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G Protein-mediated Activation of a Nonspecific Cation Current in Cultured Rat Retinal Pigment Epithelial Cells

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Abstract. We used whole-cell patch-clamp recording techniques to investigate G protein-activated currents in cultured rat retinal pigment epithelial (RPE) cells. Using 140 mM KCl intracellular and 130 mM NaCl extracellular solutions, rat RPE cells possessed both inward and outward K^+ currents. Upon addition of the nonhydrolyzable guanine triphosphate analogue, guanosine-5′-O-(3-thiophosphate) (GTPγS, 0.1 mM), to the recording electrode, a nonspecific cation (NSC) current was elicited. The NSC current had a mean reversal potential of +5.7 mV in 130 mM extracellular NaCl with Cs^+-aspartate in the pipette, and was not affected by alterations in the extracellular Ca^{2+} or Cl^- concentration. The GTPγS-activated current was found to be permeable to several monovalent cations (K^+, Na^+, choline, TRIS, and NMDG). Addition of fluoroaluminate, an activator of large molecular weight heterotrimeric GTP-binding proteins (G proteins), to the intracellular recording solution activated the NSC current. The G protein involved was pertussis toxin (PTX)-sensitive, since GTPγS failed to activate the NSC current in cells pretreated with PTX. Further investigation of second messenger molecules suggested that activation of the NSC current was not affected by alterations in intracellular Ca^{2+} or ATP. From these results, we conclude that a G protein-regulated NSC current is present in rat RPE cells. Activation of the NSC current may sufficiently depolarize RPE cells to activate outward K^+ currents. This would provide a mechanism by which these cells could rid themselves of accumulated K^+.

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Key words: Retinal pigment epithelium — G protein — Patch-clamp — Nonspecific cation channels

Introduction

The retinal pigment epithelium (RPE) is a single layer of specialized epithelial cells lying external to the neurosensory retina. The RPE serves a variety of functions which include mechanical and metabolic support for the retina and maintenance of a homeostatic flux of ions and water between the retina and choroid (Steinberg & Miller, 1979). The transport of fluid by the RPE is thought to be a significant factor in maintaining retinal attachment (Zauberman, 1979). Other functions of the RPE that are essential for the processing of incoming light rays and for the integrity and function of the neural retina include acting as a pigment barrier to absorb excess light coming through the sclera, transporting numerous metabolites to and from both the retinal cells and the choroidal circulation, phagocytosis of shed rod and cone outer segments, and the storage and transport of vitamin A (Steinberg & Miller, 1979). The RPE forms one boundary of the subretinal space and the photoreceptor outer segments and Mueller cells form the other, therefore, information on the regulation of ionic conductances in this epithelium is essential for understanding changes in ion and fluid homeostasis in the subretinal space.

Using patch-clamp techniques, a variety of ion channels have been described in freshly isolated and cultured RPE cells from various amphibian and mammalian species. Information obtained from patch-clamp studies has demonstrated that mammalian RPE cells exhibit multiple types of K^+-selective ion channels (Fox, Pfeffer & Fain,

1988; Strauss, Richard & Wienrich, 1993; Strauss, Weiser & Wienrich, 1994; Tao, Rafuse & Kelly, 1994) as well as voltage-dependent Ca^{2+} (Ueda & Steinberg, 1993; Strauss & Wienrich, 1994) and anion channels (Botchkin & Mathews, 1993; Ueda & Steinberg, 1994). A voltage-dependent tetrodotoxin-sensitive Na^+ current has also been described in cultured but not freshly isolated rat RPE cells (Botchkin & Mathews, 1994).

GTP-binding proteins are involved in the transduction of signals from a variety of receptors to effectors, such as ion channels and enzymes (*for review see* Breitwieser, 1991; Brown, 1993; Rodbell, 1992). The actions of many hormones and neurotransmitters in regulating fluid transport in exocrine cells are mediated via G protein-coupled pathways (Marty, 1987). Therefore, our purpose was to examine the role of G proteins in regulating ionic conductances in RPE cells. In this report, we describe for the first time, the presence of a nonspecific cation current (NSC) in cultured rat RPE cells and show that the activation of this current is G protein-mediated.

Part of this work has been presented in abstract form (Poyer & Kelly, 1995).

Materials and Methods

Cell Dissociation and Culture

Long Evans rats (age 8 to 12 days) were anaesthetized using halothane, sacrificed by decapitation, and the eyes enucleated. Rats were maintained and euthanized in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The RPE cells were isolated using a modification of the method of Wang, Koutz and Anderson (1993). Enucleated eyes were placed in calcium-free Hank's EDTA (CFHE; Gibco BRL, Burlington, Ontario). The connective tissue was removed and the globes bisected along the equator, discarding the anterior portion. The posterior eye cups were then placed in CFHE containing 220 U/ml hyaluronidase type III (Sigma Chemical, St. Louis) and 65 U/ml collagenase A (Boehringer Mannheim, Laval, Quebec) for 10 min at 37°C. In fresh CFHE, the neural retina was peeled from the eye cup using forceps and the remaining posterior segment incubated at 37°C for 5 min in CFHE enzyme solution. Following a second transfer to fresh CFHE, the RPE was gently removed from the remaining eyecup and collected using a fire-polished Pasteur pipette. RPE tissue was triturated through a narrow-bore fire-polished Pasteur pipette to yield a suspension of single cells and small clumps of RPE tissue. The cell suspension was washed and resuspended in 500 μl of Dulbecco's modified Eagle's medium (DMEM) plus 20% fetal calf serum, 0.5% penicillin-streptomycin (Gibco BRL, Burlington, Ontario), and gentamicin (50 μg/ml; Sigma Chemical, St. Louis). The cell viability was estimated using trypan blue exclusion and was generally greater than 90%. The cells were seeded onto glass coverslip (12 mm diameter) in 4-well culture dishes and placed in a 37°C incubator with an atmosphere of 5% CO_2/95% O_2. The media was changed 24 h following the initial plating to DMEM containing 10% fetal calf serum and antibiotics. Cells were maintained in culture for 2–5 days prior to electrophysiological recording.

Superfusion and Solutions

Cells attached to glass coverslips were placed in a shallow recording chamber (volume 2 ml) and positioned on the stage of a Nikon inverted microscope. The recording chamber was superfused (1–2 ml/min) with a variety of solutions from elevated reservoirs, and the flow rate was regulated by a series of valves. Perfusands containing $NaHCO_3$ were continuously bubbled with 5% CO_2/95% O_2. All extracellular and intracellular solutions were adjusted for pH 7.3–7.4 and the osmolarity was measured by freezing point depression (Osmette A, Fischer Scientific, Nepean, Ont.). Extracellular solutions had a final osmolarity of 330–340 mOsm and the osmolarity of intracellular solutions was between 310–320 mOsm. The use of a slightly hyperosmotic external solution was found to be effective in eliminating transient changes in ionic conductances, which occurred as a result of osmotic changes during the initial period of whole-cell recording. The Table lists the various extra- and intracellular solutions used. The standard extracellular solution consisted of (in mM): NaCl, 130; KCl, 5; Na^+-HEPES, 10; $NaHCO_3$, 10; $MgCl_2$, 1; and glucose, 10 (solution A, Table). Replacement of extracellular Na^+ was accomplished using equimolar substitution with either choline chloride, TRIS-hydrochloride, N-methyl-D-glucamine (NMDG) chloride, or KCl (solutions B-E, Table). Extracellular calcium was replaced by 0.2 mM $CaCl_2$ with 1.5 mM EGTA (solution G, Table). The extracellular calcium concentration with this substitution was estimated to be 10 nM. Calcium concentration was calculated using a software program based on the algorithm of Goldstein (1979 [provided by J. Kleinschmidt]). The standard intracellular pipette solution consisted of (in mM): KCl, 140; HEPES (free acid), 20; $MgCl_2$, 1; $CaCl_2$, 0.4; EGTA, 1; ATP, 1; and GTP, 0.1 (solution H, Table). Free Ca^{2+} in the pipette solution was estimated at 100 nM. When low chloride intracellular solutions were used, 140 mM KCl was replaced with 110 mM K^+-aspartate and 30 mM KCl (solution I, Table). Low chloride solutions were made K^+-free by substituting 100 mM Cs^+-aspartate, 10 mM Na^+-HEPES, and 30 mM NaCl for K^+-aspartate and KCl (solution J, Table). When Cl^- was reduced in the extracellular bathing medium, this was accomplished by equimolar substitution of Cl^- with aspartate ions (solution F, Table). For some experiments, GTP was replaced by 0.1 mM GDPβS, GTPγS or GppNHp. The fluoroaluminate complex, used in several studies, was formed in the low chloride, K^+-aspartate (solution I, Table) or Cs^+-aspartate (solution J, Table) intracellular solution by the addition of 1 mM NaF and 25 μM $AlCl_3$ or 10 mM NaF and 100 μM $AlCl_3$. Potassium channel blockers (barium, 4-aminopyridine [4-AP], tetraethylammonium [TEA], and quinine), the Na^+ channel blocker amiloride, and the cation channel blocker, gadolinium were added to the extracellular solution and superfused at concentrations cited in Results. All chemicals were purchased from Sigma Chemical (St. Louis).

Electrophysiological Recording Techniques

We used the whole-cell patch clamp technique to measure currents in isolated RPE cells (Hamill et al., 1981). The recording conditions used have been described previously (Tao et al., 1994). Briefly, patch electrodes were pulled from borosilicate glass micropipettes with diameters of 1.5 mm outside and 1.1 mm inside (Sutter Instruments, Novato, CA) using a two-stage vertical microelectrode puller (Narishige model PP83, Tokyo, Japan). Electrodes were coated with beeswax to reduce capacitance and had resistances of 2–3 MΩ when filled with intracellular solution and placed in the extracellular bathing solution. Offset potentials were nulled using the amplifier circuitry before seals were made on cells. Liquid junction potentials (LJP) between the bath and patch clamp electrodes were measured experimentally and defined as the potential of the bath solution with respect to the pipette solution (Barry & Lynch, 1991). For whole-cell recording the membrane potential of the cell, V_m, was then calculated as $V_m = V_p - \text{LJP}$. To confirm experimentally generated measurements, LJPs were also calculated with a software program (JPCalc, Version 2.00; P.H. Barry, Sydney, Australia) which uses the generalized Henderson equation for N polyvalent ions,

Table. Composition of extracellular and intracellular solutions

Extracellular solutions (mM)	A	B	C	D	E	F	G
NaCl	130					30	130
KCl	5	5	5	5	145	5	5
Na-HEPES	10	10	10	10	10	10	10
Glucose	10	10	10	10	10	10	10
$MgCl_2$	1	1	1	1	1	1	1
$CaCl_2$	1	1	1	1	1	1	0.2
$NaHCO_3$	10					10	10
Choline-Cl		140					
TRIS-Cl			140				
NMDG-Cl				140			
EGTA							1.5
Na^+-aspartate						100	

Intracellular solutions (mM)	H	I	J	K	L
NaCl			30	30	
KCl	140	30			140
Na-HEPES			10	10	
HEPES acid	20	20			20
$MgCl_2$	1	1	1	1	1
$CaCl_2$	0.4	0.4	0.4	0.4	0.1
EGTA	1	1	1	1	1
K^+-aspartate		110			
Cs-aspartate			100		
BAPTA					10
CsCl				110	
ATP	1	1	1	1	1
GTP	0.1	0.1	0.1	0.1	0.1

$$LJP = (RT/F)S_F\, In \left\{ \sum_{i=1}^{N} z_i^2 u_i a_i^P \,/\, \sum_{i=1}^{N} z_i^2 u_i a_i^S \right\} \quad (1)$$

where

$$S_F = \sum_{i=1}^{N} [(z_i u_i)(a_i^S - a_i^P)] \,/\, \sum_{i=1}^{N} [z_i^2 u_i (a_i^S - a_i^P)]$$

where *LJP* represents the potential of the solution (*S*) with respect to the pipette (*P*) and *u*, *a* and *z* represent the mobility, activity and valency (including sign) of each ion species (*i*); *R* is the gas constant, *T* is the temperature in *K* and *F* is the Faraday, so that $RT/Fln = 58.2$ $\log_{10}$ in mV at a temperature of 20°C.

All the data shown has been corrected for *LJP*s. The *LJP* using standard intracellular and extracellular solutions (solutions A and H, Table) was 2.5 mV. In asymmetric low chloride solution (solutions A and I, Table) the *LJP* was 9 mV. When low chloride extracellular solution and Cs^+-aspartate intracellular solution (solutions J and F, Table) was used the *LJP* was 4 mV. Under experimental conditions where $[Cl^-]_{out}$ or $[Cl^-]_{in}$ was varied the *LJP*s were −2.5 mV (solution F and H; solution *A* and H, Table) and 10 mV (solution A and J, Table) respectively. In cation substituted extracellular solutions with Cs^+-aspartate in the pipette the *LJP*s were 15 mV (solution B, C or D and J, Table) and 5 mV (solution E and J, Table). When low calcium solutions were used (solutions G and L, Table) the *LJP* was 3 mV. When data has been leak subtraction, this is noted in the figure legends. The reference electrode used was either a standard Ag/AgCl pellet in conjunction with a 3 M KCl-agar bridge or a sealed electrode/salt bridge combination (Dri-Ref-2: World Precision Instruments, Sarasota, FL).

Membrane potential and ionic currents were recorded with an Axopatch 1D amplifier (Axon Instruments, Foster City, CA). Currents were filtered with a 4-pole low-pass Bessel filter (−3 dB at 1 kHz) and digitized at a sampling frequency of 5 kHz using pCLAMP software (Axon Instruments). Current and voltages were displayed on a Gould TA240 chart recorder and were stored on both computer disk and videotape using a pulse code modulator (Medical Systems, Greenvale, NY). All experiments were conducted at room temperature (20–22°C). Values for cell capacitance were obtained from the capacitance compensation circuitry on the amplifier. Measures of series resistance were obtained from the amplifier and were always less than 20 MΩ. In most cases 80% series resistance compensation was used. Data are presented as mean ± SEM unless otherwise noted.

Permeability ratios (PB/PA) were based on experimentally measured reversal potentials and were calculated using the following derivation of the Goldman-Hodgkin-Katz equation (Hille, 1992). The ratios are stated in the text as permeability relative to Na^+:

$$\Delta V_r = V_r B - V_r A = 58\, log \frac{PB[B]_o}{PA[A]_o} \quad (2)$$

where V_r = reversal potential, A = Na^+-containing extracellular solution, B = substituted extracellular solution (*see* Table for solutions).

Results

Whole-cell Currents in Rat RPE Cells

Figure 1 shows inward and outward whole-cell currents recorded from rat RPE cells cultured for 2–5 days. With

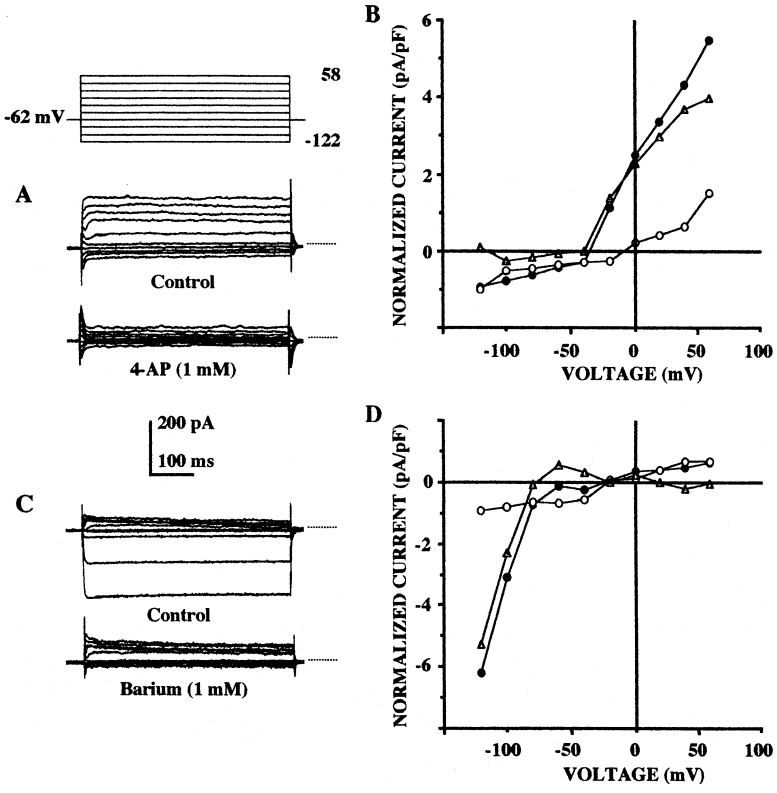

Fig. 1. Whole-cell patch-clamp recordings from two representative cells showing the effect of 1 mM 4-aminopyridine (4-AP, [*A,B*]) and 1 mM barium (*C, D*) on inward and outward currents in rat retinal pigment epithelial cells. The extracellular solution was standard 130 mM NaCl while the intracellular solution was standard 140 mM KCl with the addition of 100 μM GTP (solutions *A* and *H*, Table). The voltage protocol is shown at the top of panel *A.* Cells had a whole-cell capacitance of 25 and 20 pF for panels *A* and *B*, respectively. Data were leak subtracted at 1.6 GΩ for panel *A* and not leak subtracted for panel *B.* 4-AP virtually eliminates the outward current while barium blocks the inward current (● control; ○ blocker (4-AP or barium); △ difference current).

standard 130 mM NaCl extracellular and 140 mM KCl intracellular solutions, whole-cell currents were found to be primarily K^+-selective. Confirmation of the ion selectivity of the outward current was carried out using tail current reversal analysis with varying extracellular K^+ concentrations (*not shown*). The mean (±SEM) reversals for tail currents were −72, −37 ± 2, and −26 ± 2 mV when cells were superfused with 5, 25, and 50 mM extracellular K^+, respectively ($n = 3$ cells). This is a 47 mV change in reversal potential per 10-fold change in extracellular K^+ concentration, approaching the Nernst predicted value of 58 mV per 10-fold change in extracellular K^+ concentration and indicates that these currents are largely carried by K^+. The most frequently observed outward K^+ current in rat RPE cells was a voltage- and time-dependent outward current (I_K) which resembled the delayed rectifier K^+ current described in fresh and cultured RPE cells from a variety of amphibian and mammalian species (Hughes & Steinberg, 1990; Strauss et al., 1993; Wen et al., 1993; Strauss et al., 1994; Tao et al., 1994). I_K was blocked (70 ± 8%, $n = 3$) by 1 mM 4-AP (Fig. 1, panel *A*) and was significantly reduced (42 ± 4%, $n = 4$) by 5 mM TEA (*not shown*). Figure 1, panel *B* shows the current-voltage plot for the currents in panel *A,* before and after superfusion with 1 mM 4-AP. Also shown is the difference current for the 4-AP sensitive current, obtained by digital subtraction of the 4-AP blocked current from the control current. Inwardly rectifying K^+ current (I_{Ki}) was also present in 45% of the 62 cells tested (Fig. 1, panel *C*). For the cell shown in this figure, I_{Ki} was abolished (>90% block) by 1 mM barium leaving only a leak conductance of 3 GΩ. The current-voltage plot for the control, barium block, and difference currents is shown in Fig. 1, panel *D.* The barium-sensitive current shows inward rectification at potentials negative to −82 mV, reverses at −78 mV (−73 ± 2 mV, $n = 3$), and has a negative slope conductance between its reversal potential and −62 mV.

Hydrolysis-resistant GTP Analogues Activate a Cation Current in Rat RPE Cells

Upon introduction of the nonhydrolyzable GTP analogue, GTPγS (100 μM) into the cytoplasm of cultured

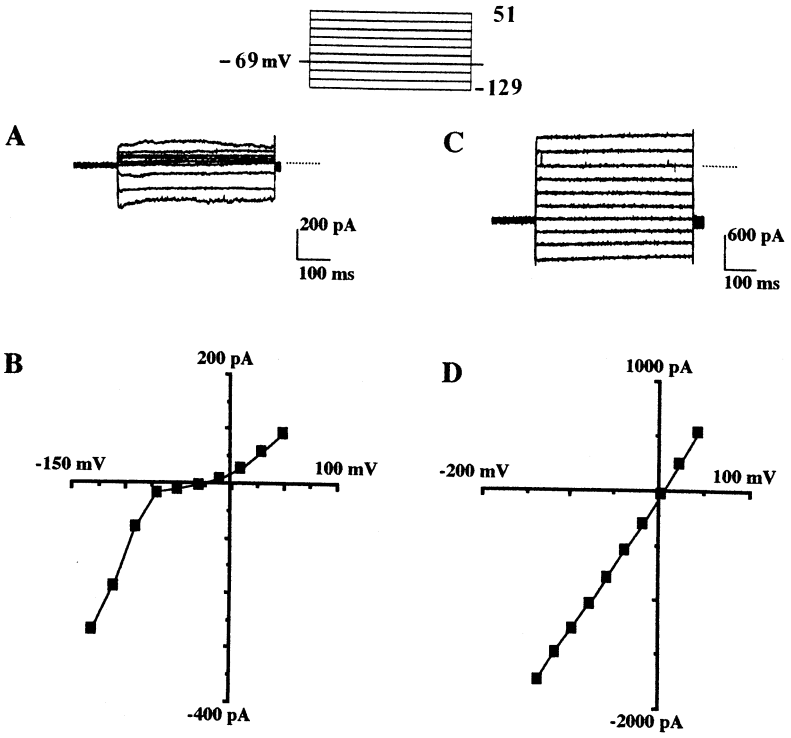

Fig. 2. (*A,B*) Patch-clamp recording from a representative cell (whole-cell capacitance of 28 pF) taken 1 min after assuming the whole-cell configuration. The voltage protocol is shown at the top of the figure. Data were not leak subtracted. The extracellular solution was standard 130 mM NaCl (solution A, Table) while the intracellular solution was 110 mM K^+-aspartate (solution I, Table) with the addition of 100 μM GTPγS. Both inwardly and outwardly rectifying potassium currents are present in this cell. (*C,D*) Same cell as shown in panel *A*, 15 min after assuming the whole-cell configuration. The activation of a cation current is apparent.

rat RPE cells, whole-cell recordings indicated the activation of a large noninactivating current. Figure 2, panel *A*, shows macroscopic currents recorded from a cell 1 min after assuming the whole-cell configuration with extracellular standard 130 mM NaCl (solution A, Table) and low chloride, K^+-aspartate solution (solution I, Table) with 100 μM GTPγS in the pipette. The asymmetric low chloride solutions were used to differentiate between chloride currents reversing at a potential of −38 mV from cation currents reversing at more positive potentials. The current-voltage plot for the whole-cell currents shown in panel *A* demonstrates that, immediately after break-in, the cell exhibits inwardly rectifying current at potentials negative to −75 mV and outwardly rectifying current at more positive potentials. These currents resemble the K^+ currents normally seen under control conditions in the absence of GTPγS. Figure 2, panel *B* shows currents recorded from the same cell 15 min after break-in with GTPγS in the pipette. Large noninactivating currents are apparent at potentials hyperpolarized and depolarized to the holding potential (−69 mV). The current-voltage relationship for the currents shown in *B* (*see* Fig. 2*D*) is linear and reverses at +5 mV (+5.5 ± 1.3 mV, $n = 22$), suggesting that GTPγS activates a cation conductance.

In RPE cells cultured for 2–5 days, the GTPγS-activated current occurred in 53 of 67 cells tested (80%) within a 15-min period following cytosolic introduction of GTPγS. In contrast, the GTPγS-activated current was rarely seen in cells cultured for more extended periods of time (>5–7 days in culture), suggesting that expression and regulation of the channels giving rise to the GTPγS-activated current may be altered in RPE cells proliferating in culture. Thus, data shown in subsequent figures represents recordings made only from cells within the first 2–5 days in culture. At this time in culture, the majority of cells have not yet begun to proliferate and remain rounded and heavily pigmented. In many cases, truncated microvillous processes were still apparent on the apical cell surface.

Like GTPγS, another hydrolysis-resistant GTP analogue GppNHp (100 μM), also activated a cation current in 7 cells tested. The GppNHp-activated current had a comparable time course of activation and was similar in magnitude to the GTPγS-activated current. To confirm the specificity of the GTPγS and GppNHp effects, we also tested the effects of GTP and GDPβS, a hydrolysis-resistant guanine nucleotide that does not activate G proteins. Figure 3*A* shows the time course for currents recorded in four representative cells using standard extra-

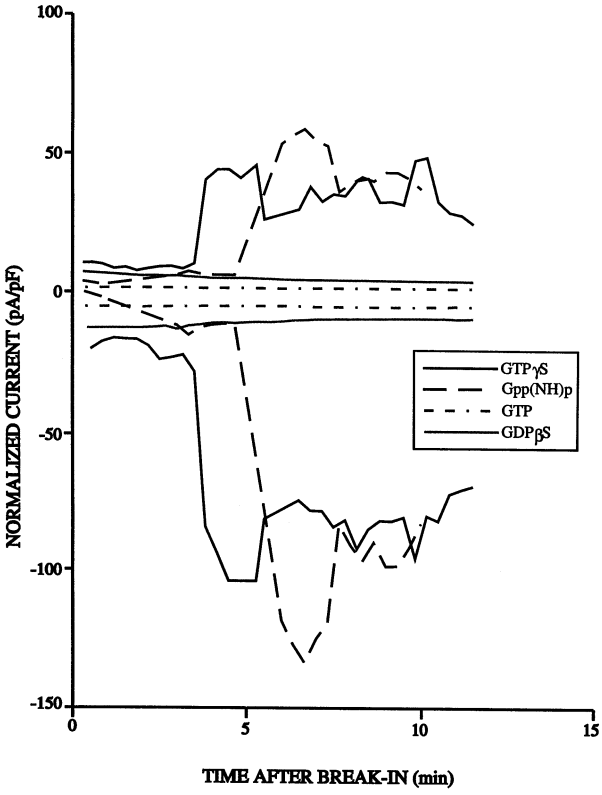

Fig. 3. Time course for the activation of the cation current by GTPγS and GppNHp. Current was measured every 20 sec following a holding potential of −69 mV using 100 msec pulses to +51 and −129 mV. Four separate representative cells are shown for intracellular solutions containing the addition of either 100 μM GTPγS, GppNHp, GTP, or GDPβS to a K^+ aspartate pipette solution (solution I, Table). The extracellular solution in all cases was standard 130 mM NaCl (solution A, Table). The whole-cell capacitances were 24, 30, 38, and 12 pF for GTPγS-, GppNHp-, GTP-, and GDPβS-containing cells, respectively. Neither GTP nor the inactive GDP analog GDPβS elicited any current change, while GTPγS and GppNHp activated a cation current.

cellular 130 mM NaCl (solution A, Table) and K^+-aspartate intracellular solution (solution I, Table) containing either GTP, GTPγS, GppNHp or GDPβS, respectively. Currents were measured every 20 sec following 100 msec voltage steps +51 and −129 mV. Neither GTP nor the inactive GDP analogue, GDPβS, elicited any change in the outward or inward currents. However, GTPγS and GppNHp induced similar increases in both outward and inward currents within 5 min of break-in. The mean activation time for the GTPγS- and GppNHp-activated currents measured in 14 and 7 cells, respectively was 5.7 ± 0.9 and 6.0 ± 1.5 min. Peak current amplitudes measured for GTPγS- and GppNHp-activated currents was 134 ± 51 and −97 ± 20 pA/pF and 95 ± 20 and −240 ± 43 pA/pF for command potentials of +51 and −129 mV. Some decline was observed in the GTPγS- and GppNHp-activated current in the 10–15 min following activation, however once activated, the whole-cell current did not return to values measured immediately after break-in.

We also attempted to determine if inward and outward rectifying K^+ currents were still present or were suppressed by GTPγS either prior to or following GTPγS-activation of the cation current. For the cell shown in Fig. 4*A*, GTPγS activated a cation current which reversed at −2 mV. Subsequent application of 5 mM extracellular Ba^{2+} produced a small reduction in both inward and outward current. The Ba^{2+}-difference current, obtained by subtracting the whole-cell current obtained in the presence of Ba^{2+} from the current prior to Ba^{2+} exposure, revealed an inwardly rectifying K^+ current and a small outward K^+ current. The inward K^+ current was increased by 65% over values recorded immediately after attaining the whole cell configuration. This suggests that K^+ currents were still present following GTPγS dialysis and that GTPγS may enhance inwardly rectifying K^+ current in rat RPE cells.

Other agents tested included the K^+ channel blocker quinine (0.5 mM), which has also been reported to inhibit NSC currents in some cell types (Gogelein & Pfannmuller, 1989), the epithelial Na^+ channel blocker, amiloride (100 μM), and the nonspecific cation channel blocker, gadolinium (100 μM). None of these compounds were effective in significantly reducing the GTPγS-activated cation current measured at +51 and −129 mV in 4–7 cells tested (*see* Fig. 4*B*). However, quinine slightly reduced currents measured at 0 mV (13 ± 5%, $n = 4$), a potential at which outward K^+ currents would be apparent but little cation current would be present.

Ion Selectivity of the GTPγS-activated Cation Current

To investigate the ionic selectivity of the GTPγS-activated current for monovalent cations, we used intracellular and extracellular solutions designed to minimize Cl^- conductance and obliterate K^+ currents. Figure 5*A* shows currents recorded in a cell with Cs^+-aspartate intracellular solution (solution J, Table) plus 100 μM GTPγS and Na^+-aspartate bathing solution (solution F, Table). Under these recording conditions, initially very little whole-cell current is apparent (Fig. 5*A*). Current recordings made at 15 min after break-in (Fig. 5*B*), however show that cytosolic superfusion of the cell with GTPγS, as before, activates a large noninactivating conductance. Figure 5*C* shows the *I-V* relationship for the currents shown in Fig. 5*A* and *B*. The linear current-voltage characteristics and positive reversal potential of this current (+5.7 ± 1.4 mV, $n = 18$), under conditions where contributions from K^+ currents and chloride currents are reduced, identifies the GTPγS-activated current as a nonspecific cation (NSC) current.

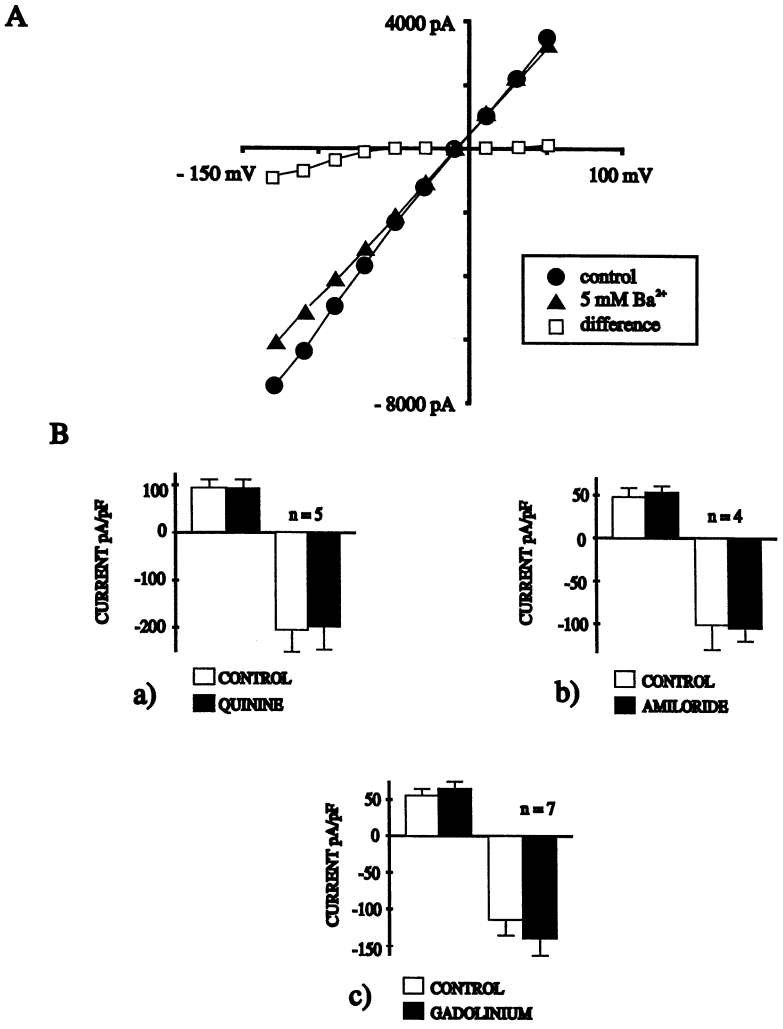

Fig. 4. GTPγS may enhance an inwardly rectifying K^+ current. Current-voltage plot (*A*) from a representative cell. The extracellular solution was low chloride (solution F, Table) while the intracellular solution was 110 mM K^+-aspartate (solution I, Table). The Ba^{2+} difference current (open squares) revealed a small outward K^+ current and an inwardly rectifying K^+ current that was increased 65% over initial values recorded immediately after attaining the whole cell configuration. (*B*) Quinine, amiloride and gadolinium do not block the GTPγS-activated cation current. GTPγS-activated current (mean ± SD) measured at −129 mV and +51 mV before and after 15 min of superfusion with 0.5 mM quinine (a), 0.1 mM amiloride (b) or 0.1 mM gadolinium (c).

Further confirmation that the GTPγS-activated current was not permeable to anions, was obtained by examining the amplitude and reversal potential for the induced current under experimental conditions where $[Cl^-]_{out}$ (solutions A and F, Table) or $[Cl^-]_{in}$ (solutions I and K) was varied. Reduction of extracellular $[Cl^-]$ from 140 mM to 40 mM did not significantly affect the amplitude of the GTPγS-induced current measured at +51 mV (75 ± 2 pA/pF in 140 mM $[Cl^-]_{out}$ and 77 ± 4 pA/pF in 40 mM $[Cl^-]_{out}$) in 7 cells tested (Fig. 6*A*). Figure 6*B* shows the measured reversal potential of the GTPγS-activated current plotted against the theoretical E_{Cl}. The least-squares best fit of the data (broken line) has a slope of 0.007. Also plotted (continuous line) is the relationship for a Cl^--selective current with a slope of 1. Thus, the GTPγS-activated conductance appears relatively impermeable to Cl^- ions, since neither the amplitude nor the reversal potential of the current is significantly affected by alterations in the Cl^- concentration gradient.

We then investigated the cation selectivity of the GTPγS-activated cation current by substituted Na^+ in the extracellular solution with other monovalent cations. Figure 7*A* shows current-voltage plots for currents recorded in a representative cell in 130 mM NaCl extracellular solution (solution A, Table) with 100 mM Cs^+-aspartate solution in the pipette (solution I, Table). Substitution of extracellular Na^+ with TRIS, or NMDG (solutions B–E, Table) shifted the reversal potential of the NSC current to more negative potentials and reduced the current amplitude measured at −40 mV (E_{Cl} = −38

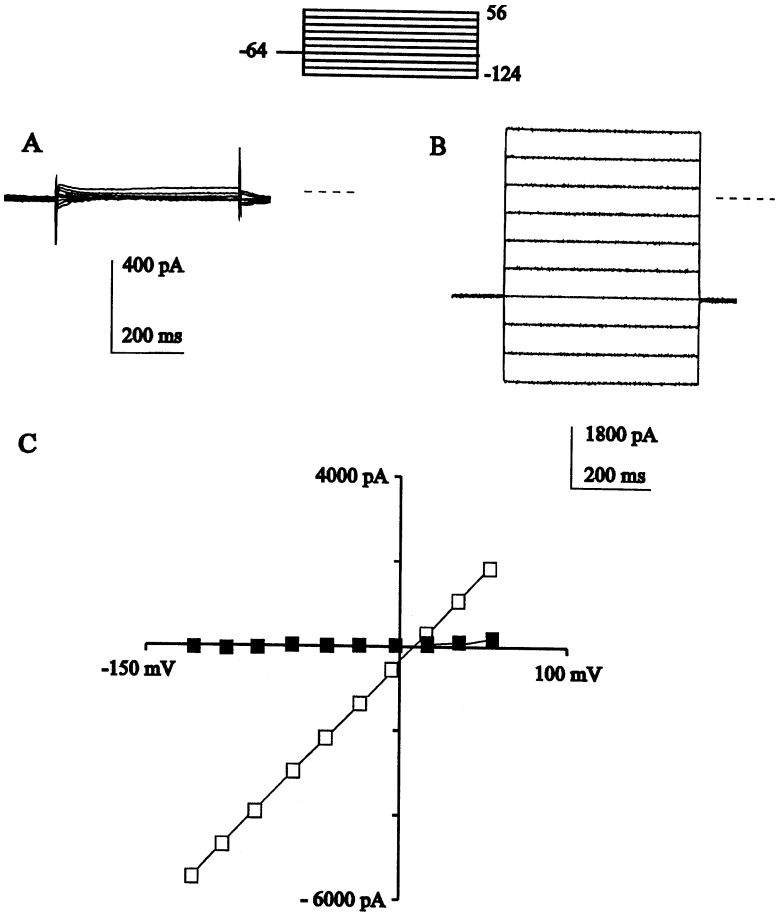

Fig. 5. The GTPγS-activated current is an NSC current. Whole-cell recordings (*A*) and current-voltage plots (*B*) from a representative cell with low chloride, K^+-free cesium aspartate intracellular solution containing 100 μM GTPγS (solution J, Table). The extracellular solution was Na^+ aspartate Ringers (solution F, Table). The voltage protocol is shown at the top of the figure. The whole-cell capacitance was 14 pF. Data were not leak subtracted. The GTPγS current is an NSC current since it was still activated in K^+-free and low Cl^- conditions and reverses closes to 0 mV.

mV for the solutions used) from −411 pA/pF to −162 pA/pF and −60 pA/pF for TRIS solutions and NMDG solutions, respectively. Figure 7*B* is a bar graph summarizing the mean reversal potentials measured from 4–11 cells in the different cation-substituted extracellular solutions. Permeability ratios (relative to Na^+ and shown in parenthesis) for K^+ (1.07), choline (0.51), TRIS (0.42) and NMDG (0.34) were calculated using Eq. (2) (*see* Materials and Methods). Thus, the permeability sequence for the rat RPE NSC channel is $K^+ = Na^+ \gg$ choline > TRIS > NMDG. Selectivity of the NSC channel to divalent cations was not investigated, however, reduction of extracellular Ca^{2+} concentration from 1 mM to <10 nM did not alter the mean reversal potential or significantly reduce the magnitude of the GTPγS-activated current in three cells tested. This indicates that external Ca^{2+} is not required for the activation of the NSC current (*see also below*).

Investigation of G Protein Characteristics and Second Messenger Pathways

Fluoroaluminate (AlF_4^-), as with the nonhydrolyzable GTP analogues, has proved to be a useful tool for the activation of heterotrimeric G proteins as opposed to the low molecular weight GTP binding proteins (Kahn, 1991; Hall, 1992). To confirm the type of G protein involved in GTPγS and GppNHp-mediated activation of the NSC current we dialyzed RPE cells with AlF_4^-. In 20 cells tested, inclusion of NaF (1 mM) and $AlCl_3$ (25 μM) in either Cs^+-aspartate (solution J, Table) or K^+-aspartate pipette solution (solution I, Table) containing Mg^{2+}, failed to activate a sustained cation current. However, increasing the concentration of NaF to 10 mM and $AlCl_3$ to 100 μM, resulted in robust activation of a large conductance in 5/5 cells tested. The AlF_4^--activated current,

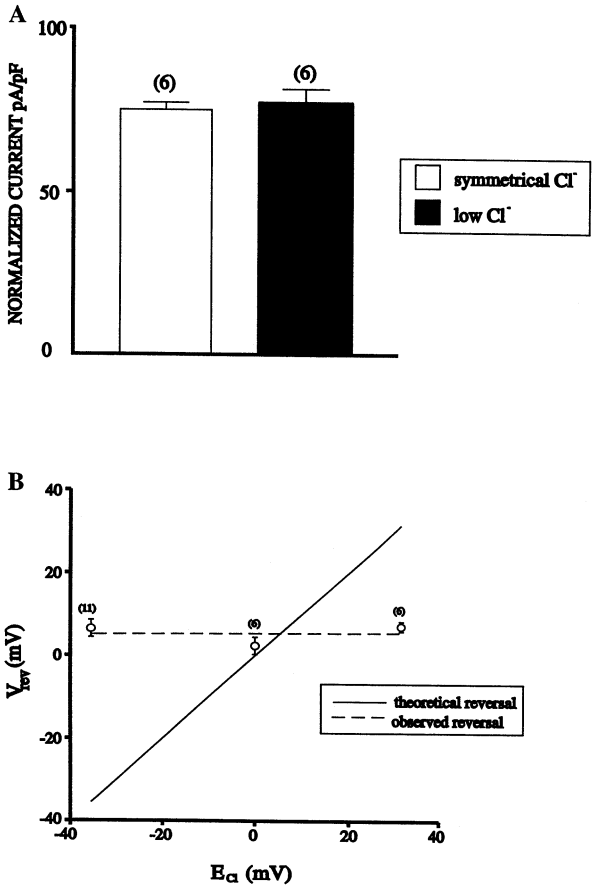

Fig. 6. The GTPγS-activated cation current is not affected by alterations in the $[Cl^-]$ concentration gradient. (*A*) The amplitude of the cation current was measured at +51 mV in 7 cells with standard intracellular recording solution (solution H, Table) and in extracellular solution in which $[Cl^-]_{out}$ was altered by substitution with aspartate (solutions *A* and *F*, Table). The current amplitude was not affected by reduction in the external $[Cl^-]$ from 140 to 40 mM. (*B*) The reversal potential of the GTPγS-activated current was measured in 23 cells in which $[Cl^-]_{in}$ or $[Cl^-]_{out}$ was altered by substitution with aspartate (solutions *A,F,J,* Table) and plotted against the theoretical E_{Cl^-}. The least-squares fit of the data (broken line) had a slope of 0.007 corresponding to a 4.8 mV per 10-fold change of $[Cl^-]_{in}/[Cl^-]_{out}$. The continuous line has a slope of 1, predicted for a Cl^--selective current.

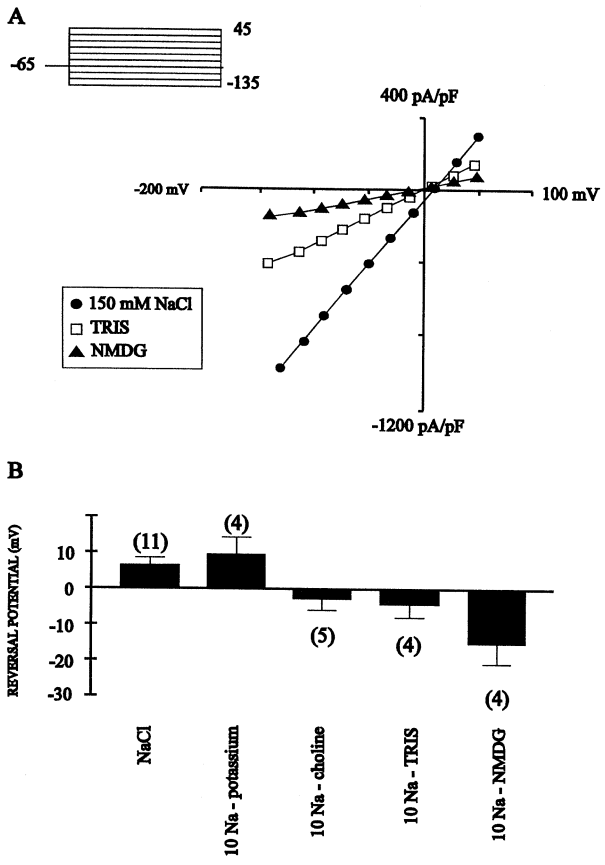

Fig. 7. Cation selectivity of the GTPγS-activated current. (*A*) Representative current-voltage curves for a rat RPE cell showing the effect of replacement of Na^+ in the extracellular solution by several monovalent cations on the GTPγS-activated current. The voltage protocol for the 150 mM Na^+ trace is the same as that shown in Fig. 5, while the protocol for the remaining traces is shown at the top of the panel. Data were not leak subtracted. The recording electrode contained solution J, Table with GTPγS substituted for GTP. Current magnitude and reversal potential were altered by replacement of all but 10 of 150 mM extracellular Na^+ (solution A, Table) with either choline, TRIS, or NMDG (solutions *B-D,* Table, respectively). (*B*) Mean ± SEM reversal potential measured for (*n*) cells in the above solutions containing intracellular GTPγS.

shown in Fig. 8*A*, had properties similar to the GTPγS-activated cation current, although the activation of this current was delayed with respect to that seen with GTPγS in the pipette (*see* Fig. 2). Figure 8*B* shows that the AlF_4^--activated current had a linear *I-V* relationship and the current reversed close to 0 mV (−1.1 ± 0.4 mV, $n = 5$). Thus, the data indicate that AlF_4^- mimics the effects of GTPγS in activating the NSC current in rat RPE cells and that activation of this current is sustained in the presence of an adequate concentration of NaF. This finding is consistent with previous findings demonstrating activation of G_α subunits by AlF_4^- (*for review see* Rodbell, 1992, also Higashijima et al., 1991) and further implicates G proteins in the regulation of the NSC current.

Different G proteins can be distinguished by their susceptibility or insensitivity to pertussis toxin (PTX) inactivation. The toxin inactivates G proteins by catalyzing the ADP-ribosylation of the α subunit of G proteins (Ui, 1990). Members of the G_i/G_o family are all sensitive to PTX while members of the G_s and G_q family are insensitive to PTX modification. We investigated whether activation of the NSC current in RPE cells was mediated via a PTX-sensitive or PTX-insensitive G pro-

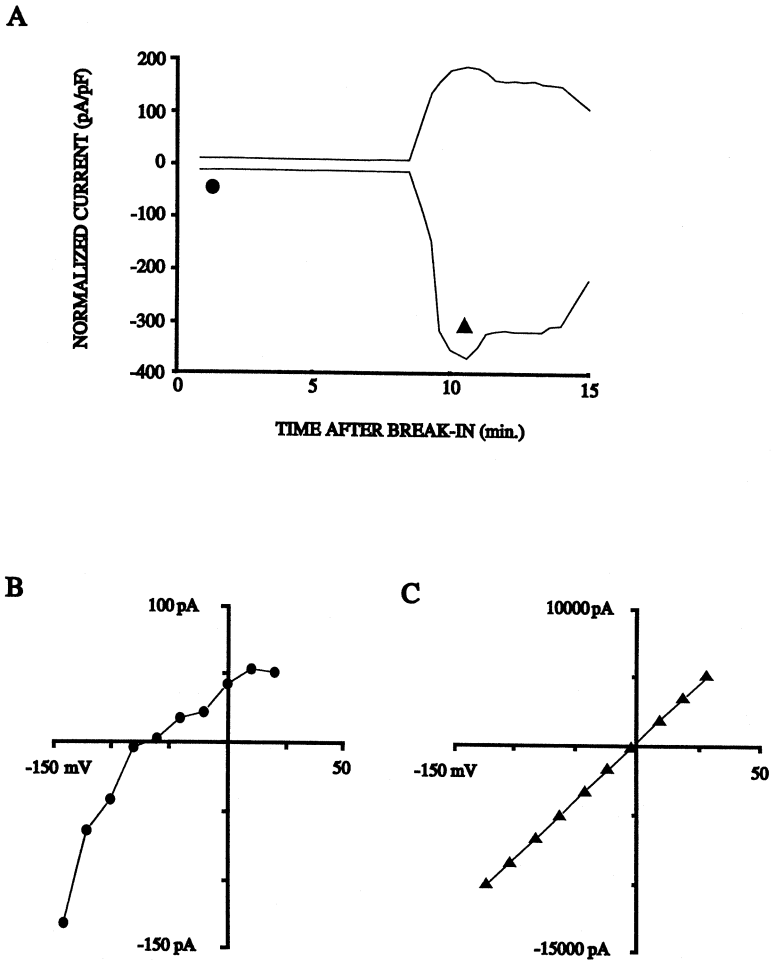

Fig. 8. Fluoroaluminate activates the NSC current. (*A*) Whole-cell current recorded in a rat RPE cell with K^+ aspartate pipette solution (solution J, Table) and 130 mM NaCl bathing solution (solution A, Table). Dialysis of the cell interior with 10 mM NaF and 100 μM $AlCl_3$ activated both inward and outward current at 9 min after breaking into the whole-cell recording configuration. *I-V* relationships obtained at 2 min (●) and 10 min (▲) indicate that initially both inward and outward K^+ currents are apparent (Fig. 8*B*), however, after 10 min a large current is activated (Fig. 8*C*) which reverses at 0 mV and has similar *I-V* characteristics to the GTPγS activated NSC current. The capacitance of the cell shown in 22 pF.

tein by incubating cells with PTX (500 ng/ml) for 24 hr before recording. In all PTX-treated cells tested ($n = 6$), GTPγS failed to activate the NSC current (Fig. 9), suggesting that the G protein involved in the activation of the NSC current is of the G_i/G_o class. In another two cells tested from the same culture, but without PTX-pretreatment, GTPγS-activated the NSC current and the activated current had a comparable amplitude (−282 and −296 pA/pF at −129 mV and 122 and 158 pA/pF at +51 mV) to the current recorded previously in other cells.

Many NSC channels are sensitive to activation by second messengers such as cytosolic Ca^{2+}. We tested the involvement of $[Ca^{2+}]_{in}$ in the activation of the NSC current in rat RPE cells by dialyzing cells with GTPγS in 10 mM BAPTA pipette solution (solution L, Table free Ca^{2+} < 10 nM) and nominally Ca^{2+}-free (solution G, Table) bath solution. Under these conditions, GTPγS still activated the NSC current. The activated current recorded in low Ca^{2+} conditions had a similar amplitude (−382 pA/pF and 199 pA/pF at −129 and +51 mV, $n = 5$) to that recorded with standard pipette solution ($[Ca^{2+}]_{in} \approx 10^{-7}$ M). Thus, the GTPγS-activated NSC current did not appear to require an increase in $[Ca^{2+}]_{in}$ for activation.

Since some NSC channels are sensitive to the modulatory effects of internal ATP (Rae et al., 1990), we also investigated whether exclusion of ATP (10^{-4} M) from the pipette solution affected the magnitude of the NSC conductance in rat RPE cells. In the three cells tested the GTPγS-activated NSC conductance was not significantly affected by exclusion of ATP from the pipette solution.

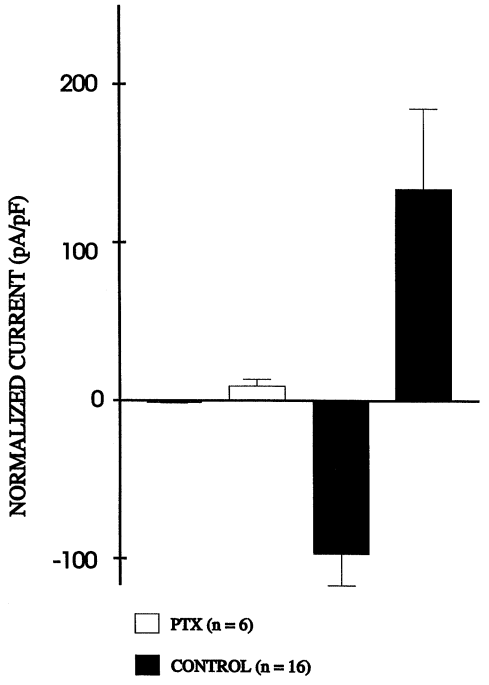

Fig. 9. PTX blocks the activation of the GTPγS current. Mean (± SEM) current amplitudes measured at +51 and −129 mV (n = 6) in cells pretreated for 24 hr with 500 ng/ml of PTX indicate that GTPγS failed to activate the NCS current. In control cells without PTX treatment GTPγS activated an NSC current with a comparable amplitude at +51 and −129 mV to the currents previously recorded in other cells (n = 14). The mean (± SEM) for all control cells were compiled (n = 16).

Discussion

This study describes the properties and the regulation of a G protein-activated NSC current present in cultured rat RPE cells. We show that, using standard whole-cell recording conditions, the membrane conductance of rat RPE cells is normally dominated by K^+ conductances. Rat RPE cells display both outwardly rectifying and, in 45% of cells tested, inwardly rectifying K^+ currents. The presence of similar K^+ currents has been demonstrated in cultured and fresh RPE cells from several amphibian and mammalian species: frog (Hughes & Steinberg, 1990), rat (Strauss, 1994), rabbit (Tao et al., 1994), monkey (Wen et al., 1993), and human (Strauss et al., 1993; Wen et al., 1993). These K^+ conductances, which are located on both the apical and basolateral membranes of the RPE, are thought to play a major role in transepithelial ion transport and contribute to K^+ homeostasis in the subretinal space (Steinberg & Miller, 1979).

Our results demonstrate that inclusion of GTPγS, GppNHp in the pipette activates a large cation current in rat RPE cells. In the presence of GTPγS or GppNHp, the NSC current activated rapidly after a delay of 5–6 min (after assuming the whole-cell configuration). The delay of 5–6 min before onset of GTPγS-induced activation of the cation conductance in RPE cells suggests an indirect effect of a G protein on the cation channel. A similar time course has been reported for GTPγS-mediated activation of NSC channels in guinea pig chromaffin cells (Inoue et al., 1991). In contrast to the effects of GTPγS and GppNHp, inclusion of GDPβS in the pipette did not activate the cation current. Since GDPβS is a hydrolysis-resistant GDP analogue that competes with GTP for binding sites on G proteins, the lack of activation of the cation current by GDPβS confirms the involvement of a G protein in regulation of this current.

A variety of different agents are known to have blocking effects on NSC channels, however studies of the function of these channels has been complicated in that no selective blockers are yet identified. Agents which have been reported to have some blocking actions on NSC channels include K^+ channel blockers, such as quinine (Gogelein & Pfannmuller, 1989) and the lanthanide compound gadolinium, which has been found to block the activation of stretch-activated cation channels in several different cell types (Yang & Sachs, 1989; Davis, Meiningert & Zawieja, 1992; Naruse & Sokabe, 1993). When we tested quinine and gadolinium on the GTPγS-activated current in rat RPE cells we found that both these compounds were without effect, since neither of these drugs reduced the amplitude of the whole-cell currents or altered the time course of activation. In addition to the above agents, the epithelial Na^+ channel blocker amiloride (Jacob, Bangham & Duncan, 1985; Benos et al., 1995) was also without effect on the GTPγS-activated current.

The presence of nonspecific cation (NSC) channels have now been described in numerous cell types including heart cells (Colquhon et al., 1981), IMCD kidney epithelial cells (Ono et al., 1994), macrophages (Lipton, 1986), rat and mouse pancreatic acinar cells (Maruyama & Petersen, 1982), lacrimal acinar cells (Marty, Tan & Trautmann, 1984), and subsequently in every type of exocrine gland which have been investigated (*for review see*, Marty, 1987). In ocular tissues, NSC channels have been described in human (Cooper et al., 1990) and frog (Cooper, Tang & Rae, 1986) lens epithelial cells and rabbit corneal endothelium (Rae et al., 1990). Characteristics of the multitude of NSC channels which have been reported vary, depending on both the cell system and the investigational approach used. The common denominator, as its name implies, is the selectivity of this channel for small monovalent cations, coupled with the inability to discriminate between these cations. The GTPγS-activated current in rat RPE cells was a NSC current, since in the presence of K^+ channel blockade it showed virtually no discrimination between Na^+ and K^+ ions with a P_K/P_{Na} of 1.07 and had a V_r close to 0 mV in

130 mM Na^+ or K^+ extracellular solutions. In addition to almost complete nondiscrimination between Na^+ and K^+ ions, large cations such as TRIS and NMDG also appeared to be able to permeate the rat NSC channel. The channel exhibited a low permeability to Cl^- with alterations in extracellular Cl^- producing little change in current amplitude or current reversal potential.

A large nonselective channel with somewhat different properties to the NSC current recorded in rat RPE cells has been described in membrane patch recordings from cultured human RPE cells (Fox et al., 1988). This nonselective channel had a unitary conductance of 300 pS, a P_K/P_{Na} of 0.8 and showed poor selectivity between K^+ and Cl^-. Investigations of the calcium and voltage-dependence of the nonselective channel in human RPE cells revealed that this channel was not dependent on either extracellular or intracellular Ca^{2+} but was voltage-dependent, with the open probability of the channel decreasing as the membrane potential increased. Similar large-conductance channels that show selectivity for several substrates but are primarily anion selective have been described in rat Schwann cells (Gray, Bevan & Ritchie, 1983), rat muscle (Blatz & Magleby, 1983) and the apical membranes of MDCK cells (Kolb, Brown & Murer, 1985). This poor discrimination between cations and anions and the voltage-dependency sets these channels apart from the NSC channels described in this study, which are selective for cations but have limited permeability for anions.

Confirmation that GTPγS-mediated activation of the NSC current in rat RPE cells involves a heterotrimeric as opposed to a low molecular-weight (18–32 kDa) monomeric G proteins, was carried out by dialyzing cells with AlF_4^-. The AlF_4^- complex, occurring as a result of F^- complexing with $AlCl_3$, is a known activator of heterotrimeric G proteins and is able to mimic the action of GTP and thus cause dissociation of the α-subunit of G proteins (Ui, 1990). We found that AlF_4^- activated a cation current with similar properties to the GTPγS-activated NSC current. In the case of fluoroaluminate, however, the time course of activation of the NSC current was somewhat slower than that found with GTPγS. This finding is supported by other studies that demonstrate that sodium fluoride acts in a concentration-dependent manner with a K_d for G protein activation of 0.47 mM (Higashijima et al., 1991).

Although fluoroaluminate has been shown to stimulate heterotrimeric G proteins in many different tissues, the results of these studies indicate that the fluoride-evoked effects were not generally mediated by a PTX-sensitive G protein (Ui, 1990). Our results are not consistent with these findings since, incubation of rat RPE cells with PTX for 24 hr blocked GTPγS-activation of the NSC suggesting that the G protein involved was PTX-sensitive and of the G_i/G_o class.

Nonspecific cation channels in many cell types are sensitive to second messenger molecules such as Ca^{2+} and nucleotides such as ATP (*for review see* Partridge & Swandulla, 1988). In rabbit corneal endothelial cells the apical membrane contains a cation-selective channel which is activated by internal Ca^{2+} levels above 10^{-4} M and inhibited by ATP when the internal concentration of this nucleotide exceeds 10 μM (Rae et al., 1990). Investigation of second messenger molecules which may regulate the activity of NSC channels in rat RPE cells revealed that this current did not appear to require an increase in $[Ca^{2+}]_{in}$ for activation and was not affected by the inclusion or exclusion of ATP in the pipette solution.

Macroscopic current recording does not provide information on the location of the NSC channel to either the RPE apical or basolateral membrane of RPE cells, therefore, it is difficult to propose a role for this channel in relation to current models of ion transport for this epithelium (Steinberg & Miller, 1979; Joseph & Miller, 1991; Edelman, Lin & Miller, 1994). However, the activation of NSC channels would allow Na^+ influx, causing a depolarization of the cell relative to the resting potential. The ensuing depolarization, if sufficient, could activate other ion channels such as outwardly rectifying K^+ channels, which may provide an efflux pathway for K^+ which enters the cell via the apical Na^+-K^+-$2Cl^-$ cotransporter or the Na^+-K^+ pump, or it may activate Cl^- conductances.

In summary, this study has demonstrated that the NSC current in rat RPE cells is G protein activated. The activation of the NSC current with fluoroaluminate and block of the current activation following pretreatment with PTX suggests that the G protein involved is a heterotrimeric G protein of the PTX-sensitive G_i/G_o class. Further studies are needed to further clarify the characteristics of the G protein involved in regulating the NSC channel in rat RPE cells and to screen for factors responsible for the physiological activation of these channels.

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References

Barry, P., Lynch, J. 1991. Liquid junction potentials and small cell effects in patch-clamp analysis. *J. Membrane Biol.* **121:**107–117

Benos, D.J., Awayda, M.S., Ismailov, I.I., Johnson, J.P. 1995. Structure and Function of Amiloride-sensitive Na^+ Channels. *J. Membrane Biol.* **143:**1–18

Blatz, A.L., Magleby, K.L. 1983. Single voltage-dependent chloride-selective channels of large conductance in cultured rat muscle. *Biophysical J.* **43:**237–241

Botchkin, L.M., Matthews, G. 1993. Chloride current activated by swelling in retinal pigment epithelium cells. *Am. J. Physiol.* **265:**C1037–C1045

Botchkin, L.M., Matthews, G. 1994. Voltage-dependent sodium channels develop in rat retinal pigment epithelial cells in culture. *Proc. Natl. Acad. Sci.* **91:**4564–4568

Breitwieser, G.E. 1991. G Protein-mediated ion channel activation. *Hypertension* **17:**684–692

Brown, A.M. 1993. Membrane-delimited cell signalling complexes: direct ion channel regulation by G proteins. *J. Membrane Biol.* **131:**93–104

Colquhoun, D., Neher, E., Reuter, H., Stevens, C.F. 1981. Inward current channels activated by intracellular calcium in cultured cardiac cells. *Nature* **294:**752–754

Cooper, K.E., Tang, J.M., Rae, J.L. 1986. A cation channel in frog lens epithelia responsive to pressure and calcium. *J. Membrane Biol.* **93:**259–269

Cooper, K., Rae, J.L., Gates, P., Dewey, J. 1990. Electrophysiology of cultured human lens epithelial cells. *J. Membrane Biol.* **117:**285–298

Davis, M.J., Meininger, G.A., Zawieja, D.C. 1992. Stretch-induced increases in intracellular calcium of isolated vascular smooth muscle cells. *Am. J. Physiol.* **263:**H1292–H1299

Edelman, J.L., Lin, H., Miller, S.S. 1994. Potassium-induced chloride secretion across the frog retinal pigment epithelium. *Am. J. Physiol.* **266:**C957–C966

Fox, J.A., Pfeffer, B., Fain, G. 1988. Single-channel recordings from cultured human retinal pigment epithelium. *J. Gen. Physiol.* **91:** 193–222

Fox, J.A., Steinberg, R.H. 1992. Voltage-dependent currents in isolated cells of the turtle retinal pigment epithelium. *Pfluegers Arch.* **420:**451–460

Gogelein, H., Pfannmuller, B. 1989. *Pfluegers Arch.* **413:**287–298

Goldstein, D.A. 1979. Calculation of the concentrations of free cations and cation-ligand complexes in solutions containing multiple divalent cations and ligands. *Biophys. J.* **26:**235–242

Gray, P.T.A., Bevan, S., Ritchie, J.M. 1983. High conductance anion-selective channels in rat cultured Schwann cells. *Proc. Royal Soc. London* **221:**395–409

Hall, A. 1992. Small GTP-binding proteins- A new family of biologic regulators. *Am. J. Respir. Cell Mol. Biol.* **6:**245–246

Hamill, O.P., Marty, A., Neher, E., Sakmann, B., Sigworth, F.J. 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pfluegers Arch.* **391:**85–100

Higashijima, T., Graziano, M.P., Suga, H., Kainosho, M., Gilman, A.G. 1991. 19F and 31P NMR spectroscopy of G protein alpha subunits. Mechanism of activation by Al^{3+} and F^{-}. *J. Biol. Chem.* **266:**3396–3401

Hille, B. 1992. Selective permeability: independence. *In:* Ionic Channels of Excitable Membranes, 2nd Ed., pp. 337–361. Sinauer Associates, Sunderland, MA

Hughes, B.A., Steinberg, R.H. 1990. Voltage-dependent currents in isolated cells of the frog retinal pigment epithelium. *J. Physiol.* **428:**273–297

Inoue, K., Kuriyama, H. 1991. Muscarinic receptor is coupled with a cation channel through a GTP-binding protein in guinea-pig chromaffin cells. *J. Physiol.* **436:**511–529

Jacob, T.J.C., Bangham, J.A., Duncan, G. 1985. Characterization of a cation channel on the apical surface of the frog lens epithelium. *Quart. J. Exp. Physiol.* **70:**403–421

Joseph, D.P., Miller, S.S. 1991. Apical and basal membrane ion transport mechanisms in bovine retinal pigment epithelium. *J. Physiol.* **435:**439–463

Kahn, R.A. 1991. Fluoride is not an activator of the smaller (20–25 kDa) GTP-binding proteins. *J. Biol. Chem.* **266:**15595–15597

Kolb, H.A., Brown, C.D.A., Murer, H. 1985. Identification of a voltage-dependent anion channel in the apical membrane of a Cl^{-} secretory epithelium (MDCK). *Pfluegers Arch.* **403:**262–265

Lipton, S.A. 1986. Antibody activates cationic channels via second messenger Ca^{2+}. *Biochimica et Biophysica Acta.* **856:**59–67

Marty, A., Tan, Y.P., Trautmann, A. 1984. Three types of calcium-dependent channels in rat lacrimal glands. *J. Physiol.* **357:**293–325

Marty, A. 1987. Control of ionic currents and fluid secretion by muscarinic agonists in exocrine glands. *Trends Neurol. Sci.* **10:**373–377

Marty, A. 1989. The physiological role of calcium-dependent channels. *Trends Neurol. Sci.* **12:**420–424

Maruyama, Y., Petersen, O.H. 1982. Single channel currents in isolated patches of plasma membrane from basal surface of pancreatic acini. *Nature* **299:**159–161

Naruse, K., Sokabe, M. 1993. Involvement of stretch-activated ion channels in Ca^{2+} mobilization to mechanical stretch in endothelial cells. *Am. J. Physiol.* **264:**C1037–1044

Ono, S., Mougouris, T., DuBose, T.D., Sansom, S.C. 1994. ATP and calcium modulation of nonselective cation channels in IMCD cells. *Am. J. Physiol.* **267:**F558–565

Partridge, L.D., Swandulla, D. 1988. Calcium-activated nonspecific cation channels. *Trends Neurol. Sci.* **11:**69–72

Poyer, J.F., Kelly, M.E.M. 1995. G protein regulation of ionic currents in the retinal pigment epithelium of the rat. *Invest. Ophthalmol. Vis. Sci.* **36(ARVO Suppl.):**2723

Rae, J.L., Dewey, J., Cooper, K., Gates, P. 1990. A nonselective cation channel in rabbit corneal endothelium activated by internal calcium and inhibited by internal ATP. *Exp. Eye Res.* **50:**373–384

Rodbell, M. 1992. The role of GTP-binding proteins in signal transduction: From the sublimely simple to the conceptually complex. *Curr. Topics Cell Reg.* **32:**1–47

Steinberg, R.H., Miller, S.S. 1979. Transport and membrane properties of the retinal pigment epithelium. *In:* The retinal pigment epithelium. pp. 205–225. K.M. Zinn, M.F. Marmor, editors. Harvard University Press, Cambridge

Strauss, O., Richard, G., Wienrich, M. 1993. Voltage-dependent potassium currents in cultured human retinal pigment epithelial cells. *Biochem. Biophys. Res. Commun.* **191:**775–781

Strauss, O., Wienrich, M. 1993. Cultured retinal pigment epithelial cells from RCS rats express an increased calcium conductance compared with cells from non-dystrophic rats *Pfluegers Arch.* **425:**68–76

Strauss, O., Weiser, T., Wienrich, M. 1994. Potassium currents in cultured cells of the rat retinal pigment epithelium. *Comp. Biochem. Physiol.* **109:**975–983

Tao, Q.-P., Rafuse, P.E., Kelly, M.E.M. 1994. Potassium currents in rabbit retinal pigment epithelial cells. *J. Membrane Biol.* **141:**123–138

Ueda, Y., Steinberg, R.H. 1993. Voltage-operated calcium channels in fresh and cultured rat retinal pigment epithelial cells. *Invest. Ophthalmol. Vis. Sci.* **34:**3408–3418

Ueda, Y., Steinberg, R.H. 1994. Chloride currents in freshly isolated rat retinal pigment epithelial cells. *Exp. Eye Res.* **58:**331–342

Ui, M. 1990. Pertussis toxin as a valuable probe for G-protein involvement in signal transduction. *In:* ADP-Ribosylating Toxins and G Proteins. Insights into Signal Transduction. J. Moss and M. Vaughan, editors, pp. 45–77. American Society for Microbiology Press, Washington, DC

Wang, N., Koutz, C.A., Anderson, R.E. 1993. A method for the isolation of retinal pigment epithelial cells from adult rats. *Invest. Ophthalmol. Vis. Sci.* **34:**101–107

Wen, R., Lui, G.M., Steinberg, R.H. 1993. Whole-cell K^+ currents in fresh and cultured cells of the human and monkey retinal pigment epithelium. *J. Physiol.* **465:**121–147

Yang, X-C., Sachs, F. 1989. Block of stretch-activated ion channels on *Xenopus* oocytes by gadolinium and calcium ions. *Science* **243:** 1068–1071

Zauberman, H. 1979. Adhesive forces between the retinal pigment epithelium and sensory retina. *In:* The retinal pigment epithelium. K.M. Zinn, and M.F. Marmor, editors. pp. 192–204. Harvard University Press, Cambridge