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Transformation of human ciliary epithelial cells by simian virus 40: Induction of cell proliferation and retention of β_2-adrenergic receptors

(glaucoma/human cell line/[^{125}I]iodopindolol binding/vimentin phosphorylation)

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ABSTRACT **Ciliary epithelial cells derived from human eye were successfully propagated through many generations after transformation with simian virus 40. The cell clone 8-SVHCE was isolated and characterized by immunoprecipitation and pharmacological studies that demonstrated the presence of several functional properties observed in the parent cells of this tissue. Immunoprecipitation revealed the presence of large tumor (T) antigen, and Southern blot analysis showed the incorporation of viral DNA into high molecular weight ciliary epithelial cell DNA. The presence of β-adrenergic receptors was demonstrated by direct binding of a radiolabeled antagonist, [^{125}I]iodopindolol, to membrane preparations of 8-SVHCE cells (K_d = 41.8 pM and B_{max} = 67.1 fmol/mg of protein). Competition experiments with [^{125}I]iodopindolol and selective drugs suggested that the receptors are of the β_2-adrenergic subtype. Studies of catecholamine-stimulated cellular cAMP production and of isoproterenol-dependent protein phosphorylation of vimentin in 8-SVHCE indicated the functional conservation of β-adrenergic receptor-mediated processes that are thought to be important in the regulation of aqueous humor production by the ciliary epithelium *in vivo*.**

Ciliary epithelium (CE) in the mammalian eye is thought to play an essential role in the formation of aqueous humor. The composition of aqueous humor is largely dependent on the mechanism of active transport of plasma proteins and electrolytes in these cells. The rate of formation of aqueous humor is modulated by the sympathetic (adrenergic) system (1). The stroma in the ciliary processes is innervated by adrenergic fibers whose terminations remain close to the CE (2). Two structural features of these cells *in vivo* are (*i*) a structural polarity where the apical domain of a nonpigmented epithelial (NPE) cell faces the apical domain of a pigmented epithelial (PE) cell and (*ii*) a physiological, enzyme-based active transport system, Na^+,K^+-ATPase, located in the basolateral domains in both cell types. The relationship of the structural and physiological polarity in the CE to the regulation of ion transport (i.e., fluid flow from the stroma to the posterior chamber) is unclear.

Previous studies (3–5) using crude membrane preparations from whole ciliary processes have indicated the presence of β-adrenergic receptors in the ciliary epithelium of eyes from a variety of mammalian species. It is possible, however, that vascular endothelial cells from microvessels in the stroma of ciliary processes may have contributed to the stimulation of adenylate cyclase by β-adrenergic receptors that was observed in these preparations. Attempts have been made to dissociate the CE cells from the stroma tissue to measure the properties and characteristics of the β-adrenergic receptor/adenylate cyclase system in a mixed population of NPE and PE cells (6), after their separation in a density gradient (7), and in isolated NPE cells (8). In these studies, β-adrenergic receptors were found in both cell types. In addition, several attempts have been made to grow CE in tissue culture media after its dissociation and separation (9) from ciliary processes. Some of the differentiated characteristics of human CE cells, such as their structural polarity and epithelial cell type, are retained in primary tissue cultures and have been studied by morphological and immunological criteria (9, 10). Activation of adenylate cyclase in human CE cells by catecholamines results in increased intracellular cAMP and significant stimulation of protein phosphorylation, particularly of vimentin (a major cytoskeletal component) (11).

In this paper we report the use of simian virus 40 (SV40) as a tool to induce proliferation of human CE cells in tissue culture. Previous attempts to establish a CE cell line have been unsuccessful, primarily due to the short life span of these cells in primary culture. Human cells are semipermissive to SV40, and although eventually the virus may proliferate and result in the death of the transformed cells, this occurrence is uncommon before the passage of many cell generations (12). We have isolated and characterized one clone, 8-SVHCE, derived from transformed CE cells. The functional preservation of the β-adrenergic receptor/adenylate cyclase system in 8-SVHCE cells was demonstrated by high-affinity direct binding of the radioligand [^{125}I]iodopindolol to membranes of 8-SVHCE and by cAMP production after stimulation by catecholamine hormones. In addition, we believe that isoproterenol-dependent protein phosphorylation of vimentin supports the contention that membrane-bound β-adrenergic receptor function in human CE cells is preserved after viral transformation.

MATERIALS AND METHODS

Virus and Cells. Normal human CE cells were derived from dissected ciliary processes from two eyes of a deceased 8-month-old baby. A primary culture with these cells was prepared as described (10) and grown in 25-cm^2 Primaria flasks (Falcon no. 3813, Becton Dickinson). When the cells were at semiconfluence, they were infected with wild-type SV40 (generously provided by P. K. Ghosh, Yale University) at multiplicities of infection of ≈25 plaque-forming units per cell. Transformation assays were carried out according to standard procedures. The medium was replaced with Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS) and garamycin, and the cells were inspected for the formation of transformed foci

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Abbreviations: CE, ciliary epithelium; PE, pigmented epithelium; NPE, nonpigmented epithelium; SV40, simian virus 40; T antigen, large tumor antigen; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; kb, kilobase(s).

during the following 2–3 weeks. Individual foci were then selected manually from transformed CE cells, transferred to Primaria flasks, and maintained in DMEM containing 5% FBS and garamycin at 37°C in a 5% CO_2 atmosphere. Transformed cells in early passages from foci were retained and saved frozen at −70°C to replace old cultures.

Immunoprecipitation of SV40 Antigens from 8-SVHCE Cells. Immunoprecipitation was carried out as described (13), with some modifications. Extracts of [^{35}S]methionine-labeled cells were centrifuged at 100,000 × *g* at 4°C for 1 hr. An aliquot of supernatant was incubated with anti-large tumor (T) antigen antiserum, generously provided by M. Garcia-Blanco (Yale University) (14). Normal hamster serum was used as control. Immune complexes were adsorbed to protein A-Sepharose CL-4B (Sigma), eluted as described (15), and subjected to NaDodSO$_4$/PAGE in 5–18% acrylamide gradient slab gels, using the discontinuous buffer system of Laemmli (16). The gels were then fixed, stained, soaked in EN^{3}HANCE (New England Nuclear) before drying, and subjected to autoradiography (X-Omat AR film; Eastman Kodak).

Immunofluorescence Microscopy. 8-SVHCE cells were grown on glass coverslips, fixed, and stained by indirect immunofluorescence (9). The primary reagent was undiluted hybridoma medium containing a monoclonal antibody directed against desmosomal proteins from bovine muzzle epidermis and designated M$_m$DGI-1 (17), which was kindly provided by M. S. Steinberg and C. Blanck (Princeton University). Indirect immunofluorescence was carried out by incubation for 30 min at 37°C with fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Miles). The coverslips were mounted and photographed as described (9).

Southern Blot Analysis. Integrated viral DNA was analyzed by the Southern blot technique, essentially as described (18–20).

cAMP Assay of Response to Catecholamine Hormones. Intracellular cAMP was assayed by means of a radioimmunoassay kit (New England Nuclear) adapted from the procedures of ref. 21. Confluent cells in 35-mm plates were washed with phosphate-buffered saline (PBS) at 37°C. The cultures were incubated for 15 min at 37°C with 2 ml of PBS containing theophylline with or without a β-adrenergic agonist and nonspecific β-adrenergic antagonist.

Crude Membrane Preparation. 8-SVHCE cells were grown to confluence, washed three times with Ca^{2+}- and Mg^{2+}-free PBS, and lysed with ice-cold hypotonic buffer (1 mM Hepes/2 mM EDTA). After 10 min the cells were scraped from the flasks with a rubber policeman and centrifuged at 20,000 × *g* for 10 min. The pellet was resuspended in isotonic buffer (20 mM Hepes, pH 7.4/145 mM NaCl) and homogenized with a Brinkmann Polytron (five times for 1 sec at setting 6–7) prior to repeat centrifugation as indicated above. The final pellet (crude membrane) was resuspended in the isotonic buffer immediately prior to the binding assay.

Preparation of Radioligand. [^{125}I]Iodopindolol was prepared by a modification of the method of Barovsky and Brooker (22), as described by Wolfe and Harden (23). Typically, 20–30% of the ^{125}I was incorporated into the radioligand. The ligand (specific activity 2200 Ci/mmol; 1 Ci = 37 GBq) was stored at −20°C and was used within 3 weeks of preparation.

Assay of β-Adrenergic Receptors. Properties of β-adrenergic receptors were assessed in triplicate by adding aliquots of crude membranes to assay tubes containing, in a final volume of 0.25 ml, 0.0004% bovine serum albumin, 1.1 mM ascorbic acid, 12 mM Hepes buffer (pH 7.4), 0.54% NaCl, and 100 μM GTP. Specific binding was defined as the amount of [^{125}I]iodopindolol bound in the absence of a competing ligand minus the amount bound in the presence of 100 μM (−)-isoproterenol. Equilibrium binding was carried out for 20 min at 37°C in disposable polypropylene tubes (Walter Sarstedt, Princeton, NJ). Reactions were terminated by dilution with 10 ml of buffer (10 mM Tris·HCl/154 mM NaCl, pH 7.5) at room temperature. Samples were then filtered over Schleicher & Schuell glass-fiber filters (no. 30) on a Millipore vacuum filter manifold. The filters were washed with 10 ml of buffer at room temperature and dried with suction. Radioactivity was determined in a Beckman 4000 gamma counter. To determine the density of binding sites, the amount of specifically bound [^{125}I]iodopindolol was determined at six concentrations of radioligand ranging from 10 to 400 pM. Protein was determined by the method of Lowry *et al.* (24), with bovine serum albumin as the standard.

Drugs. The following were gifts: betaxolol (Synthelabo, Paris), ICI 118,551 and 89,406 (Imperial Chemical Industries America, Wilmington, DE), and timolol (Merck Sharp & Dohme). The active isomers of isoproterenol, epinephrine, and norepinephrine were purchased from Sigma.

Data Analysis. The interaction of a nonselective radioligand with receptors is modeled as a simple bimolecular reaction obeying the laws of mass action. Saturation data were analyzed by the method of Scatchard (25) to provide an estimate of the density of receptors and the affinity of the receptor (K_d) for [^{125}I]iodopindolol.

Data from indirect binding studies, in which binding of a fixed concentration of a nonselective radioligand is inhibited by various concentrations of a competing selective or nonselective ligand, were analyzed by a computer-aided curve-fitting program using the mathematical modeling program MLAB, available on the National Institutes of Health-sponsored PROPHET system. Results were analyzed by a method of nonlinear regression analysis of untransformed data (26) to identify the presence of one or two classes of binding sites by comparing the sum of squares of the residuals for one- and two-site models. The data from all competition curves were better fit to a one-site model. K_d values for the binding of a competing drug were calculated from the IC_{50} values by the method of Cheng and Prusoff (27).

RESULTS

We have used SV40 to induce cell proliferation of a starting or primary culture of a mixed population of human PE and NPE cells. After their transformation with SV40, we isolated and characterized a clone, 8-SVHCE. The 8-SVHCE cells described and characterized here have been grown over a period of 7 months (26 passages). The epithelial nature of transformed human CE cells in culture (the 8-SVHCE clone) was confirmed histologically by indirect immunofluorescence using the monoclonal antibody M$_m$DGI-1, which recognizes desmosomal protein components of M_r 145,000–155,000 from bovine muzzle epidermis (17) and which shows crossreaction with desmosomal proteins from 8-SVHCE cells (Fig. 1*A*). This method allows epithelial cells to be distinguished from fibroblasts or endothelial cells (28, 29).

Properties of Clone 8-SVHCE. The growth rate for 8-SVHCE in medium containing 5% FBS was compared with that of untransformed CE cells. Transformed CE cells have the ability to grow in medium containing 0.3% agar supplemented with 10% FBS. After 20–30 days in agar, transformed cells produced visible colonies, whereas no growth was observed with untransformed cells. We also tested transformed cells for continued growth in DMEM with 1% FBS and compared them with normal cells under the same conditions. We observed that normal cells required higher concentrations of FBS and stopped growing after 4–5 days in low-serum medium, whereas transformed cells continued to grow after day 5. Thus, serum requirements differ for normal and transformed cells. Saturation density of 8-SVHCE was 1.2×10^5 cells per cm^2, as opposed to 4×10^4 for primary culture CE cells.

Presence of SV40 T Antigen in 8-SVHCE. An autoradiogram of [^{35}S]methionine-labeled proteins immunoprecipitated from

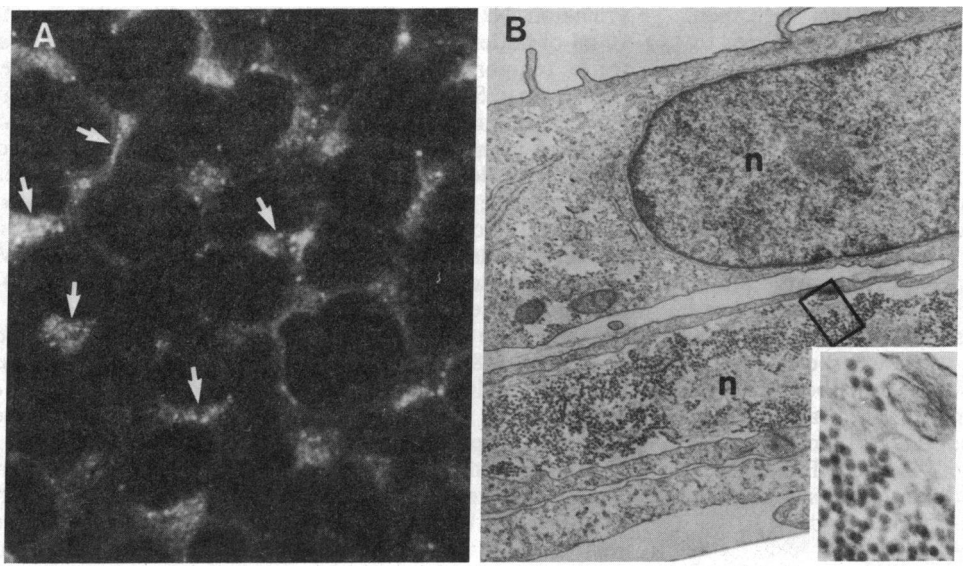

FIG. 1. (*A*) Immunofluorescence microscopy of confluent 8-SVHCE cells showing desmosome-specific staining using monoclonal antibody M_mDGI-1. Typical punctate fluorescent staining (desmosomal plaques) is denser between cells (arrows). (×320.) (*B*) Electron microscopy of transverse thin sections of 8-SVHCE cells at passage 18, grown in multilayers. (×12,000.) Two nuclei (n) of two cells are shown, the upper has nuclear membrane intact, and the other contains free virus particles (see *Insert*, ×48,000).

extracts of 8-SVHCE with anti-SV40 tumor serum (lane T) is shown in Fig. 2. The large T antigen, with M_r estimated to be between 88,000 and 100,000 (13), reacted with hamster anti-SV40 tumor serum. As expected, normal hamster serum did not react with large T antigen (lane N). The immunoprecipitation of the large T antigen was not detected when the additional control was performed using anti-SV40 tumor serum with nontransformed human CE cells (data not shown).

We also used hamster anti-SV40 tumor serum to examine the steady-state levels of T antigens by indirect immunofluorescence. Whereas transformed cells (>90% were T antigen-positive) exhibited a nuclear fluorescence, only background levels were observed in nontransformed cells and when nonimmune serum was used (data not shown).

Southern Blot Analysis. To test for the presence of integrated viral DNA in 8-SVHCE cells, cellular DNA was prepared and digested with the restriction endonuclease *Eco*RI. This restriction enzyme cleaves once within the SV40 genome. The pattern of SV40-specific fragments was analyzed by Southern blot analysis using nick-translated wild-type SV40 DNA as a probe (Fig. 3). A major fragment of 5.2 kilobases (kb) was detected with the same mobility as linear SV40 DNA (Fig. 3, lane 1). When the cellular DNA was digested with *Bgl* II, which does not cleave the SV40 genome but cleaves cellular sequences adjacent to integrated viral DNA, SV40-specific fragments migrating above the position of open circular (II) or linear (L) SV40 DNA were found (Fig. 3, lane 2). SV40 DNA is integrated in high molecular weight cellular DNA, and the number of SV40-specific fragments present in the cell DNA reflects the number of sites where SV40 DNA is integrated. However, we also found SV40-specific DNA of the sizes of closed circular (I) and open circular (II) forms, indicating that these fragments may be derived from free viral DNA (Fig. 3, lane 2). The finding of free viral DNA that was not integrated into the high molecular weight cellular DNA (Fig. 3) is accounted for by the appearance of viral particles in the cell nuclei observed by electron microscopy (Fig. 1*B*). This finding confirms the semipermissive nature of human transformed CE cells.

β-Adrenergic Receptor/Adenylate Cyclase System Responses. The responsiveness of 8-SVHCE cells to catecholamine hormones was shown by the production of intracellular cAMP (Table 1). At 1 μM isoproterenol, the intracellular cAMP level was ≈40% and 170% higher, respectively, than that at 1 μM epinephrine or norepinephrine. The nonspecific β-adrenergic antagonist timolol inhibited cAMP production by the β-adrenergic agonists. No differences were found in the order and level of potency of β-adrenergic agonists when compared with untransformed human CE cells (data not shown). The functional integrity of the β-adrenergic receptor/adenylate cyclase system in 8-SVHCE cells was further demonstrated by the ability of isoproterenol to enhance the phosphorylation of the intermediate-size filament of vimentin type, as shown by the incorporation of ^{32}P into vimentin upon activation (Fig. 4, compare lanes 1 and 2). Similar results have been observed previously in nontransformed human CE cells (11). The addition of timolol blocked the isoproterenol-induced phosphorylation of vimentin (Fig. 4, lane 3). This

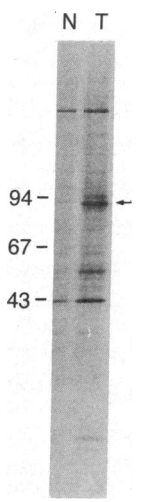

FIG. 2. Immunoprecipitation of viral T antigen in SV40-transformed human cell line 8-SVHCE. [^{35}S]Methionine-labeled cell extracts were immunoprecipitated with normal hamster serum (N) or anti-SV40 tumor serum (T). Immunoprecipitates were analyzed by $NaDodSO_4$/5–18% PAGE followed by fluorography. Positions and molecular weights ($M_r \times 10^{-3}$) of standards run in parallel are at left. Arrow indicates the position of T antigen.

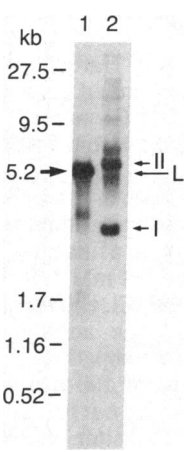

FIG. 3. Southern blot analysis of SV40 DNA in 8-SVHCE cells. High molecular weight DNA from 8-SVHCE cells was digested with *Eco*RI (lane 1) or *Bgl* II (lane 2), separated by electrophoresis in 0.7% agarose gels, transferred to nitrocellulose paper, and probed with ^{32}P-labeled SV40 DNA (specific activity 2.3×10^8 cpm/μg). Arrows at right represent the mobility of superhelical (I), linear (L) 5.2 kb, and open circular (II) SV40 DNA forms. Sizes in kb were determined by comparison with several fragments of SV40 and bacteriophage λ DNA electrophoresed in the same gel.

Table 1. Intracellular cAMP levels in 8-SVHCE cells exposed to β-adrenergic agonists (1 μM) in the presence or absence of antagonist (10 μM timolol)

Treatment	cAMP level	
	pmol/mg of protein	Relative to control
None (control)	16.2 ± 0.5	1.0
Isoproterenol	56.7 ± 3.5	3.5
Isoproterenol + timolol	19.6 ± 2.4	1.2
Epinephrine	40.5 ± 3.2	2.5
Epinephrine + timolol	15.6 ± 2.7	1.0
Norepinephrine	22.4 ± 1.9	1.4
Norepinephrine + timolol	19.8 ± 0.5	1.2

cAMP measurement was done in the presence of 1 mM theophylline. This experiment represents the mean ± SD of triplicate determinations.

suggests that a cAMP-dependent protein kinase is responsible for the β-adrenergic receptor/adenylate cyclase-mediated effect on vimentin phosphorylation in the clone 8-SVHCE.

Equilibrium Binding of [^{125}I]Iodopindolol. Membrane homogenates of 8-SVHCE cells were incubated with [^{125}I]-iodopindolol at concentrations ranging from 10 to 400 pM. The observed binding of the radioligand was saturable and of high affinity [K_d = 41.8 ± 10 pM (mean ± SEM, n = 5)] (Fig. 5). Scatchard analysis revealed a linear plot of transformed direct binding data, indicating a single class of binding sites with an estimated receptor density of 67.1 ± 24 fmol/mg of protein (Fig. 5*A*).

Indirect Analysis of Binding. The ability of several agonists and antagonists to compete for [^{125}I]iodopindolol binding sites was determined (Fig. 5 *B* and *C*). In each case, inhibition curves were best explained by the presence of one population of binding sites. Table 2 lists the affinities of competing antagonists and agonists for the β-adrenergic receptors in 8-SVHCE cell membranes in decreasing order of potency. The pharmacological order of potencies suggests that the β-adrenergic receptors are of the β_2 subtype.

DISCUSSION

The lack of a tissue culture system to maintain secondary cultures of human CE cells, specifically of the NPE layer, has seriously handicapped the study of the physiological properties and biochemical responses of each cell type of the CE. When activated by a variety of pathways (30), the β-adrenergic receptor/adenylate cyclase system in the secretory tissue of the eye, the ciliary process epithelia, reduces the net rate of aqueous humor inflow and the level of intraocular pressure. Specifically, drugs such as β-adrenergic antagonists and carbonic anhydrase inhibitors are known to

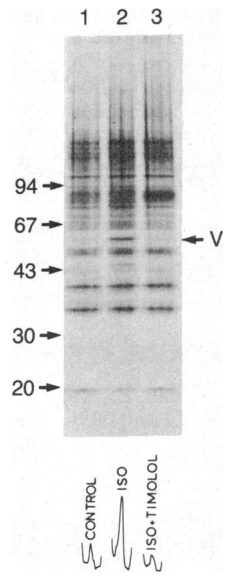

FIG. 4. Effect of isoproterenol on phosphorylation of vimentin in 8-SVHCE cells. ^{32}P-labeled cells were incubated for 60 min with 1 μM isoproterenol (lane 2) or 1 μM isoproterenol plus 10 μM timolol (lane 3). At the end of treatment, cellular lysates were prepared and equal amounts of protein were analyzed by NaDodSO$_4$/5–18% PAGE and autoradiography. Molecular weight ($M_r \times 10^{-3}$) markers at left show positions of standards run in the same gel. The position of phosphorylated vimentin (V) in the gel is indicated and was position-identified as described (11). At the bottom, a comparison of the densitometric scanning of phosphorylated vimentin in the above autoradiogram is shown. The integrated area of the ^{32}P-labeled vimentin peak observed after treatment of cells with isoproterenol alone (lane 2) was about 3 times that of control (lane 1).

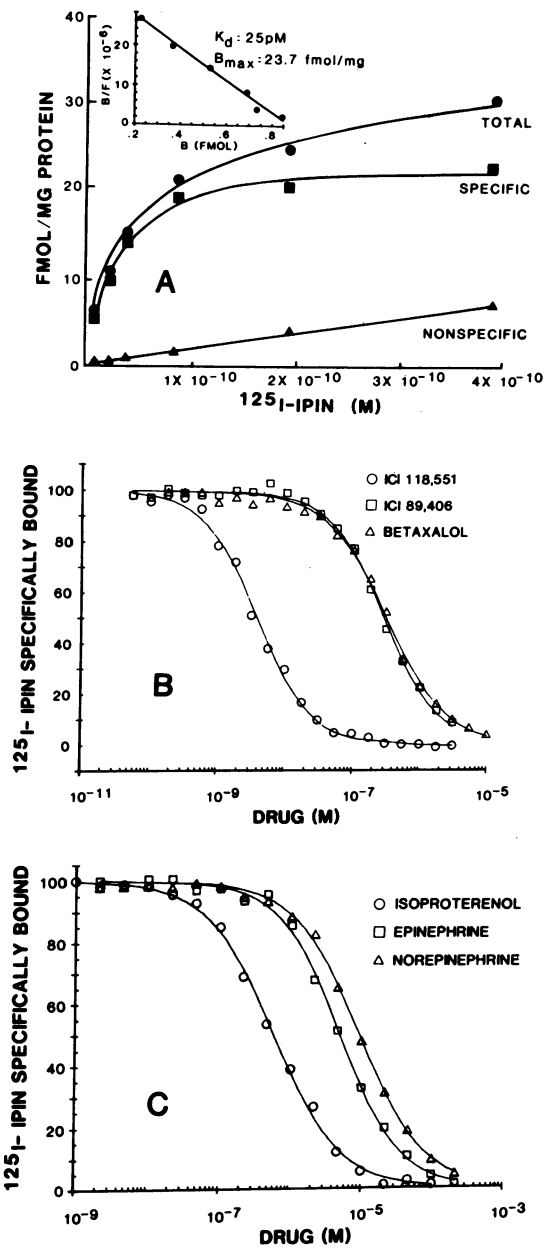

FIG. 5. (*A*) Representative saturation curve for binding of [^{125}I]iodopindolol (^{125}I-IPIN) to 8-SVHCE cell membranes. Total binding (●), specific binding (■), and nonspecific binding (▲) are shown in addition to Scatchard transformation (*Inset*). The K_d and B_{max} values of five determinations in triplicate were 41.8 ± 10 pM and 67.1 ± 24 fmol/mg of protein, respectively (mean ± SEM). (*B*) Inhibition, by selective antagonists, of [^{125}I]iodopindolol binding to 8-SVHCE cell membranes. Assays were carried out with 150 pM [^{125}I]iodopindolol. The IC_{50} values for ICI 118,551 (3.0 nM), ICI 89,406 (0.35 μM), and betaxolol (0.33 μM) are as listed in Table 2 and represent the average of 2 experiments done in triplicate. (*C*) Representative experiment shows the inhibition, by selective agonists, of [^{125}I]iodopindolol (150 pM) binding to 8-SVHCE cell membranes. The IC_{50} values for isoproterenol (0.59 μM), epinephrine (5.0 μM), and norepinephrine (9.4 μM) are the means of triplicate determinations.

Table 2. Apparent equilibrium dissociation constants for drugs acting at β-adrenergic receptors in binding assays with [^{125}I]iodopindolol and 8-SVHCE cell membranes

Drug	Selectivity	K_d, nM	n^H
Antagonists			
ICI 118,551	β_2	0.8	1.0
Betaxolol	β_1	81.0	0.98
ICI 89,406	β_1	93.5	1.05
Agonists			
Isoproterenol	Nonselective	203	0.89
Epinephrine	Nonselective	1440	1.04
Norepinephrine	β_1	2380	0.91

Competition for [^{125}I]iodopindolol binding sites was assayed as described in *Materials and Methods*. All assays were performed in the presence of 100 μM GTP, and the results represent the average of two experiments performed in triplicate. The order of potency is characteristic of the β_2 subtype of β-adrenergic receptors.

have *in vivo* effects on the CE that result in the desirable lowering of the intraocular pressure in glaucomatous eyes. Thus, the study of these cells may be greatly facilitated by the establishment of a viable, immortalized line of CE cells for *in vivo* studies.

A major goal of pharmacology is to define the molecular mechanisms through which an agonist can elicit a biological response. Membrane-bound, β-adrenergic receptor occupancy by agonists usually entails an increase in cAMP levels that are thought to mediate the cellular and physiological changes that occur after the activation of the enzyme adenylate cyclase. We have demonstrated the maintenance of functional β-adrenergic receptors in broken-cell preparations of 8-SVHCE cells and the functional integrity of the β-adrenergic receptor/adenylate cyclase system in whole-cell preparations as assessed by the level of intracellular cAMP following catecholamine stimulation *in vitro*.

β-Adrenergic receptors can be divided into two subtypes, based on the pharmacological specificity of adrenergic receptor-mediated responses to selective drugs (31, 32). In our experiments the rank order of potency to agonists, as assessed by [^{125}I]iodopindolol competition experiments with membrane-bound β-adrenergic receptors of 8-SVHCE cells, is consistent with β-adrenergic receptors of the β_2 subtype. The high affinity of 8-SVHCE β-adrenergic receptors for the β_2-selective antagonist ICI 118,551 and the lower affinity of these receptors for the β_1-selective antagonists betaxolol and ICI 89,406 are consistent with the order of potencies observed for the agonists isoproterenol, epinephrine, and norepinephrine. The pharmacological order of potencies for agonists was similar to that obtained by radioligand-binding experiments when 8-SVHCE cells were functionally assessed by measuring cAMP levels after exposure of the cells to these same drugs. Further, catecholamines enhanced phosphorylation of vimentin in 8-SVHCE cells with the same order of potency as described for normal human CE cells (11).

PE cells contain melanin granules, which serve as structural markers at the light microscopic level and are absent in NPE cells. Once in culture, however, the melanin granules and tyrosinase activity in PE cells are lost, making them phenotypically indistinguishable from NPE cells in secondary cultures (9). Although we do not know whether the isolated 8-SVHCE clone used for our studies is derived from a PE or an NPE cell, we have reported the initial successful establishment of a cell line that retains at least several of the physical and functional properties of the human CE from which it was derived. The establishment of a clone of human CE cells will benefit the study of cellular processes and events that underlie the active component of aqueous humor production. Although undifferentiated with respect to pigment in the cell from which they are derived, these cells may potentially be differentially separated by use of hormonally defined media and identified by specific cellular markers. 8-SVHCE can also be grown as a polarized epithelial monolayer on permeable substrates such as Millipore filters (M.C.-P., unpublished results), and the formation of desmosomes by 8-SVHCE in culture (Fig. 1*A*) can be confirmed by indirect immunofluorescence using antibodies recognizing desmosome plaques, as previously described for bovine CE (9). The successful preservation of cellular identity and function of the 8-SVHCE cells reported here is encouraging in the pursuit to establish differentiated CE cells in culture.

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