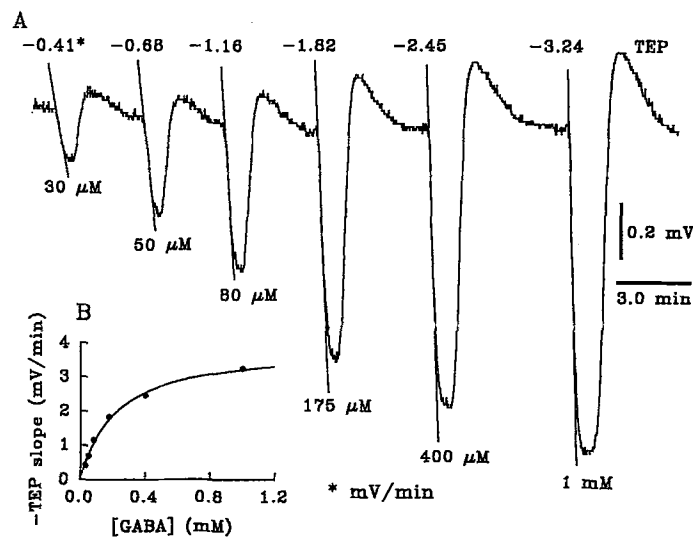


FIGURE 12.  The concentration dependence of TEP on successively larger concentrations of apical GABA followed first-order Michaelis-Menten kinetics. (A) As shown under the continuous TEP trace, varying concentrations of GABA (30 μM to 1 mM) were added to the apical bath while the TEP was continuously monitored. The duration of each GABA pulse was 30 s. The concentration dependence was determined by the initial rate change of TEP, $d(TEP)_{ini}/dt$ (see text). The line representing $d(TEP)_{ini}/dt$ is drawn in and its value in units of mV/min is shown. (B) Filled circles represent a plot of $d(TEP)_{ini}/dt$ as a function of GABA concentration from the results shown in A. The continuous trace represents a first-order Michaelis-Menten equation with an apparent $K_m$ of 210 μM and a $V_{max}$ of 3.88 mV/min. Values for $K_m$ and $V_{max}$ were determined using nonlinear regression analysis.

*Calculations of the GABA-induced Current*

By using the values for $R_t$ and $a$ (reported above) before the addition of GABA, along with the GABA-elicited $\Delta V_A$ and $\Delta V_B$, the values for $R_A$, $R_B$, and $R_s$ can be calculated using Eqs. 3 and 4 (see Methods): $R_A = 310 \pm 60$ Ω·cm², $R_B = 690 \pm 210$ Ω·cm², and $R_s = 400 \pm 140$ Ω·cm² ($n = 21$). These values can be used in Eq. 5 to calculate the current generated by the transporter, $I_{gaba} = 17 \pm 4$ μA/cm². Since this value for $I_{gaba}$ was determined from tissues exposed to 500 μM GABA, this infor-
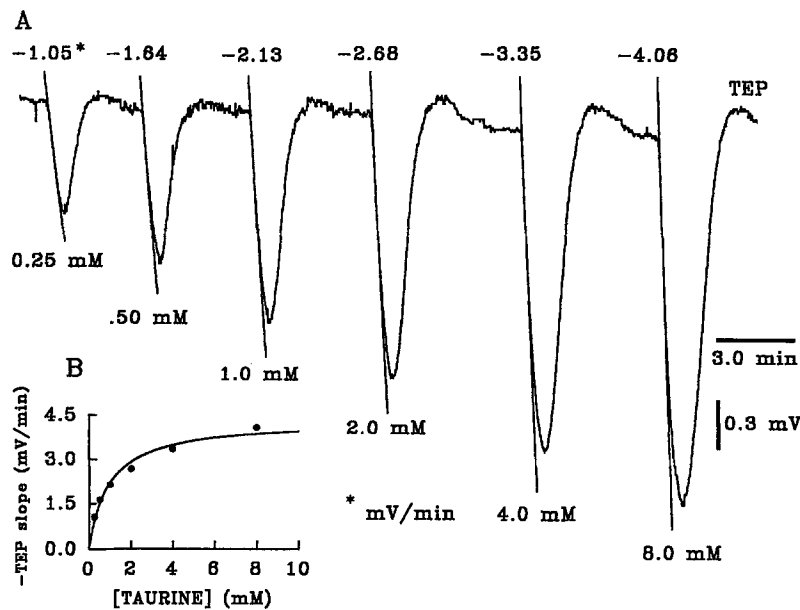
FIGURE 13. The concentration dependence of TEP on apical taurine followed first-order Michaelis-Menten kinetics. The results of this figure were generated in a manner similar to that of Fig. 12. (*A*) Apical taurine concentrations were varied from 250 $\mu$M to 8 mM. In general, a fivefold larger concentration of taurine was required to elicit TEP changes comparable to those of GABA. (*B*) The continuous trace in the bottom left figure represents a first-order Michaelis-Menten equation with a $K_m$ of 0.96 mM and a $V_{max}$ of 4.31 mV/min. The differences in $V_{max}$ between Figs. 12 and 13 are within the variations in $V_{max}$ observed.

mation can be used with the apparent $K_m$ of 160 $\mu$M to calculate the maximum transporter-associated current, $I^m_{gaba}$ = 22 ± 5 $\mu$A/cm$^2$, which is approximately half of the magnitude of the current produced by the apical membrane Na/K ATPase (Miller et al., 1978). This large GABA transporter-associated current indicates the presence of a high capacity uptake mechanism.

### Steady State GABA Flux

Previous studies have demonstrated a net absorptive flux (apical to basolateral; *A→B*) of taurine, glutamate and aspartate across frog and bovine RPE (Miller and Steinberg, 1976 and 1979; Pautler and Tangerdy, 1986). Therefore it seemed possible that the RPE could clear GABA from the subretinal space by actively transporting this amino acid from the retinal (apical) to choroidal (basal) side of the tissue. Unidirectional *A→B* and *B→A* fluxes of [2,3-$^3$H(N)]-GABA were measured in paired tissue from the same eye. Fig. 15 *A* shows that after two hours, the unidirectional *A→B* GABA fluxes (*filled circles*) reached a steady state value of approximately 35 nEq/cm$^2$·h (*solid line*). During this period the unidirectional *B→A* flux (*open circles*) was ∼4 nEq/cm$^2$·h (*dashed line*). The mean net flux for two pairs of tissues was 31 ± 1 nEq/cm$^2$·h (mean ± SEM). These results provide direct evidence for net ab-
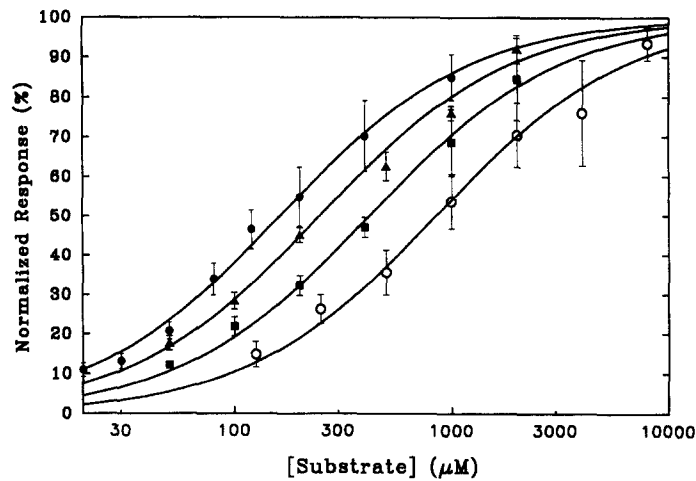
FIGURE 14. Results summarizing the concentration dependence of TEP responses to GABA ( filled circles), β-alanine ( filled triangle), nipecotic acid ( filled squares), and taurine (open circles). Y-axis shows the normalized $d(TEP)_{ini}/dt$ response and the x-axis shows a logarithmic plot of substrate concentration. Error bars represent SEM. The apparent $K_m$ and sample sizes are as follows: GABA (160 μM; n = 8–14); β-alanine (250 μM; n = 4); nipecotic acid (420 μM; n = 4); taurine (850 μM; n = 7–12).

sorptive flux of GABA from the retinal-to-choroidal direction, and strongly suggest that the RPE can actively transport GABA out of the subretinal space and into the blood supply via an uptake mechanism at the apical membrane.

### GABA-elicited Stimulation of the Na/K ATPase

In a variety of epithelia including the RPE, activation of Na-dependent transporters has been associated with an increase in Na/K ATPase activity (Miller and Steinberg, 1979; Albus, Lippens, and van Heukelom, 1983; Trunheim, Thompson, and Schultz, 1987; Scharschmidt et al., 1988). The RPE Na/K ATPase is located on the apical membrane, and its activity can be activated in isolation from other $[K]_o$-dependent membrane transport mechanisms by first adding $Ba^{2+}$ (K channel blocker) and bumetanide (Na,K,2Cl cotransporter inhibitor) to the apical perfusate, and then making a step change in $[K]_o$ from 0.5 mM to 5.0 mM. This step change in $[K]_o$ increases pump activity and hyperpolarizes $V_A$ (Griff, Shirao, and Steinberg, 1985). To determine if activation of the GABA transporter causes a stimulation in pump activity, we performed this step change in $[K]_o$ in the absence and presence of apical GABA.

The left panel of Fig. 16 shows a series of $V_A$ responses to this step change in $[K]_o$. These measurements were made from the same cell. Traces 1 and 3 are control responses (GABA-free Ringer) and trace 2 was made in the presence of 200 μM apical GABA. Trace 4 was made in the presence of 200 μM ouabain (GABA-free). Trace 1 shows that $V_A$ hyperpolarized by 7.7 mV during this step change in $[K]_o$, and is consistent with an increase in pump activity. This effect was blocked by ouabain (trace 4), which further supports the notion that the Na/K ATPase is the
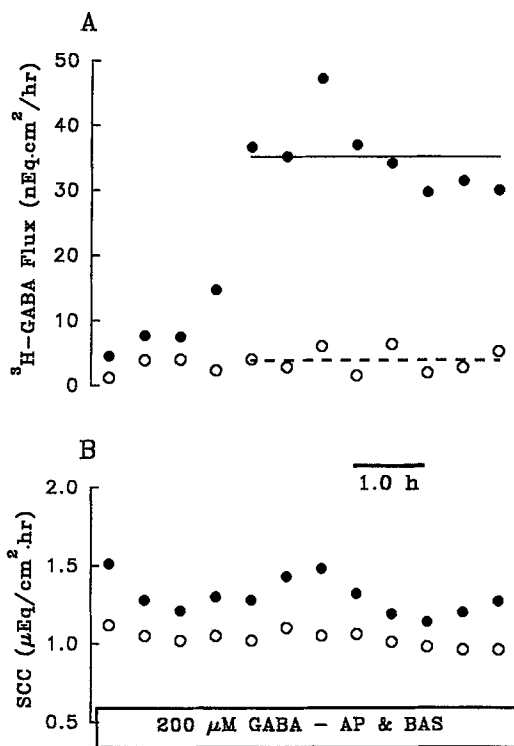
FIGURE 15. Unidirectional fluxes of GABA in paired tissues. Each tissue was bathed in control Ringer containing 200 μM cold GABA, and 15 μCi [2,3-$^3$H(N)]-GABA was added to the apical bath in one tissue for determination of unidirectional apical-to-basal flux ($A{\to}B$; filled circles) and added to the basal bath in the paired tissue for determination of unidirectional basal-to-apical flux ($B{\to}A$; unfilled circles). Samples from the cold side was taken every 30 min under open-circuit conditions, and the short-circuit current (SCC) was monitored. (A) The $A{\to}B$ steady state fluxes occurred after 2 h and was significantly larger than the $B{\to}A$ fluxes after this time ($P < 0.001$). (B) SCC plotted during open circuit is equal to TEP/$R_t$ and remained relatively constant throughout the experiment.

source of the voltage responses observed in trace 1. The small depolarization of $V_A$ in trace 4 may be due to incomplete blockage of K conductances by apical $Ba^{2+}$.

GABA (200 μM) was added to the apical bath prior to the results shown in trace 2. Trace 2 shows that in the presence GABA, this $[K]_o$ step change hyperpolarized $V_A$ by 9.8 mV. In the absence of GABA, this $\Delta[K]_o$-induced voltage response was significantly smaller ($\Delta V_A$ is 7.7 mV in trace 1 and 6.5 mV in trace 3). If step changes in $[K]_o$ produced $\Delta V_A$ responses in the left panel of Fig. 16 (traces 1-3) that are solely due a change in the Na/K pump activity (i.e., a pure current source), then these $\Delta[K]_o$-induced change in TEP should be directly proportional to $\Delta V_A$ (Miller and Steinberg, 1977) and should provide a very sensitive measure of the $V_A$ changes. This expectation was tested by comparing the $\Delta[K]_o$-induced changes in TEP and $V_A$. In traces 1 and 2, these $\Delta$TEP and $\Delta V_A$ responses both increased by ~23% in the presence of apical GABA. The subsequent removal of GABA decreased both $\Delta V_A$ and $\Delta$TEP by approximately 35% (traces 2 and 3). This monotonic relationship between $\Delta$TEP and $\Delta V_A$ was observed in all tissues examined ($n = 5$); we can therefore use TEP measurements to provide a more accurate quantification the GABA-evoked stimulation of the pump. In five tissues, the $\Delta[K]_o$-induced stimulation of the Na/K pump current was $21 \pm 5\%$ larger in the presence of 200 μM GABA. We conclude that activation of the GABA transporter significantly increases Na/K pump activity, presumably because GABA transport raises $a^i_{Na}$.
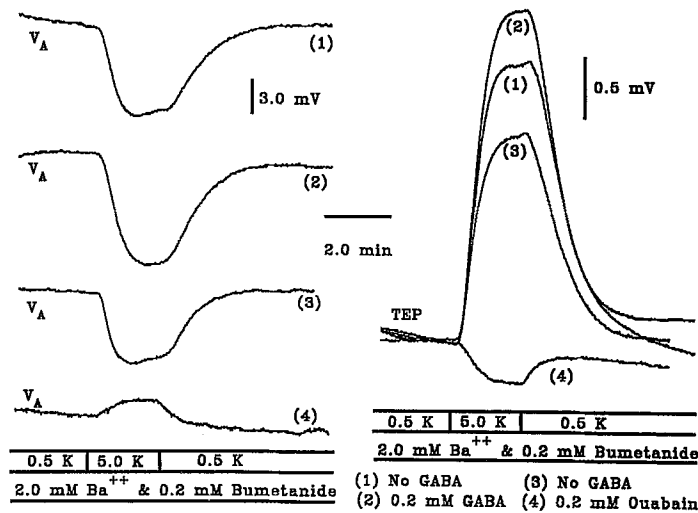
FIGURE 16. Apical GABA stimulates Na/K ATPase activity. Figure shows voltage responses of the RPE that was perfused with apical $Ba^{2+}$ (2 mM) and bumetanide (0.20 mM) to step changes in apical $[K]_o$. The four left traces represent $V_A$ recordings made from the same cell. A change in Ringer containing 0.5 mM $[K]_o$ to 5.0 mM $[K]_o$ causes a hyperpolarization of $V_A$ of 7.7 mV due to stimulation of the electrogenic Na/K ATPase, as shown in trace 1 for $V_A$. 200 μM GABA was then added to Ringer containing 0.5 mM $[K]_o$ before trace 2 and membrane potentials were then allowed to stabilize before another step change in $[K]_o$ was made in the continued presence of GABA. Trace 2 for $V_A$ shows a hyperpolarization of 9.8 mV during this step change. Before trace 3, GABA was removed from the apical Ringer and the membrane potentials were again allowed to stabilize. The following control shows that $V_A$ hyperpolizes by only 6.5 mV in Ringer containing 5 mM $[K]_o$. Before trace 4, 0.2 mM ouabain was added to the Ringer containing 0.5 mM $[K]_o$ (not shown). After the membrane potentials stabilized, a step change in $[K]_o$ caused a slight depolarization of $V_A$. The four traces on the right side of the figure represent simultaneous TEP recordings made while $V_A$ was being monitored. Traces 1, 2, and 3 show that a $[K]_o$ step change produced an increase in TEP (or ΔTEP) of 2.17 mV, 2.66 mV, and 1.68 mV, respectively. A comparison of this $[K]_o$-induced $\Delta V_A$ and ΔTEP responses in the absence and presence of GABA shows a monotonic relationship between the two quantities. For example, both $\Delta V_A$ and ΔTEP were ∼23% larger traces 2 than in trace 1. Similarly both $\Delta V_A$ and ΔTEP were ∼35% larger in trace 3 compared with trace 2.

## Effects of Basolateral $Ba^{2+}$ and DIDS on the GABA-induced Resistance Changes

The decrease in total resistance, $R_t$, and increase in apical to basolateral membrane resistance, $a$, due to apical addition of GABA in Fig. 2, A and B, are consistent with an increase in RPE basolateral membrane conductance. These GABA-induced resistance changes are surprising because activation of a GABA transporter should only increase the conductance of the apical membrane by <0.1% (Malchow and Ripps, 1990; Kavanaugh, Arriza, North, and Amara, 1992) and should therefore be undetectable. Instead the changes in $R_t$ and $a$ strongly suggest that apical GABA produces a large conductance increase at the basolateral membrane. K and Cl channels account for almost all of the conductance at the basolateral membrane in all species of RPE studied, including the frog (Miller and Steinberg, 1977; Joseph and

Miller, 1991; Quinn and Miller, 1992; la Cour, 1992; Gallemore, Hernandez, Tayya-
nipour, Fujii, and Steinberg, 1993; Bialek and Miller, 1994). To determine if the
GABA-induced changes in resistance affect either of these conductances, we added
500 μM apical GABA in the absence and presence of either the K channel blocker,
$Ba^{2+}$ (3 mM added to basal bath), or the Cl channel blocker, DIDS (1 mM added to
basal bath), while monitoring $R_t$ and $a$.

Fig. 17, *A* and *B*, shows the effects of GABA on $R_t$ and $a$ in the absence and pres-
ence of 3 mM basal $Ba^{2+}$. In $Ba^{2+}$-free Ringer, Fig. 17 *A* shows that $R_t$ decreased
from a starting value of 273 $\Omega \cdot cm^2$ to a final value of 258 $\Omega \cdot cm^2$, while $a$ increased
by 0.12 from an initial value of 0.48. Before the records shown in Fig. 17 *B*, 3 mM
$Ba^{2+}$ was added to the basal bath, which depolarized $V_A$ and $V_B$ by 6.0 mV and 6.2
mV, respectively, caused an increase of $R_t$ from an initial value of 274 $\Omega \cdot cm^2$ to 292
$\Omega \cdot cm^2$, and decreased $a$ from an initial value 0.48 to 0.26 (results not shown). The
electrical effects of basal $Ba^{2+}$ are consistent with a decrease in K conductance
(Gallemore et al., 1993). In the presence of basal $Ba^{2+}$, Fig. 17 *B* shows that the ad-
dition of GABA decreased $R_t$ to 298 $\Omega \cdot cm^2$ from an initial value of 303 $\Omega \cdot cm^2$, and
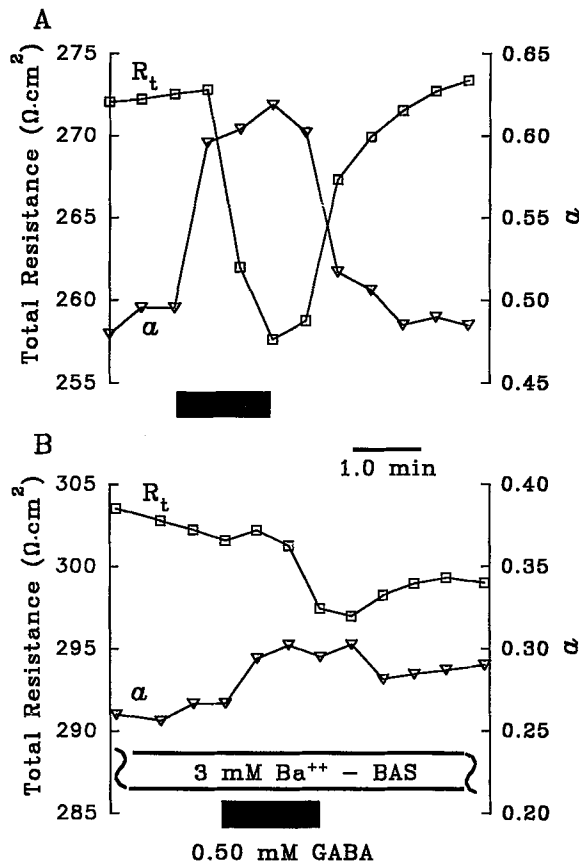increased $a$ to 0.30 from an initial value of 0.26. In five tissues, the addition of



FIGURE 17.  Effects of 3 mM baso-
lateral $Ba^{2+}$ on the GABA-medi-
ated changes in $R_t$ and $a$. (*A*) Con-
trol responses due to addition of
500 μM GABA to the apical perfu-
sate. The decrease in $R_t$ (*open
squares*) and increase in $a$ (*open tri-
angles*) are consistent with a con-
ductance decrease at the basolat-
eral membrane. (*B*) The decrease
in $R_t$ and the increase in $a$ were
substantially reduced when the ba-
solateral membrane was perfused
with $Ba^{2+}$-containing Ringer. $Ba^{2+}$
was added to the basal bath 15 min
before the addition of GABA to
the apical bath. Resistance re-
sponses were made from the same
cell. The GABA-evoked changes in
membrane potentials, $\Delta V_A$ and
$\Delta V_B$, were +4.9 and +3.2 mV (con-
trol conditions), and +5.0 and
+3.6 mV (in presence of basal
$Ba^{2+}$), respectively.

GABA in the absence of $Ba^{2+}$ decreased $R_t$ by $10.5 \pm 2.8 \ \Omega \cdot cm^2$ and increased $a$ by $0.16 \pm 0.03$ (mean $\pm$ SEM), whereas the addition of GABA in the presence of $Ba^{2+}$ decreased $R_t$ by $3.7 \pm 1.5 \ \Omega \cdot cm^2$ and increased $a$ by $0.03 \pm 0.02$ (mean $\pm$ SEM; $P < 0.05$). These results indicate that the GABA-evoked resistance changes are significantly inhibited by basal $Ba^{2+}$.

Fig. 18 $A$ and $B$ shows the effects of GABA on $R_t$ and $a$ in the absence and presence of 1 mM basal DIDS. Fig. 18 $A$ shows that $R_t$ decreased from an initial value of $224 \ \Omega \cdot cm^2$ to $216 \ \Omega \cdot cm^2$ in the presence of GABA, and $a$ increased from 0.54 to 0.71. DIDS (1 mM) was added to the basal bath before the records shown in Fig. 18 $B$ and hyperpolarized $V_A$ and $V_B$ by 3.3 mV and 11.5 mV respectively, caused an in-
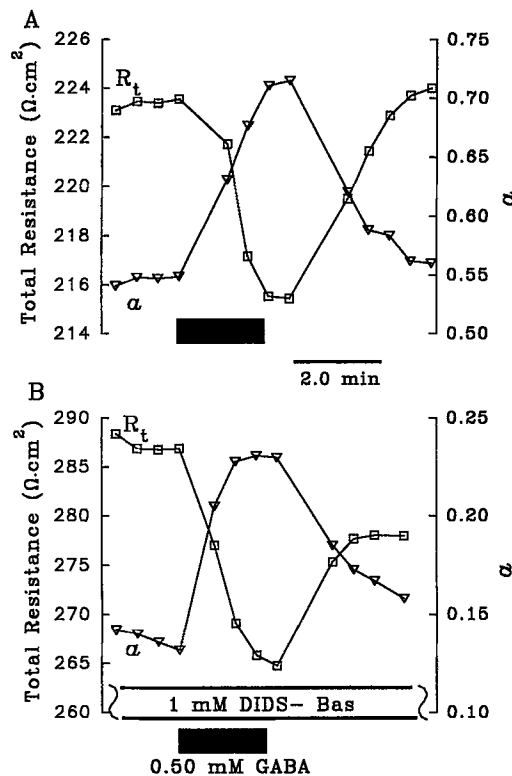


Figure 18. Effects of 1 mM basolateral DIDS on the GABA-induced $R_t$ and $a$ measurements. The results were generated using a similar protocol as those of Fig. 17. ($A$) Control resistance responses to apical addition of GABA. ($B$) Effects of a basolateral addition of 1 mM DIDS on the GABA-induced changes in $R_t$ and $a$. DIDS was added to the basal bath 25 min before the addition of GABA to the apical bath. These recordings show that basal DIDS did not block the GABA-elicited changes in $R_t$ and $a$. Results were obtained from the same cell. The GABA-evoked changes in membrane potentials, $\Delta V_A$ and $\Delta V_B$, were +4.4 and +3.0 mV (control conditions), and +7.2 and +5.9 mV (in presence of basal DIDS), respectively.

crease of $R_t$ from an initial value of $224 \ \Omega \cdot cm^2$ to $287 \ \Omega \cdot cm^2$, and decreased $a$ from an initial value 0.59 to 0.14 (results not shown). These voltage and resistance responses to basal DIDS are consistent with a decrease in basolateral membrane Cl conductance (Gallemore and Steinberg, 1989; Joseph and Miller, 1991). In the presence of 1 mM DIDS in the basal perfusate, Fig. 18 $B$ shows that apical addition of GABA caused a decrease in $R_t$ from an initial value of $287 \ \Omega \cdot cm^2$ to $265 \ \Omega \cdot cm^2$, while $a$ increased from 0.14 to 0.22. Similar observations were made in three other tissues. These results suggest that basal DIDS does *not* block the GABA-induced changes in resistance.

An estimation of the GABA-induced change in $R_B$ in the absence and presence of basal $Ba^{2+}$ or DIDS can be made from an analysis based on the previously calculated values of $R_A$ (310 ± 60 $\Omega \cdot cm^2$), $R_B$ (690 ± 210 $\Omega \cdot cm^2$) and $R_t$ (400 ± 140 $\Omega \cdot cm^2$) and the GABA-induced changes in $a$ and $R_t$. If we assume that the GABA-evoked increase in $a$ reported earlier (i.e., an increase from 0.45 to 0.59) is due *solely to a decrease in $R_B$*, from 690 $\Omega \cdot cm^2$ to 530 $\Omega \cdot cm^2$, we can calculate $R_t$ to be ~271 $\Omega \cdot cm^2$ in the presence of GABA. This value for $R_t$ is very similar to the measured value of 275.5 ± 4.2 $\Omega \cdot cm^2$. Therefore, the GABA-induced resistance changes are consistent with a conductance increase *only* at the basolateral membrane. This observation can be used to determine GABA-evoked $\Delta R_B$ in the absence and presence of either basal $Ba^{2+}$ or DIDS. In five tissues, the addition of GABA in the absence and presence of $Ba^{2+}$ decreased $R_B$ by 121 ± 20 $\Omega \cdot cm^2$ and 35 ± 13 $\Omega \cdot cm^2$, respectively (mean ± SEM; $P < 0.01$). This difference in $\Delta R_B$ was not due to a decrease in $I_{gaba}$ in the presence of basal $Ba^{2+}$ ($I_{gaba}$ = 17 ± 1 $\mu A/cm^2$ (mean ± SEM) in control Ringer and 16 ± 2 $\mu A/cm^2$ in $Ba^{2+}$-containing Ringer). In four tissues, the addition of GABA in the absence and presence of basal DIDS decreased $R_B$ by 128 ± 15 $\Omega \cdot cm^2$ and 450 ± 77 $\Omega \cdot cm^2$, respectively (mean ± SEM; $P < 0.02$). In the absence of basal DIDS, $I_{gaba}$ = 19 ± 2 $\Omega \cdot cm^2$ (mean ± SEM), and in the presence of basal DIDS, $I_{gaba}$ = 30 ± 4 $\mu A/cm^2$. This significant increase in $I_{gaba}$ due to the presence of basal DIDS may account for some the increase in $\Delta R_B$ from the addition of GABA. Because the GABA-induced $\Delta R_B$ is significantly reduced in the presence of basal $Ba^{2+}$, but *not reduced* in the presence of basal DIDS, we conclude that apical GABA increases basolateral membrane K conductance (see Discussion).

### $[K]_o$ Regulation of GABA Transport

Previous work in the frog has demonstrated that subretinal space K concentration ($[K]_o$) is ~4 mM in the dark and decreases to 1–2 mM ~30 s after light onset (Oakley and Steinberg, 1982). This small, physiological decrease in $[K]_o$ in vivo can hyperpolarize $V_A$ by 10–15 mV. These $[K]_o$ changes in vitro significantly alter transport across the RPE (Joseph and Miller, 1991; Edelman, Lin, and Miller, 1994). The subretinal space change in $[K]_o$ following a dark/light transition can be mimicked in vitro with a solution change of apical Ringer containing 5 mM $[K]_o$ to Ringer containing 2 mM $[K]_o$ (Lin and Miller, 1991; Bialek and Miller, 1994). Because of the electrogenic nature of the GABA transporter, we were interested to determine if the GABA-induced currents calculated above could be affected by Ringer containing either 2 mM or 5 mM $[K]_o$.

Fig. 19, *A* and *B*, shows the membrane resistance responses to 500 $\mu M$ GABA in Ringer containing 2 mM $[K]_o$ and in Ringer containing 5 mM $[K]_o$. Fig. 19, *C* and *D*, shows the membrane voltage responses under these conditions. These data can be used to calculate the GABA-induced current, $I_{gaba}$, in the two cases. In 2 mM $[K]_o$ Ringer, $R_t$ and $a$ before addition of GABA were 208 $\Omega \cdot cm^2$ and 0.54, respectively, and $\Delta V_A$ and $\Delta V_B$ upon addition of GABA were 3.4 mV and 2.2 mV, respectively. In 5 mM $[K]_o$ Ringer, the $R_t$ and $a$ before addition of GABA were 188 $\Omega \cdot cm^2$ and 0.92, respectively, and $\Delta V_A$ and $\Delta V_B$ upon addition of GABA were 2.5 mV and 1.3 mV, re-

spectively. Based on the results in Fig. 19, we calculate $I_{gaba}$ to be 17 $\mu$A/cm$^2$ in 2 mM [K]$_o$ and 13 $\mu$A/cm$^2$ in 5 mM [K]$_o$.

The same protocol was used on a total of seven tissues. In Ringer containing either 2 mM or 5 mM [K]$_o$, we calculated $I_{gaba}$ to be 17.2 ± 1.2 $\mu$A/cm$^2$ and 12.4 ± 0.7 $\mu$A/cm$^2$ (mean ± SEM), respectively ($P < 0.01$; paired $t$ test). In each of these tissues, apical addition of GABA was first done in Ringer containing 2 mM [K]$_o$ followed by the addition of GABA in Ringer containing 5 mM [K]$_o$. Membrane potentials were
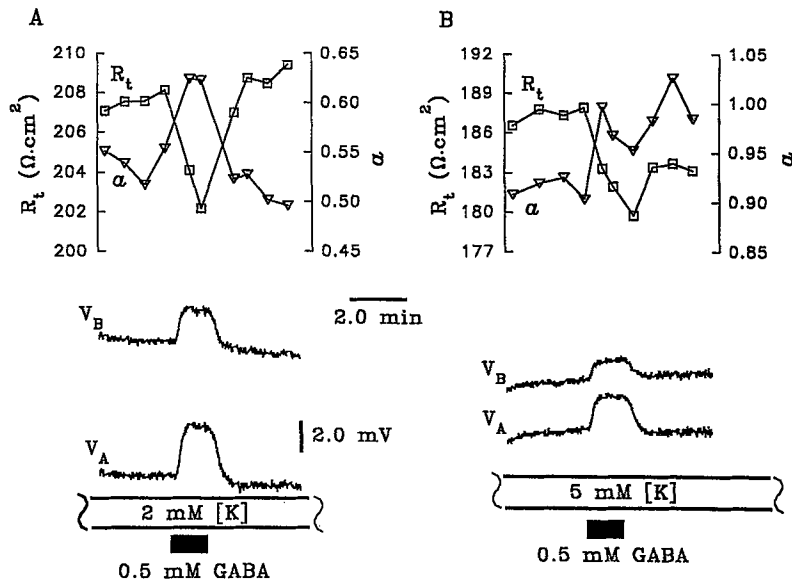


FIGURE 19. Membrane voltage and resistance responses to 1 min apical addition of 500 $\mu$M GABA in Ringer containing either 2 mM [K]$_o$ ($A$) or Ringer containing 5 mM [K]$_o$ ($B$). Values of $R_t$ and $a$ before the addition of GABA as well as $\Delta V_A$ and $\Delta V_B$ during exposure to GABA were used to calculate the GABA-induced currents, $I_{gaba}$, in these different [K]$_o$-containing Ringer solutions. Recordings were made from the same cell. The results from this experiment show that $I_g$ is ~40% larger than 2 mM [K]$_o$ Ringer than in 5 mM [K]$_o$ Ringer. (See text for details.) $V_A = -82.2$ mV before the addition of GABA in $A$. The solution change from 2 mM [K]$_o$ to 5 mM [K]$_o$ depolarized $V_A$ by $V_B$ by 13.8 and 7.5 mV, respectively, decreased $R_t$ by 19 $\Omega$·cm$^2$, and increased $a$ by 0.50 (voltage and resistance traces not shown).

allowed to stabilize before perfusing GABA-containing Ringer in the apical bath. In four of these tissues, a following control was performed in 2 mM [K]$_o$, and the calculated values for $I_{gaba}$ in these conditions were 95 ± 9% of the initial control values. These results demonstrate that the GABA-mediated currents are significantly smaller in Ringer containing 5 mM [K]$_o$ than in Ringer containing 2 mM [K]$_o$. The transporter activity is therefore significantly reduced in conditions that approximate [K]$_o$ in the SRS following prolonged darkness, during which extracellular

GABA levels are thought to be higher (Yang and Wu, 1989; Stockton and Slaughter, 1991).

DISCUSSION

*Pharmacology*

The present data show that GABA depolarizes the apical membrane of the frog retinal pigment epithelium by activating a Na-dependent, electrogenic transporter. This conclusion is supported by a number of observations: (*a*) the voltage effects of GABA were not blocked by the GABA-receptor antagonists, picrotoxin, bicuculline, and phaclofen, and not mimicked by the ionotropic GABA-receptor agonists, muscimol and phenobarbital; (*b*) nipecotic acid, a competitive inhibitor of GABA uptake, elicited electrical responses similar to those of GABA and inhibited the effects of GABA; (*c*) removal of Na completely blocked the electrical effects of GABA; (*d*) addition of GABA significantly increased intracellular Na levels; and (*e*) net apical-to-basal transport of [$^3$H]-GABA was observed in radiotracer flux studies. The small voltage effects of the GABA$_B$-receptor agonist, baclofen, suggest that the carrier also recognizes baclofen, but at a lower affinity. A surprising finding of the present study was that GABA and nipecotic acid significantly inhibited the electrical effects of a previously reported β-alanine sensitive taurine transporter (Lake, Marshall, and Voaden, 1977; Voaden, Lake, Marshall, and Morjaria, 1977; Miller and Steinberg, 1976 and 1979; Ostwald and Steinberg, 1981; Scharschmidt et al., 1988).

A number of observations support the presence of a single low affinity carrier for GABA, nipecotic acid, β-alanine and taurine. We showed, for example, that the voltage effects of 500 μM apical GABA inhibited the voltage effects of 10 mM and 1 mM taurine by 79 and 93%, respectively. In addition, 1 mM apical nipecotic acid inhibited the effects of 10 and 1 mM taurine by 74 and 90%, respectively. Given the $K_m$ values determined for GABA (160 μM), taurine (850 μM), and nipecotic acid (420 μM) and assuming a single, competitive transport mechanism for all three substrates, one can calculate the predicted percentage inhibition of these taurine responses by either GABA or nipecotic acid. These calculations show that GABA (500 μM) should inhibit the responses to 10 and 1 mM taurine by 80 and 91%, respectively; nipecotic acid (1 mM) should inhibit the responses to 10 and 1 mM taurine by 74 and 85%, respectively. These predicted values are in good agreement with the observed effects (see Figs. 10 and 11). The dose dependence of the voltage responses to GABA, taurine, nipecotic acid, and β-alanine all showed first-order Michaelis-Menten kinetics and provides additional support for a single, saturable transport system for each substrate. The results of this study strongly suggest that this transporter should be classified as a β-alanine and nipecotic acid sensitive GABA carrier that additionally transports taurine.

Several studies report a common transport system for GABA and taurine in other neural and glial cell types, but these systems were either characterized by a much higher affinity for either or both substrates, or a considerably greater difference in specificity for GABA or taurine than the observed effects in the bullfrog RPE (Bowery, Brown, White, and Yamini, 1979; Martin and Shain, 1979; Kontro and Oja,

1981, 1983; Holopainen, Kontro, Frey, and Oja, 1983; Tasaka, Sakai, Tosaka, and Yoshihama, 1989). A unique pharmacological property of the present carrier is that it transports GABA and taurine with relatively similar affinities.

### Ionic Dependence

Substitution of apical Na with NMDG completely and reversibly abolished the voltage effects of GABA, indicating an external requirement for Na. The importance of the Na gradient in providing the driving force for GABA transport is shown in experiments where apical addition of ouabain significantly reduced the GABA-induced depolarization of $V_A$. Furthermore, imaging of intracellular Na levels directly demonstrated that addition of apical GABA increased intracellular Na levels. Taken together these results support a Na-dependent GABA carrier that depolarizes $V_A$ by transporting Na inward across the apical membrane.

Substitution of apical Cl with gluconate, methane sulfonate, and cyclamate did not significantly affect the magnitude of voltage changes to GABA, but did significantly reduce the GABA-evoked currents by $\sim$40%. These results suggest that this putative transporter either has a partial external requirement of Cl, or that removal of apical Cl leads to a reduction in driving force for GABA transport by increasing $a_{Na}^i$ or by changing the apical membrane potential. The latter explanations are unlikely because the effects of apical Cl removal on $a_{Na}^i$ and on $V_A$ should instead increase the driving force for GABA uptake. In the absence of Cl, the apical Na,K,2Cl cotransporter will reverse direction, transport Na out of the cell (Bialek and Miller, 1994), and reduce $a_{Na}^i$, and thereby increase the Na gradient across the apical membrane. Furthermore, removal of apical Cl hyperpolarized $V_A$ by 10.2 $\pm$ 2.0 mV, which should also stimulate GABA transport (Malchow and Ripps, 1990; Mager et al., 1993). A number of reports have indicated either a partial or absolute dependence of GABA uptake on external Cl (Borden, Smith, Hartig, Branchek, and Weinshank, 1992; Cammack and Schwartz, 1993). In terms of its Cl dependence, the GABA transporter in the RPE resembles the cloned GAT2 and GAT3 transporters from the mouse brain, where GABA uptake was inhibited by 50% (GAT-2) and 80% (GAT-3) in Cl-free Ringer (Borden et al., 1992).

### The RPE as a "Sink" for GABA

The present study implicates the RPE as a potential sink for neurotransmitters, which is a functional role of this tissue that has been explored in a number of radio-labeled uptake studies (Pautler and Tangerdy, 1986; Sivakami et al., 1992; Salceda and Saldana, 1993; Miyamoto and Del Monte, 1994). GABA uptake mechanisms at the RPE apical membrane may be particularly important in nonmammalian systems because Müller cells do not take up GABA (with the exception of the skate retina). The apical processes in the bullfrog RPE are anatomically poised to respond to changes in GABA concentration in the subretinal space (SRS) because they extend 60–100 $\mu$M from the cell body into the inner photoreceptor layer (Nilsson, 1964).

The determination of $I_{gaba}^m$ and $K_m$ (Results) can be used to calculate the theoretical time course of clearance of GABA from the SRS given a step input of various

GABA concentrations from the retina. The volume of the SRS associated with a 1 cm$^2$ area of RPE can be estimated by multiplying the depth ($\sim$100 $\mu$m) of the photoreceptor layer and the percent (7%) extracellular space (Faber, 1969; Ogden and Ito, 1971). Removal of GABA from this SRS volume can be modeled using a differential equation based on a first-order Michaelis Menten equation:

$$\frac{d(\text{GABA})}{dt} = \frac{d(\text{GABA})_{max}}{dt} \cdot \frac{[\text{GABA}]_o}{K_m + [\text{GABA}]_o} \tag{6}$$

where $d(\text{GABA})\,dt$ = rate of GABA removal from SRS (nmol/s); $d(\text{GABA})_{max}/dt$ = maximal rate of GABA uptake by the transporter (=0.22 nmol/s, determined from $I_{gaba}$ for a 1 cm$^2$ patch of RPE); $[\text{GABA}]_o$ = concentration in SRS ($\mu$M); and $K_m$ = 160 $\mu$M. The rate of GABA removal was determined by assuming that each GABA molecule is transported with the inward translocation of a single positive charge. Fig. 20 shows the normalized time course for GABA removal for a step input of 100 $\mu$M (trace 1) and for a step input of 1 $\mu$M (trace 2) based on the solution of Eq. 6. Both traces indicate that GABA concentrations can be reduced by an order of magnitude in less than 1.5 s. The time scale for the 1 $\mu$M step input of GABA reveals that, despite its low affinity, this transporter has the capacity to effectively remove GABA even when starting levels are orders of magnitude lower than the transporter's $K_m$.

The present data can be also used to calculate the relative density of the GABA carrier. Because invaginations of the apical membrane increase the surface area of the RPE by a factor of 30 (Miller and Steinberg, 1977), the specific GABA transporter current per unit area of apical membrane is 22 $\mu$A/cm$^2$ divided by 30, or 0.73 $\mu$A/cm$^2$. This current is the product of the charge transferred inward per translocation cycle (1.60 $\times$ 10$^{-13}$ $\mu$coul), the turnover rate (6 to 13 per second for GAT1) of the GABA carrier (Mager et al., 1993) and the density of the transporter.
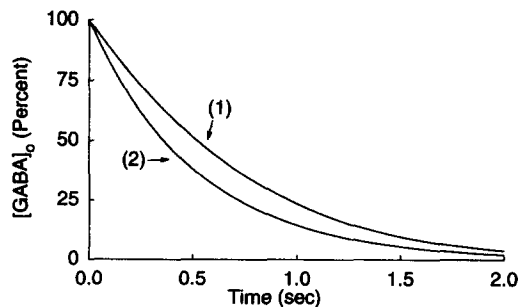


FIGURE 20. Theoretical time course for the clearance of GABA from the subretinal space given various input concentrations of GABA. The differential equation for rate of GABA uptake based on first-order Michaelis-Menten kinetics (Eq. 6) was solved using a mathematical modeling program and a theoretical plot of [GABA]$_o$ against time is shown here. Trace *1* shows the normalized (percentage) time course of a decrease in [GABA]$_o$ assuming an initial concentration of 100 $\mu$M. Trace *2* shows the time course assuming [GABA]$_o$ is 1 $\mu$M. In both cases greater than 10-fold reduction of [GABA]$_o$ can be seen after 1.5 s.

This calculation gives a transporter density of 3,500 to 7,500/$\mu$m$^2$. This value is similar to the density of GAT1 expression (7,200/$\mu$m$^2$) in *Xenopus* oocytes and the density of glucose transporter expression in the intestine (Mager et al., 1993; Peerce and Wright, 1984).

The radiotracer flux results indicate that under steady state conditions, the RPE transports GABA (200 $\mu$M) from the retina to the choroid with a net flux of approximately 31 nEq/cm$^2$·h. The net flux of GABA is a factor of 10 less than the expected flux based on the transporter current probably because the flux results reflect a steady state measurement across both membranes that includes significant recycling, whereas transporter currents were calculated based on initial uptake.

### Relationship Between GABA Carrier, Na/K ATPase, and K Conductance

GABA-evoked stimulation of the Na/K ATPase was observed by using a protocol where apical K conductance was first blocked by Ba$^{2+}$, and then step changes in [K]$_o$ were made in the absence and presence of GABA. We found that the voltage changes due to this $\Delta$[K]$_o$ manipulation was increased by approximately 20% in the presence of 200 $\mu$M apical GABA. These results are similar to that of Scharschmidt, Griff and Steinberg (1988) in which addition of apical taurine stimulated the Na/K ATPase activity in the frog RPE. The observation that GABA elevates $a^i_{Na}$ strongly suggests that the pump is stimulated by an increased occupation of the internal Na binding sites of the ATPase and a resultant increase in pump turnover rate (Sejersted, Wasserstrom, and Fozzard, 1988).

Fig. 21 summarizes the membrane transport mechanisms that contribute to the observed voltage and resistance responses to apical GABA. Activation of the GABA/taurine transporter elevates $a^i_{Na}$ and in turn stimulates the Na/K ATPase. Increased pump activity would be expected to elevate $a^i_K$ and increase basolateral K conductance, although other mechanisms may be involved in this GABA-mediated conductance increase (Joseph and Miller, 1991). The different components of the membrane voltage and resistance responses shown in Fig. 2 can be understood in terms of the interactions between the GABA transporter, the Na/K pump, and the basolateral K conductances. During the first 10–15 s after the arrival of GABA to the apical membrane, a carrier-associated current generator is activated. The electrical response during this phase is characterized by a linear and rapid depolarization of $V_A$ and $V_B$, a rapid decrease in TEP, and no measurable changes in tissue resistance. During the next phase (15–45 s) there is a much slower depolarization of $V_A$ and $V_B$, a rapid decrease in TEP, an increase in $a$ and decrease in $R_t$. This phase of the response probably represents a complex interaction between the carrier, the pump, and the basolateral K conductances. After 45 s, $V_A$ slowly hyperpolarized along with a concomitant increase in TEP, which can be attributed to an increase in pump activity that generates a larger outward current across the apical membrane. The resistance recordings during this period indicate that the basolateral K conductances remain increased. It is interesting to note that the increase in $a^i_{Na}$ (Fig. 8) follows a time course very similar to that of the increase in $a$ during the five minute pulse of GABA in Fig. 2 $B$, which implies that Na-accumulation is involved in producing the GABA-evoked resistance changes. Because an increase in pump stimulation and K conductance would hyperpolarize $V_A$ and $V_B$, their combined ef-

fects would offset the GABA transporter-associated depolarization of $V_A$. Therefore the previously calculated GABA-induced current of $I_{gaba}$ of 22 $\mu A/cm^2$ (Results) is probably significantly lower than the actual transporter-associated current.

### Functional Role during Light/Dark Transitions

Horizontal cells are the most proximal source of GABA to the apical membrane of the RPE, and release and take up GABA via an electrogenic transporter. Light offset causes a large depolarization of the horizontal cells and result in a transporter-mediated increase of extracellular GABA, whereas light onset hyperpolarizes the horizontal cells and causes a large increase in driving force for GABA uptake (Schwartz, 1982, 1987; Yazulla and Kleinschmidt, 1983; Yang and Wu, 1989; Mal-
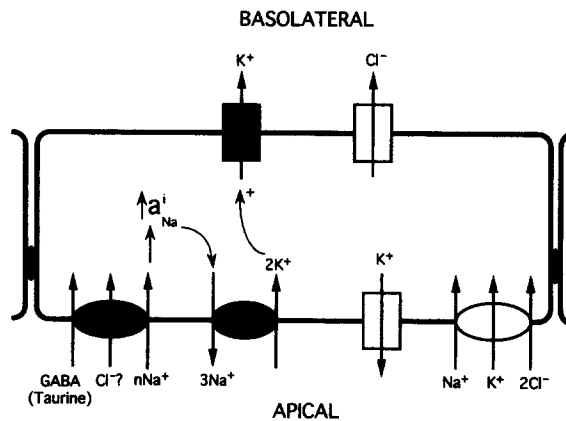


FIGURE 21. A summary of some of the important membrane transport mechanisms in the bullfrog RPE (showed as unfilled and filled icons). Putative mechanisms contributing to the GABA-elicited electrical responses are shown as filled icons. At the apical membrane are the GABA-taurine transporter, the NA/K ATPase, the bumetanide-sensitive Na,K,2Cl cotransporter, and the $Ba^{2+}$-inhibitable K conductances. The basolateral membrane contains a DIDS-inhibitable Cl conductance and a $Ba^{++}$-inhibitable K conductance. Activation of the GABA transporter causes a stimulation of the Na/K ATPase, probably through the elevation of $a^i_{Na}$ and an increase in basolateral K conductances.

chow and Ripps, 1990; Stockton and Slaughter, 1991; Kamermans and Werblin, 1992).

GABA uptake by the RPE from the SRS may also in part be regulated by light-induced changes in voltage and ionic gradients across the apical membrane. It has been demonstrated in vivo that $[K]_o$ in the subretinal space decreases from $\sim 5$ to $\sim 2$ mM following light onset (Oakley and Steinberg, 1982). In vitro this SRS change in $[K]_o$ is approximated by changing the $[K]_o$ composition of the Ringer perfusing the apical membrane from 5 to 2 mM. This in vitro reduction of $[K]_o$ from 5 to 2 mM hyperpolarized $V_A$ by 10–15 mV and significantly lowered $a^i_{Na}$ (latter observation is unpublished), both of which would significantly increase the driving force for GABA uptake. We report that the GABA transporter-associated currents were, in fact, $\sim 40\%$ larger in Ringer containing 2 mM $[K]_o$, which suggests that GABA uptake is stimulated under $[K]_o$ conditions that approximate light onset. This increased uptake would facilitate GABA removal from the SRS; conversely, in

the dark a decrease of GABA uptake would help maintain higher concentrations in the SRS.

The present study shows that the bullfrog RPE contains a single transporter for GABA, the major inhibitory retinal neurotransmitter, and for taurine, the most abundant amino acid in the retina. It is likely these two amino acids compete for uptake via this carrier in vivo, and both substrates can in turn be regulated by dark/light transitions. For example, a release of taurine from the frog rod outer segments is proposed to occur following light onset (Salceda, Lopez-Colome, and Pasante-Morales, 1977), yet during this period GABA uptake presumably increases. This transporter could also operate as a heterocarrier, in which uptake of GABA stimulates release of taurine from the RPE. GABA uptake by the RPE could thus provide a mechanism for taurine (intracellular taurine levels are $\sim$30 mM) to be transported into the SRS. Coordinated uptake and release by this carrier would likely determine the activities of GABA and taurine in the subretinal space.

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