α_1- and β_2-Adrenergic Receptor Expression in the Madin-Darby Canine Kidney Epithelial Cell Line

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ABSTRACT The Madin-Darby canine kidney (MDCK) cell line, derived from distal tubule/collecting duct, expresses differentiated properties of renal tubule epithelium in culture. We studied the expression of adrenergic receptors in MDCK to examine the role of catecholamines in the regulation of renal function. Radioligand-binding studies demonstrated, on the basis of receptor affinities of subtype-selective adrenergic agonists and antagonists, that MDCK cells have both α_1- and β_2-adrenergic receptors. To determine whether these receptor types were expressed by the same cell, we developed a number of clonal MDCK cell lines. The clonal lines had stable but unique morphologies reflecting heterogeneity in the parent cell line. Some clones expressed only β_2-adrenergic receptors and were nonmotile, whereas others expressed both α_1- and β_2-receptors and demonstrated motility on the culture substrate at low cell densities. In one clone, α- and β-receptor expression was stable for more than 50 passages. Catecholamine agonists increased phosphatidylinositol turnover by activating α-adrenergic receptors and cellular cyclic adenosine monophosphate accumulation by activating β-adrenergic receptors. Guanine nucleotide decreased the affinity of isoproterenol for the β_2-receptor but did not alter the affinity of epinephrine for the α_1-receptor. These results show that α_1- and β_2-receptors can be expressed by a single renal tubular cell and that the two receptors behave as distinct entities in terms of cellular response and receptor regulation. Heterogeneity of adrenergic receptor expression in MDCK clones may reflect properties of different types of renal tubule cells.

Catecholamines regulate a variety of cellular functions in the mammalian kidney, including tubule water and ion reabsorption, renin release, renal hemodynamics, and gluconeogenesis (20, 35). The influence of renal sympathetic nerves (which release catecholamines as neurotransmitters) on renal function has been discussed in several reviews (1, 6, 32). Catecholamines act on target cells by initially binding to cell surface receptors, and we and others (20, 41–43) have identified α_1-, α_2-, β_1-, and β_2-adrenergic receptors in membrane preparations of the kidney cortex by radioligand-binding techniques. However, the sites of action of adrenergic agents along the nephron have not been clearly defined. Determination of the location of catecholamine action within the kidney is particularly important to distinguish indirect adrenergic effects (resulting from changes in vascular tone) from direct effects on epithelial transport and metabolism. The kidney consists of segments that are anatomically and functionally distinguishable, yet each segment may contain several morphologically distinct cell types (8). To understand the regulation of renal function by catecholamines and sympathetic nerves, it therefore is important to study homogeneous populations of renal cells. Such populations may be found in the established renal cell lines, which have provided valuable information regarding kidney transport and metabolism (15).

We chose the Madin-Darby canine kidney (MDCK)[1] cell line as a model system in which to examine the actions of catecholamines on renal tubule epithelium. The MDCK cell line was established in 1958 from normal dog kidney (12). MDCK cells retain differentiated properties of renal tubule

[1] *Abbreviations used in this paper*: MDCK, Madin-Darby canine kidney cells; and PtdIns, phosphatidylinositol; ICYP, iodocyanopindolol; IHEAT, iodo-2-[β(4-hydroxyphenyl)ethylaminomethyl]tetralone.

epithelium, including transepithelial water and solute transport (28). These cells appear to be derived from distal tubule/collecting duct because of their enzyme markers (38), morphologic features (47), and antigenic determinants (16). Cells of the distal nephron are likely target sites for the catecholamines. The basolateral surface of the distal tubule is directly innervated by renal sympathetic fibers, and the distal nephron is the most responsive segment to β-adrenergic agonists as measured by cAMP accumulation (7). The presence of both α- and β-adrenergic responses in MDCK cells has been reported. In these cells, α-adrenergic agonists stimulate arachidonic acid metabolism (23) and K^+ efflux (3). β-Adrenergic agonists increase cyclic adenosine monophosphate (cAMP) levels (38, 39) and transepithelial Cl^- secretion (2). The adrenergic receptor subtype specificities of these responses have not been identified, although knowledge of the nature of these subtypes is important for understanding the mechanisms mediating target cell responses (11) and for identifying endogenous agonists and exogenous agonists and antagonists that will preferentially occupy the target cell receptors (1, 6, 20, 32, 35). Moreover, several investigators (22, 24, 37, 47) have presented evidence that MDCK is not a homogeneous cell line. We, therefore, addressed the following questions: (*a*) Are α- and β-adrenergic receptors present on renal tubule epithelium? (*b*) If so, which adrenergic subtype(s) are present? (*c*) Are α- and β-adrenergic receptors coexpressed by the same epithelial cell? (*d*) Are responses to α- and β-adrenergic agonists independently mediated?

In this paper, we describe the characterization of α- and β-adrenergic receptors in MDCK cells in terms of their ligand specificities, guanine nucleotide regulation, and intracellular second-messenger systems. We have developed a number of clonal MDCK cell lines with different morphologic features and different adrenergic receptor expression. We conclude that: (*a*) MDCK cells express α_1- and β_2-adrenergic receptors; (*b*) MDCK is a heterogeneous cell line consisting of at least two stable cell types, one with both α_1- and β_2-receptors and the other with only β_2-receptors; (*c*) in a clonal cell line derived from parent MDCK cells, α_1- and β_2-agonists mediate discrete intracellular responses that are independently regulated. Preliminary reports of some of these findings have been presented in abstract form (29, 30).

MATERIALS AND METHODS

Materials: The following compounds were received as gifts from the sources indicated: (−)-epinephrine, (+)-epinephrine, and (−)-norepinephrine (+)-bitartrate salts (Sterling Winthrop Research Institute, Rennselear, NY); (−)-propranolol HCl, (+)-propranolol HCl (Ayerst Laboratories, New York); phentolamine mesylate, clonidine (Geigy Pharmaceutical, Summit, NJ); cyanopindolol HCl (Sandoz Inc., Basel, Switzerland); prazosin (Pfizer Inc., Groton, CT); practolol (ICI Americas, Inc., Wilmington, DE); 2[β-(4-hydroxyphenyl)ethylaminomethyl]tetralone HCl (Beiersdorf AG, Hamburg, Federal Republic of Germany); and IPS 339 (Dr. Kenneth Minneman, Emory University, Atlanta, GA). The following radiochemicals were purchased from the sources indicated: carrier-free $Na^{125}I$ (New England Nuclear, Boston, MA; Amersham Corp., Arlington Hts., IL); *myo*-[^{3}H]inositol, [^{3}H]prazosin (Amersham); [^{3}H]yohimbine (New England Nuclear). Formula 946 liquid scintillation cocktail was from New England Nuclear. Cell culture media and sera were from Grand Island Biological Co., Grand Island, NY. Plasticware for cell culture was manufactured by Falcon Labware, Div. of Becton, Dickinson & Co. (Oxnard, CA), Costar (Cambridge, MA), or Lux Scientific Corp. (Naperville, IL). All other reagents were from standard sources.

Preparation of [^{125}I]Iodocyanopindolol ([^{125}I]ICYP) and [^{125}I]Iodo-2-[β(4-hydroxyphenyl)ethylaminomethyl]tetralone ([^{125}I]IHEAT): Cyanopindolol (CYP) was iodinated by minor modification of the method of Engel et al. (10). The reaction mixture contained 20 μg CYP, 10 μl 13.5 mM HCl, 20 μl potassium phosphate buffer (0.3 M, pH 7.6), 2 mCi carrier-free $Na^{125}I$, and 20 μl chloramine T in aqueous solution (0.34 mg/ml). The mixture was incubated for 5 min at room temperature, after which the reaction was stopped by the addition of 300 μl of aqueous $Na_2S_2O_3$ (6.3 mM). NaOH (10 μl of 1 N) was then added, and the reaction mixture was extracted four times with 300 μl 0.01% phenol in ethylacetate. The combined washes were concentrated under N_2 and then subjected to ascending paper chromatography at room temperature on 3-mm paper (22 × 24 cm) (Whatman Ltd., Clifton, NJ) with 0.01% phenol in 0.1 M ammonium formate (pH 8.5) as the solvent (running time ~4 h). The iodinated product migrated with an R_f of 0.09 and was extracted from the appropriate strip of the chromatogram with 0.01% phenol in methanol for ~24 h at −20°C. The [^{125}I]ICYP migrated as a single spot when subjected to thin-layer chromatography on silica gel uniplates (Analtech Inc., Newark, DE) with pyridine/glacial acetic acid/water (0.33:0.6:9.07, vol/vol/vol) as the solvent. [^{125}I]ICYP was stable for at least 12 wk when stored as a 100 nM solution in 0.01% phenol in methanol.

2[β-(4-Hydroxylphenyl)ethylaminomethyl]tetralone (HEAT) was iodinated by a modification of the method of Engel and Hoyer (9), which is the same as that described above for [^{125}I]ICYP except the reaction time was 1 min. When subjected to ascending paper chromatography, the product migrated with an R_f of 0.13. The [^{125}I]IHEAT migrated as a single spot when subjected to thin-layer chromatography on silica gel with pyridine/glacial acetic acid/water (0.33:0.6:9.07, vol/vol/vol) as the solvent (R_f = 0.7). When methanol/chloroform (30:50, vol/vol) was used as solvent, the product ran with an R_f of 0.79, and trace radiochemical impurities with R_fs of 0.25 and 0.92 were detected. The [^{125}I]IHEAT was stable for at least 4 mo when stored at −20°C as a 100 nM solution in 0.01% phenol in methanol; the amounts of the radiochemical impurities did not increase during storage.

Cell Culture: Low-passage MDCK cells (catalog No. CCL34, passage 53) were obtained from the American Type Culture Collection (ATCC, Rockville, MD). ATCC-derived cells of a later passage number were obtained from Dr. Milton Saier, Department of Biology, University of California, San Diego. The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 7.5% heat-inactivated horse serum, 2.5% heat-inactivated fetal calf serum, 15 mM HEPES, and 20 μg/ml gentamycin. Serum-free medium was prepared according to the formulation of Taub et al. (46). Confluent cells were subcultured once weekly with a trypsin-EDTA solution and were inoculated at dilution ratios between 1:4 and 1:10 into 250-ml culture flasks containing 20 ml of medium. The cells were maintained in a humidified 37°C incubator in an atmosphere of 90% air and 10% CO_2. Clonal cell lines were obtained by limiting dilution (<0.5 cell/ml) of single-cell suspensions prepared from subconfluent cultures into 24-well plates (1 ml/well; 2 cm^2 surface area/well). Clonal growth was confirmed by frequent microscope inspection of the wells. Cell lines were stored at −70°C after detachment with trypsin-EDTA and resuspension in growth medium containing 10% dimethyl sulfoxide (vol/vol).

Membrane Preparation: Cell membranes were prepared by a hypotonic lysis method from MDCK cells grown to confluence (3–6 d) in 150-mm culture dishes. Each dish was washed twice with 5 ml of ice-cold lysis buffer (1 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.5) and incubated with 10 ml of lysis buffer for 10 min at 4°C. The lysed cells were removed from the dishes by scraping with a Costar cell scraper. The dishes were washed once with 5 ml of lysis buffer, and the pooled lysate was centrifuged at 30,000*g* for 10 min at 4°C. The pellet was washed two more times with lysis buffer and resuspended in ice-cold incubation buffer (145 mM NaCl, 20 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.5). This procedure resulted in uniform lysis of the cells. The resulting preparation consisted of cell membrane "ghosts" and all of the nuclei could be stained by the addition of trypan blue. The cell concentration in the suspension was determined for each experiment by mixing 100 μl of suspension and 100 μl of 0.4% trypan blue and then counting the intact, trypan blue–stained nuclei in a hemacytometer. Membrane protein was determined by the method of Lowry (25) using bovine serum albumin standards. The protein content was 0.146 ± 0.03 mg/10^6 lysed cells and was ~15% higher for subconfluent cells than for confluent cells.

Radioligand Binding Assays: Binding assays were performed in duplicate or triplicate by incubating 0.125–0.2 ml of freshly prepared membrane suspension (0.5–3 × 10^6 cells/tube) with 0.025 ml of [^{125}I]ICYP or [^{125}I]IHEAT or 0.05 ml of [^{3}H]prazosin and 0.025–0.1 ml of various drugs in a final volume of 0.25 ml (0.5 ml for [^{3}H]prazosin) in polypropylene test tubes (16 × 105 mm, Walter Sarstedt, Inc., Princeton, NJ). Assays were initiated by the addition of membrane and were carried out in incubation buffer (145 mM NaCl, 20 mM Tris-HCl, 2 mM $MgCl_2$, pH 7.5). For equilibrium binding studies, membranes were incubated for 60 min at 37°C in a shaking water bath (~80 cycles/min). The incubation was terminated by the addition of 10 ml of 37°C incubation buffer. Bound and free radioligand were then separated by rapid (<10 s) filtration over Whatman GF/C glass fiber filters (Whatman Ltd.) on a Millipore filtration manifold (Millipore Corp., Bedford, MA), and the filters were washed

with 10 ml of 37°C incubation buffer. Radioactivity retained on the filters was determined using a Searle gamma counter (Searle Analytic, Inc., Des Plaines, IL) at 63% efficiency (^{125}I), or with 4.5 ml of scintillation cocktail in a Beckman scintillation counter (Beckman Instruments, Inc., Fullerton, CA) at 30% efficiency (^{3}H). Nonspecific binding was defined with the following agents: for [^{3}H]prazosin and [^{3}H]yohimbine, 10 μM phentolamine; for [^{125}I]IHEAT, 0.5 or 1.0 μM prazosin; and for [^{125}I]ICYP, 1.0 μM (±)propranolol. Specific binding was determined by subtraction of nonspecific binding from total binding. The specific binding was routinely the following percents of the total binding at concentrations of the radioligands near their dissociation constants: >60% for [^{3}H]prazosin, >70% for [^{125}I]IHEAT and >75% for [^{125}I]ICYP. Specific binding of the radioligands was linear with cell number. When catecholamines were used in binding experiments, ascorbic acid was included at a final concentration of 0.5 mg/ml to prevent drug oxidation. Replicate data points varied by <10%.

Data Analysis: The dissociation constant (K_D) and maximum number of binding sites (B_{max}) were determined from Scatchard analysis (40) of saturation binding isotherms. The line of the Scatchard plot was fitted by linear regression analysis.

Competitive binding curves were analyzed by a computer program (LIGAND) that performs iterative nonlinear regression (33).

Phosphatidylinositol Assay: MDCK-D cells were seeded in 35-mm culture dishes containing 3 ml of medium and grown to confluence (2 d). Assays were performed with triplicate dishes in a humidified 37°C incubator with a 5% CO_2 atmosphere. Experiments were initiated by rinsing each dish twice with 20 ml of 37°C Krebs-Henseleit buffer (KHB: 118 mM NaCl, 4.7 mM KCl, 3.0 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 0.5 mM EDTA, 25 mM $NaHCO_3$, and 10 mM glucose, pH 7.4) and then adding 750 μl of *myo*-[^{3}H]inositol (6.6 Ci/mmol, ~4.5 μCi/dish) in KHB. 60 min later, 7.5 μl of drug or control solution was added, and the incubation was continued for an additional 30 min. Ascorbic acid (5 mg/ml) was included in the freshly prepared epinephrine stock solutions to prevent drug oxidation before addition to the cells (final concentration, 0.05 mg/ml). Incubations were terminated by removal of the incubation mixture by aspiration followed by three washes with 2 ml of ice-cold saline. Cold saline (0.5 ml) was then added, and the cells were removed from each dish by scraping. Each dish was washed two more times with 0.5 ml of cold saline, and the pooled washes were centrifuged for 30 s in a microfuge. The supernatant was removed, and 0.75 ml of ice-cold chloroform/methanol (1:2) was added to the pellet. The termination procedure was completed in <3 min.

Phosphatidylinositol (PtdIns) was extracted by the addition of 0.2 ml of 2 M KCl to each sample (final proportions of chloroform/methanol/2 M KCl, 10:5:4) followed by sonication using a Kontes cell disrupter in three 10-s bursts. A two-phase system was formed by the addition of 0.25 ml of chloroform and 0.25 ml of 2 M KCl followed by centrifugation at 1,600*g* for 20 min at 4°C. The aqueous upper phase and any interfacial material were removed, the organic lower phase was decanted, and the remaining tissue pellet was discarded. The lower phase was washed twice with 0.5 ml of chloroform/methanol/water (3:47:48) and dried under N_2, and the tritium content was determined in 3.5 ml of OCS scintillation cocktail (Amersham)/Triton X-100 (2:1). Control experiments showed that <0.05% of the free *myo*-[^{3}H]inositol contaminated the lower phase and that 100 ± 1% of the total lower phase counts were recovered as PtdIns, as determined by lipid separation by two-dimensional thin-layer chromatography (first dimension: chloroform/methanol/water/18 M NH_4OH, 130:70:8:0.5; second dimension: chloroform/acetone/methanol/acetic acid/water, 10:4:2:2:1) on 20 × 20-cm silica gel glass plates (Merck & Co., Rahway, NJ) (36).

cAMP Assay: MDCK cells were grown to confluence (1–3 d; ~5 × 10^5 cells/well) in 24-well culture dishes. The medium was removed by aspiration, and the cells were washed twice with 1 ml of Hanks' balanced salt solution. Incubation medium (0.4 ml) consisting of a freshly prepared solution of drugs in HBSS was then added, and the dishes were incubated at 37°C for 5 min. At the conclusion of the incubation, 100 μl of 40% trichloroacetic acid was added to each well. After a 10-min incubation at room temperature, the trichloroacetic acid extracts were removed from each well and transferred to 0.5 ml Dowex AG 1 × 8 columns. The columns were washed with 2.5 ml of water, and the cAMP was eluted with 4 ml water. Recovery of the cAMP was 90–95% as determined in control columns using [^{3}H]cAMP. The cAMP eluate was dried with a Speedvac concentrator (Savant Instruments, Inc., Hicksville, NY). Cyclic AMP was measured by a competitive binding protein assay using aliquots of the dried sample fractions resuspended in sodium acetate buffer (18, 19).

RESULTS

MDCK Cell Lines

The results of our initial radioligand binding experiments with MDCK membranes indicated that MDCK cells possessed both α_1- and β_2-adrenergic receptors. These receptors are described in subsequent sections. We found, however, that the expression of α_1-receptors varied in MDCK cell lines obtained from different sources. Our first experiments were conducted using MDCK cells derived from ATCC stock culture that had been maintained in the laboratory of Dr. Saier. These cells had approximately equal numbers of α_1- and β_2-receptors (Table I). When we obtained cells directly from ATCC, we found that early passage cultures had more

TABLE I

Summary of the Morphologic and Adrenergic Receptor Properties of MDCK Parental and Clonal Cell Lines

Cell Line	Origin	Morphology	Domes	Adrenergic Receptors	
				Alpha$_1$	Beta$_2$
				sites/cell	
Parental:					
ATCC	ATCC, p53	M and NM	+	1,200	1,100 (p56)
				1,100	2,300 (p60)
				0	3,100 (p75)
ATCC	M. Saier	M, F	+	4,600	3,900
Clonal:					
C	ATCC, p59	NM, F	+	0	1,800 (p7)
D	ATCC, p59	M, F	±	7,900	470 (p7)
H	ATCC, p68	NM, C	−	0	970 (p7)
L	ATCC, p68	NM, C	±	0	6,300 (p7)
M	ATCC, p68	NM, F	−	0	740 (p8)
N	ATCC, p68	NM, F	−	0	960 (p7)
Q	SF	M, F	+	15,000	1,600 (p5)
R	SF	M, F	+	13,000	2,500 (p6)
S	SF	M, F	+	15,000	3,100 (p5)
T	SF	M, F	+	22,000	3,200 (p4)
U	SF	M, F	+	19,000	1,100 (p5)

The origin column indicates the cell line from which each cell line was derived. The morphology column refers to the appearance of the cells when seeded at low density. "Domes" refers to the presence or absence of multicellular domes (or hemicysts) in confluent monolayers as observed over ~20 passages of continuous growth. The number of sites for both adrenergic receptor subtypes was determined in parallel at the indicated passage number by Scatchard analysis of radioligand-binding isotherms as described in the text. *ATCC*, American Type Culture Collection; *SF*, ATCC stock from Saier's laboratory grown in serum-free medium for 6 mo and then returned to serum-containing medium; *NM*, nonmotile; *M*, motile; *F*, flattened; *C*, cuboid; *p*, passage number, for clonal cell lines this refers to the number of passages after cloning.

β_2-receptors than α_1-receptors (Table I) and that the number of α_1-receptors decreased with increasing passage number. The two cell lines were maintained in our laboratory under identical culture conditions. To resolve the problem of variable α_1-receptor expression, we cloned early-passage ATCC cells (MDCK-ATCC) by limiting dilution and obtained several clonal cell lines. In addition, we cloned cells that we had originally obtained from Dr. Saier's laboratory but that had been grown in serum-free medium for ~6 mo and then returned to serum-containing medium (MDCK-SF).

Phase-contrast micrographs of several representative clonal lines are shown in Fig. 1. When ATCC cells were seeded at low density, cells with different morphologies were observed within the population (Fig. 1*A*). Some colonies consisted of cells that were elongated and flattened, while others contained more cuboid cell types. Clones derived from parent populations were homogeneous but exhibited distinct morphologic features resembling different elements of the parent population. The morphologic differences between clones were most pronounced in subconfluent cultures and were not as obvious in cells seeded at near-confluent densities or in confluent cultures. Clone R and other clones derived from MDCK-SF had a typical epithelioid appearance (Fig. 1, *B* and *C*). At low density, these clones were extended, flattened, and motile. Upon reaching confluency, the cultures formed multicellular "domes," which is indicative of apical to basolateral fluid transport (22) (Fig. 1 *C*). These clones were extremely resistant to detachment from their culture substrate by trypsin-EDTA at confluency. A clone with this same general morphology was also obtained from early passage MDCK-ATCC (clone D; Fig. 1 *D*). However, clone D seldom formed domes under routine culture conditions. Other clonal lines obtained from MDCK-ATCC had strikingly different morphologies. These cells were nonmotile, as evidenced by their growth in discrete, compact colonies at low cell densities. Some, such as clone L (Fig. 1 *F*), were cuboid and did not spread on the culture substrate. Others, such as clone M (Fig. 1 *E*), were more flattened but were similarly nonmotile. The majority of the nonmotile clones did not form structures that could be identified as domes by phase-contrast light microscopy. In addition, these clones were relatively easily detached from their substrate with trypsin-EDTA, even after attaining confluency. When cultures were initiated from clonal lines that had been stored at −70°C for several months, the morphologies of the thawed cells were identical to those of the original clone, indicating that the clonal morphologies were stable phenotypic features.

Clonal MDCK cell lines were screened for their expression of α_1- and β_2-adrenergic receptors by radioligand binding techniques (described in the following sections). Scatchard analyses of radioligand binding isotherms were performed at least once with each clone for both α_1- and β_2-receptors. As summarized in Table I, we found that the nonmotile clones did not express α_1-receptors, as shown by their lack of detectable specific binding of the iodinated α_1-receptor probe, [^{125}I] IHEAT. The lower limit of detection was ~100 α_1-receptors per cell. The motile cell lines consistently expressed α_1-receptors, and all cell lines expressed β_2-receptors. There were 8,000–20,000 α_1- and 500–6,000 β_2-receptors per cell in receptor-bearing cells. Growth of Saier's ATCC cells in serum-free defined medium for 2 mo had no acute effect on receptor expression, since α_1- and β_2-receptor numbers on serum-free cells (4,600 α_1- and 4,000 β_2-receptors/cell) were comparable to those of cells grown in serum-containing medium (5,000 α_1- and 3,900 β_2-receptors/cell) when assayed in parallel. Therefore, we attribute the high frequency of α_1-receptor–

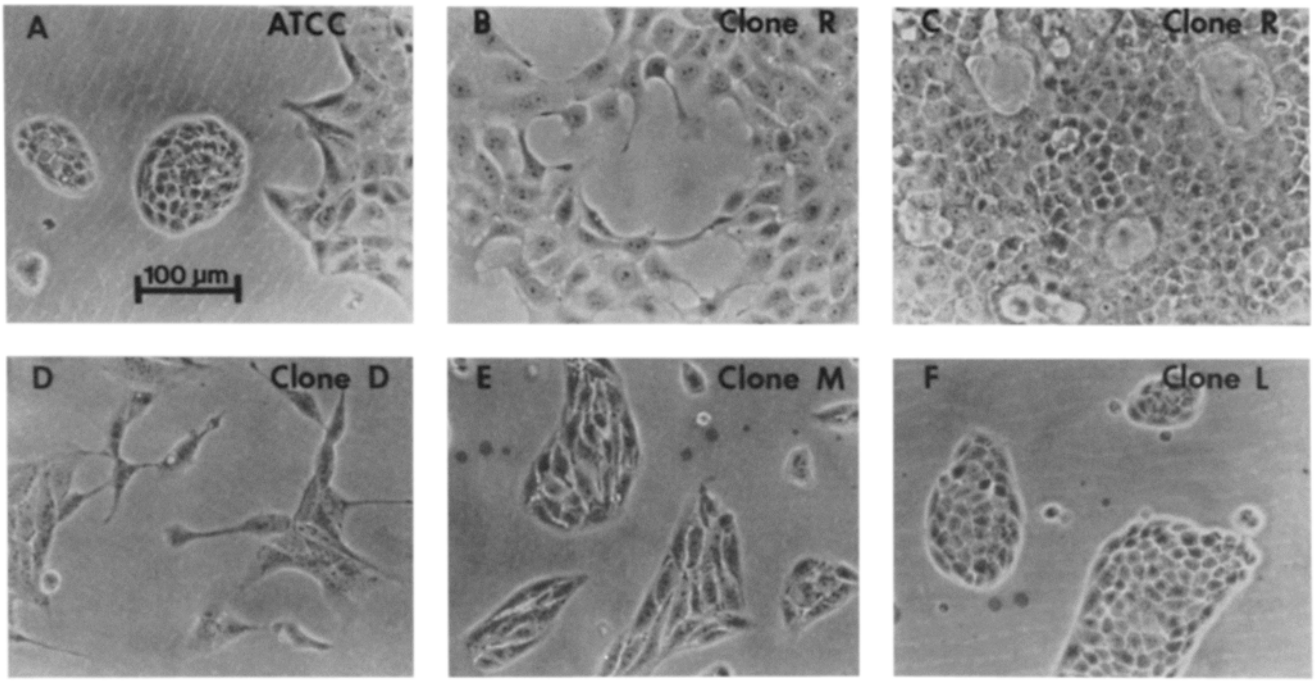

FIGURE 1 Phase-contrast photomicrographs of MDCK parental and clonal lines. MDCK cells were seeded at low density in plastic culture dishes and then photographed 1–3 d later with a Nikon inverted microscope equipped with phase-contrast optics, courtesy of Dr. Silvio Varon, University of California, San Diego. (*A*) MDCK-ATCC, passage 76. (*B*) Clone R, subconfluent, passage 6. (*C*) Clone R, confluent with dome formation, passage 6. (*D*) Clone D, passage 22. (*E*) Clone M, passage 7. (*F*) Clone L, passage 7. The passage numbers refer to the number of passages after cloning, except for the ATCC cells in which the ATCC passage number applies. × 1,400.

bearing clones in MDCK-SF primarily to the composition of the parent cell population. However, after long-term (5 mo) growth in serum-free medium, the MDCK-SF cells had 13,000 α_1-receptors and 2,400 β_2-receptors per cell. Therefore, we cannot rule out the possibility that the increased frequency of α_1-receptor–bearing cells resulted, in part, from selective pressure caused by long-term growth in serum-free medium.

To study both α_1- and β_2-adrenergic receptors in a homogeneous cell population, we used clone D. This clone was chosen because it was derived from the early-passage ATCC stock and was therefore most likely to reflect properties of the renal cell from which it was derived. The morphology and adrenergic receptor expression of clone D have been stable for over 50 passages since initial cloning. In the following sections we will describe adrenergic receptor properties in both parental (MDCK-ATCC) and clonal (MDCK-D) lines.

α_1-Adrenergic Receptor Characterization

Two subtype-selective α-adrenergic antagonists, [^{3}H]prazosin and [^{3}H]yohimbine, which we have found useful in identifying renal α_1- and α_2-receptors, respectively (42), were used in initial binding experiments with parental MDCK cells. No specific binding of [^{3}H]yohimbine was detected in several experiments, indicating that MDCK cells did not express α_2-adrenergic receptors. In contrast, specific binding of [^{3}H]prazosin was consistently observed, suggesting that α_1-receptors were present on MDCK.

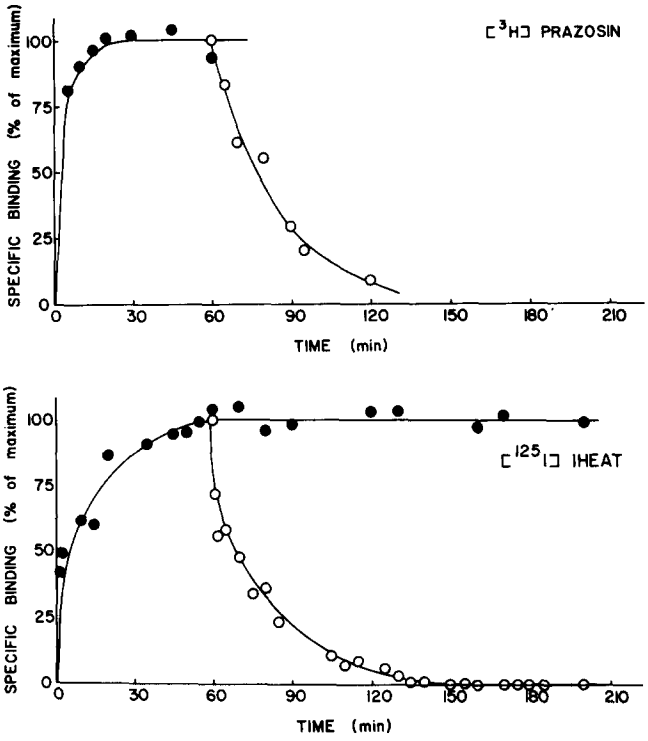

FIGURE 2 Kinetic analyses of [^{3}H]prazosin and [^{125}I]IHEAT binding to MDCK membranes. Specific binding of 0.50 nm [^{3}H]prazosin (*upper* panel) to MDCK and 0.13 nM [^{125}I]IHEAT (*lower* panel) to MDCK-D membranes at 37°C was followed as a function of time (filled circles). After 60 min, the rate of dissociation of the radioligands (circles) was followed subsequent to the addition of phentolamine to a final concentration of 10 μM (for [^{3}H]prazosin, *upper* panel) or prazosin to a final concentration of 1 μM (for [^{125}I]IHEAT, *lower* panel). Each data point represents the mean of duplicate samples. The data are expressed as percent of maximum specific binding as defined in the text.

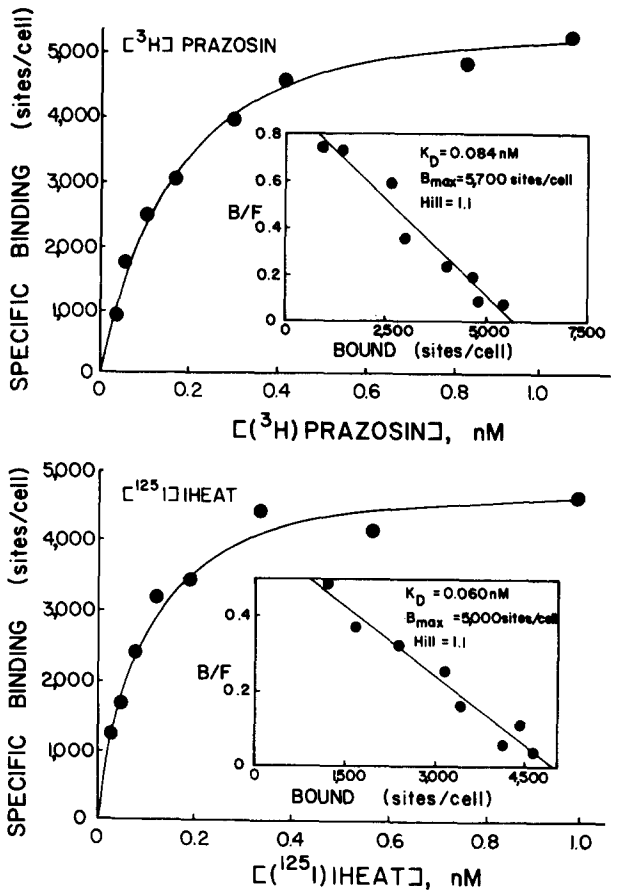

FIGURE 3 Saturation binding isotherms for [^{3}H]prazosin and [^{125}I]IHEAT on MDCK membranes. MDCK membranes were incubated with the indicated concentrations of [^{3}H]prazosin (*upper* panel) or [^{125}I]IHEAT (*lower* panel) for 60 min at 37°C. Conditions for the binding assay and the determination of specific binding are described in the text. The *insets* show the Scatchard analyses of the binding data used to determine the equilibrium dissociation constants (K_D), binding site numbers (B_{max}), and Hill coefficients. These data were obtained in a single experiment using the same membrane preparation for both radioligands. Each data point represents the mean of triplicate determinations.

Further characterization of the specific [^{3}H]prazosin binding sites showed that these sites demonstrated properties expected for α_1-adrenergic receptors. A kinetic analysis of [^{3}H]prazosin binding to MDCK membranes is shown in Fig. 2 (*upper* panel). Specific binding reached equilibrium rapidly at 37°C, with saturation occurring in 20 min. Upon addition of 10 μM phentolamine (the α-adrenergic antagonist used to define nonspecific binding), specific [^{3}H]prazosin binding dissociated with a half-time of 18 min and was totally reversible. The specific binding of [^{3}H]prazosin was saturable, as shown in Fig. 3 (*upper* panel). In this representative experiment, the maximum number of binding sites (B_{max}) was 5,700 sites per cell. Scatchard analysis of the equilibrium binding isotherm indicated the presence of a single class of binding sites with a K_D of 0.74 ± 0.3 nM (n = 4). The noncooperative nature of the binding sites was indicated by a Hill coefficient of unity (1.08 ± 0.09). When [^{3}H]prazosin binding assays were performed in the presence of competing adrenergic ligands, the results were consistent with binding to a pure population of α_1-receptors (Fig. 4). Indoramin, a classic α_1-antagonist, had

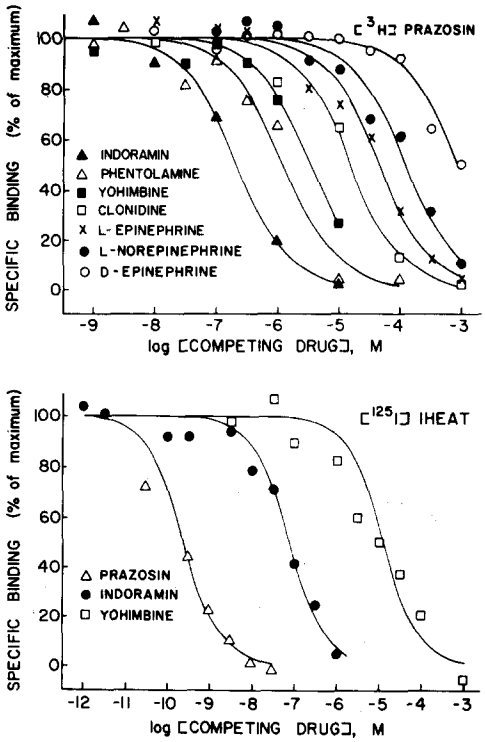

FIGURE 4 Competition for [^{3}H]prazosin and [^{125}I]IHEAT binding sites by adrenergic agonists and antagonists. The binding of 0.6 nM [^{3}H]prazosin (*upper* panel) or 0.4 nM [^{125}I]IHEAT (*lower* panel) to MDCK membranes was measured in the presence of the indicated concentrations of competing ligands. Data are expressed as percent of maximum specific binding as defined in the text. The curves were fitted to one-site models by the LIGAND program. Each data point represents the mean of triplicate determinations, and each panel shows data obtained for different ligands using the same membrane preparation.

an affinity for the [^{3}H]prazosin binding sites 19 times higher than did the α_2-antagonist yohimbine. Using the LIGAND curve-fitting program, we were able to determine whether competing drugs bound to a single site with one affinity ("one-site fit") or whether the binding was better described by two (or more) sites with different affinities ("two-site fit") (33). The subtype-selective antagonists bound to a single population of receptors, inasmuch as fits of the competition binding data for these drugs based on a two-site model were not significantly improved over fits to a one-site model. [^{3}H] Prazosin bound to stereoselective sites, as evidenced by the severalfold higher affinity of (−)-epinephrine as compared with (+)-epinephrine; this result is as expected for α-adrenergic receptors (42).

α-Adrenergic binding sites were also identified with the iodinated α_1-selective antagonist, [^{125}I]IHEAT (9, 13). The higher specific activity of this ligand compared to [^{3}H]prazosin allowed binding experiments to be performed with fewer MDCK cells. [^{125}I]IHEAT bound to α_1-receptors that had the same properties as the receptors identified with [^{3}H]prazosin. Binding of [^{125}I]IHEAT at 37°C was saturable, achieved equilibrium in 50 min, and was totally reversible upon the addition of excess unlabeled prazosin (0.5 μM, the concentration used to define specific binding) with a half-time of dissociation of 8 min (Fig. 2, *lower* panel). Scatchard analysis of [^{125}I] IHEAT binding is shown in Fig. 3 (*lower* panel). This assay was conducted in parallel with the [^{3}H]prazosin binding assay shown in Fig. 3 (*upper* panel) with the same membrane preparation. [^{125}I]IHEAT bound with high affinity (K_D = 0.151 ± 0.07 nM [n = 8]) to a single class of noncooperative binding sites (Hill coefficient = 1.03 ± 0.04). Comparison of the upper and lower panels of Fig. 3 shows that similar numbers of sites were identified with [^{125}I]IHEAT and [^{3}H] prazosin (5,000 vs. 5,700 sites/cell). The results of competitive binding assays are shown in Fig. 4 and are summarized in Table II. The finding that the affinity of unlabeled HEAT was 18 times lower than that of [^{125}I]IHEAT confirms a similar observation by Glossman et al. (13). Prazosin was more potent than yohimbine, and (−)-epinephrine was more potent than (+)-epinephrine. These data indicate that both [^{3}H]prazosin and [^{125}I]IHEAT are suitable ligands for the identification of α_1-adrenergic receptors of MDCK cells.

β_2-Adrenergic Receptor Characterization

β-Adrenergic receptors were identified on MDCK membranes with [^{125}I]ICYP, a high-affinity β-antagonist that binds with equal affinity to both β_1- and β_2-receptor subtypes (10). The kinetics of association and dissociation of [^{125}I]ICYP from MDCK membranes is shown in Fig. 5. [^{125}I]ICYP specific binding reached equilibrium in 40 min at 37°C and was slowly dissociable ($t_{1/2}$ ~ 300 min) upon the addition of 1 μM propranolol. [^{125}I]ICYP specific binding was saturable, with maximum binding to 2,100 sites/cell in this representative experiment. Scatchard analysis of the equilibrium binding isotherm (Fig. 6) showed a single class of noncooperative binding sites with a K_D of 0.051 ± 0.03 nM (n = 12) and a Hill coefficient of 1.00 ± 0.09. The results of competitive binding assays are shown in Fig. 7 and are summarized in Table II. The [^{125}I]ICYP binding sites were determined to be β_2-receptors on the basis of the dissociation constants of catecholamine agonists (isoproterenol < epinephrine ≪ norepinephrine. The subtype-selective antagonists practolol (β_1) and IPS 339 (β_2) each bound to a single class of binding sites, since their competition binding data were adequately described by one-site models. From these results, we conclude that MDCK cells have a pure population of β_2-receptors and have no detectable β_1-receptors. (−)-Propranolol was 210 times more potent than (+)-propranolol, and (−)-isoproterenol was 23 times more potent than (+)-isoproterenol, indicating that the [^{125}I]ICYP-binding sites had the stereoselectivity expected of β-adrenergic receptors.

Stability of Receptor Expression in Clone D

The presence of both α_1- and β_2-receptors on a number of clonal MDCK lines showed that these two receptor types were expressed on the same cell. The pharmacologic properties of the α_1- and β_2-receptors of clone D were similar to those of the parent cell lines (Table II). A subclone of clone D (D-1) also coexpressed α_1- and β_2-receptors, confirming that these receptors were present on a single cell. To use clone D as a model system in which to examine these receptors, we required evidence that the receptor expression was stable with time in culture. Scatchard analyses of radioligand binding to α_1- and β_2-receptors in early- and late-passage MDCK-D cells are shown in Fig. 8. Receptor numbers were compared between cells that had been maintained for 53 passages since initial cloning from the parent line and cells that had been grown from frozen early-passage stock and had been main-

TABLE II

Summary of Equilibrium Dissociation Constants for Adrenergic Ligands in MDCK

Radioligand	Competing Ligand	K_D ATCC	K_D Clone D
		nM	
I. Alpha			
A. [^{3}H]prazosin	—	0.074 ± 0.03	
Antagonist:	Indoramin	20	—
	phentolamine	140	—
	yohimbine	380	—
Agonist:	clonidine	1,400	—
	(−)-epinephrine	6,200	—
	(−)-norepinephrine	18,000	—
	(+)-epinephrine	120,000	
B. [^{125}I]IHEAT	—	0.151 ± 0.07	0.186 ± 0.03
Antagonist:	prazosin	0.048	0.076
	HEAT	—	2.8
	indoramin	—	24
	phenoxybenzamine	—	46
	phentolamine	640	—
	yohimbine	1,100	4,100
Agonist:	(−)-epinephrine	2,100	2,200
	(+)-epinephrine	—	87,000
II. Beta-adrenergic receptors			
A. [^{125}I]ICYP	—	0.051 ± 0.03	0.031 ± 0.02
Antagonist:	IPS 339	0.19	—
	(−)-propranolol	0.36	—
	(+)-propranolol	77	—
	practolol	7,200	—
Agonist:	(−)-isoproterenol	35	23
	(−)-epinephrine	44	280
	(+)-isoproterenol	800	—
	(−)-norepinephrine	3,300	3,000

K_Ds for the radioligands were determined by Scatchard analysis of equilibrium binding isotherms; K_Ds for unlabeled agonists and antagonists were determined from the concentrations that reduced specific radioligand binding by 50% and in all cases were estimated by the LIGAND program using a one-site model to fit the data. Each value was derived from a single experiment in which several other ligands were concomitantly tested, so that their rank orders of potency might be easily compared.

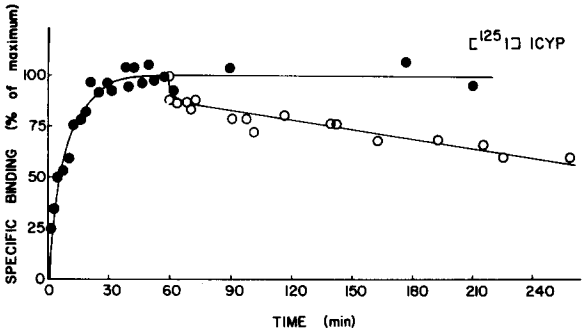

FIGURE 5 Kinetic analysis of [^{125}I]ICYP binding to MDCK membranes. Specific binding of 0.07 nM [^{125}I]ICYP to MDCK membranes at 37°C was followed as a function of time (filled circles). After 60 min, the rate of dissociation of [^{125}I]ICYP (circles) was followed subsequent to the addition of (±)-propranolol to a final concentration of 1 μM. Each data point represents the mean of duplicate determinations. The data are expressed as percent of maximum specific binding as defined in the text.

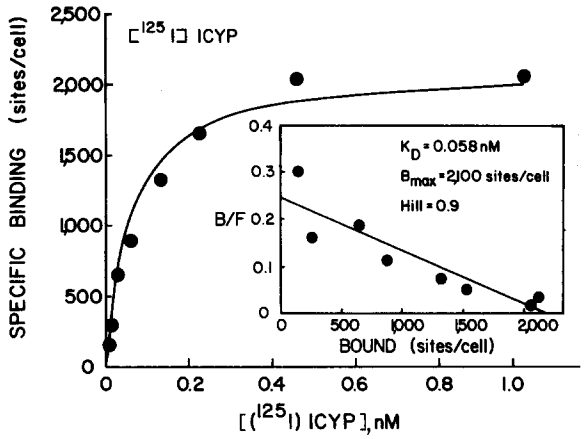

FIGURE 6 Saturation binding isotherm of [^{125}I]ICYP to MDCK membranes. MDCK-D membranes were incubated with the indicated concentrations of [^{125}I]ICYP for 60 min at 37°C, and specific binding was determined as described in the text. The *inset* shows a Scatchard analysis of the equilibrium binding data, which was used to calculate the K_D, B_{max}, and Hill coefficient. Each data point represents the mean of triplicate determinations.

tained for 11 passages since cloning. The early- and late-passage cells, when grown and assayed under identical conditions, expressed identical numbers of α_1- and β_2-adrenergic receptors, indicating that receptor expression in MDCK-D was not altered during prolonged maintenance in culture.

Guanine Nucleotide Modulation of Agonist Binding

Guanine nucleotides are known to lower the affinity of β-adrenergic agonists for their receptors (44) and have been

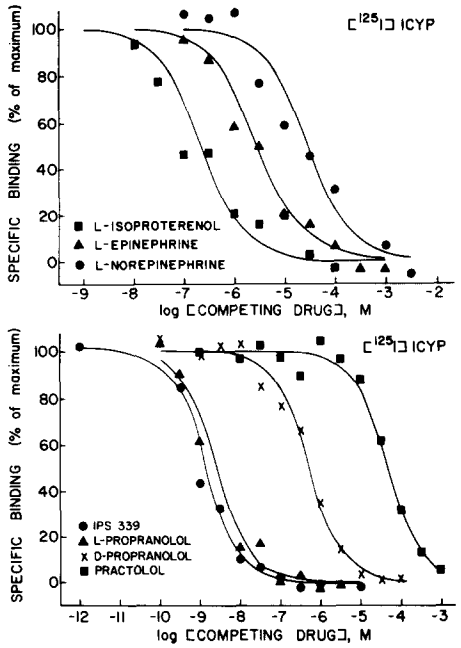

FIGURE 7 Competition for [^{125}I]ICYP binding to MDCK β-adrenergic receptors by adrenergic agonists and antagonists. The binding of 0.3 nM [^{125}I]ICYP to MDCK membranes was measured in the presence of the indicated concentrations of competing agonists (*upper* panel) and antagonists (*lower* panel). The data were fitted to one-site models by the LIGAND program and are expressed as percent of maximum specific binding as defined in the text. Each data point represents the mean of triplicate determinations, and each panel shows data obtained for different ligands using the same membrane preparation.

shown to have a similar but less pronounced effect at renal α_1-adrenergic receptors (42). We investigated guanine nucleotide effects on agonist binding to MDCK-D membranes to see whether the receptors were differentially sensitive to the nucleotide.

The effects of the nonhydrolyzable guanine nucleotide guanylyl-5′-imidodiphosphate (Gpp(NH)p) on agonist-binding to MDCK-D α_1- and β_2-receptors are shown in Fig. 9. In the *lower* panel of Fig. 9, the effect of Gpp(NH)p on the affinity of isoproterenol for MDCK-D β_2-receptors is shown. The control curve shown is the two-site fit, which was statistically superior to the one-site fit ($P = 0.003$) for these data. In this representative experiment, the high-affinity site represented 76% of the total sites, with a K_D for isoproterenol of 15.5 nM. The low-affinity site (24% of total sites) had a K_D of 490 nM. In the presence of guanine nucleotide, the data were described adequately by a one-site fit ($P > 0.1$ for two-site vs. one-site fit), with a K_D for isoproterenol of 470 nM. These data are similar to observations in other β-receptor systems (44). The effect of guanine nucleotides is thought to reflect heterogeneity of agonist binding sites, in which some of the agonist-receptor complexes interact with a GTP-binding regulatory protein that mediates the coupling between receptor and adenylate cyclase (44). In the absence of added guanine nucleotide, this portion of the agonist-bound receptor population is in a high-affinity state that converts to a low-affinity state upon the addition of guanine nucleotide.

As shown in the *upper* panel of Fig. 9, Gpp(NH)p did not alter the affinity of (−)-epinephrine for MDCK-D α_1-receptors (K_D = 11,000 nM for control and 8,700 nM with Gpp(NH)p). This experiment was repeated several times with both MDCK-D and MDCK ATCC cells and with both [^{3}H]prazosin and [^{125}I]IHEAT, yet no consistent effect of Gpp(NH)p was observed. The data shown are fit to a one-site model, because

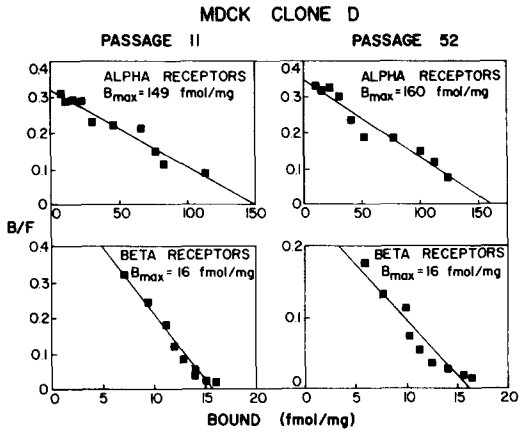

FIGURE 8 Comparison of α_1- and β_2-adrenergic binding sites in low- and high-passage MDCK-D cells. Membranes prepared from clone D cells that had been frozen at passage 6 and then grown to passage 11 (*left* panels) were analyzed for adrenergic radioligand binding in the same experiment with membranes prepared from clone D cells grown for 52 continuous passages since cloning (*right* panels). The Scatchard analyses of the [^{125}I]IHEAT and [^{125}I]ICYP binding isotherms are shown. The data are expressed as sites per milligram of protein to correct for any differences in cell size (both cultures were ~90% confluent). Each data point represents the mean of triplicate determinations.

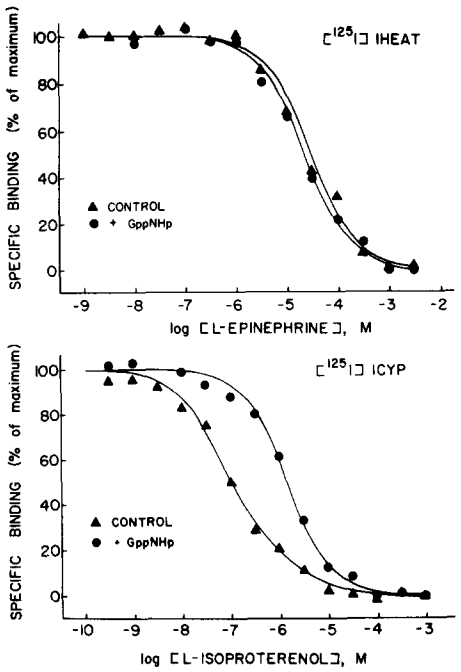

FIGURE 9 Effects of guanine nucleotide on agonist binding to α_1- and β_2-adrenergic receptors in MDCK-D. The competition of (−)-epinephrine for 0.24 nM [^{125}I]IHEAT sites (*upper* panel) and of (−)-isoproterenol for 0.13 nM [^{125}I]ICYP sites (*lower* panel) was assessed in the presence (circles) and absence (filled circles) of 100 μM Gpp(NH)p using membranes prepared from MDCK clone D. The data shown are LIGAND-determined one-site fits for all curves except for the isoproterenol control, in which the data were better described by the two-site fit. Each data point represents the mean of triplicate determinations.

fits to a two-site model were not significantly better ($P > 0.1$ for two-site vs. one-site fit). These results show that MDCK α_1- and β_2-receptors are not affected in the same way by guanine nucleotides.

Phosphatidylinositol Turnover

The effect of epinephrine on the incorporation of [^{3}H] inositol into phosphatidylinositol (PtdIns) was examined to determine whether α_1-receptors in MDCK cells are functionally coupled to this putative second-messenger system, as has been observed for α_1-receptors in other tissues (11). MDCK-D cells were prelabeled with *myo*-[^{3}H]inositol for 60 min, exposed to various concentrations of (−)-epinephrine for an additional 30 min, and the incorporation of the label into cellular PtdIns was determined (Fig. 10). In the presence of epinephrine the rate of incorporation of [^{3}H]inositol increased to a maximum of 92% above the baseline levels seen in the absence of epinephrine (Fig. 10). A half-maximum effect (EC_{50}) occurred with 1.8 μM epinephrine, a concentration similar to the K_D of epinephrine in the binding studies (Table 2). The response to epinephrine was totally blocked by the simultaneous addition of 1 μM prazosin (Fig. 10), whereas prazosin alone had no effect on inositol incorporation (data not shown). Epinephrine stimulation of *myo*-[^{3}H]inositol incorporation into PtdIns was also observed when cells were prelabeled with *myo*-[^{3}H]inositol for 30 min, rather than 60 min, before drug addition (data not shown). These results indicate that α_1-receptor occupation stimulates PtdIns synthesis in MDCK cells.

Cyclic AMP Accumulation

The effect of isoproterenol on cyclic AMP accumulation in MDCK cells was studied to determine whether the β_2-receptors were coupled to adenylate cyclase. When ATCC-derived cells were incubated with isoproterenol, a rapid increase in cAMP accumulation was observed. In the presence of 0.5 mM isobutylmethylxanthine, a phosphodiesterase inhibitor, the cAMP attained a maximum level after 5 min of isoproterenol exposure (data not shown). The dose-response relationship for this effect (Fig. 11, *left*) indicated that the EC_{50} for isoproterenol was 0.1 μM. Epinephrine also increased

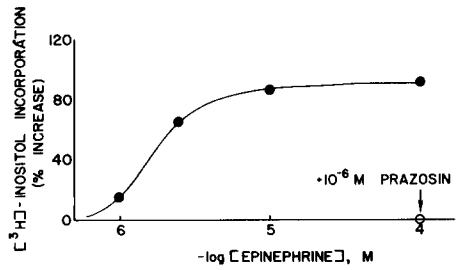

FIGURE 10 Stimulation of phosphatidylinositol turnover in MDCK-D by epinephrine. MDCK clone D cells were incubated with *myo*-[^{3}H]inositol for 60 min at 37°C. The indicated concentrations of epinephrine were then added, and the incubation was continued for 30 min. The assay was terminated, and PtdIns was extracted as described in the text. The data are expressed as the percent increase in *myo*-[^{3}H]inositol incorporation into PtdIns compared with control. The data obtained when 1 μM prazosin was added concomitantly with 10^{-4} M epinephrine are indicated on the figure by the circle. Each data point represents the mean of triplicate determinations.

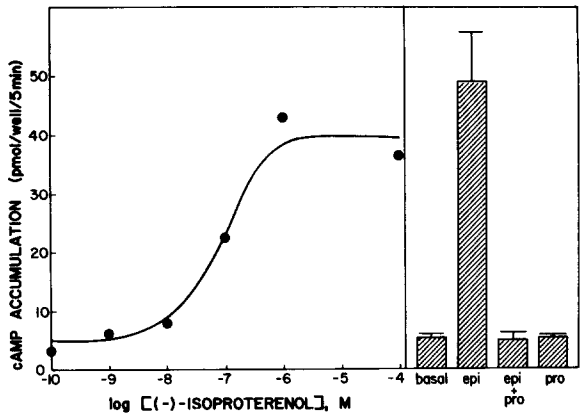

FIGURE 11 Stimulation of cAMP accumulation in MDCK by isoproterenol and epinephrine. MDCK cells were incubated with the indicated concentrations of (−)-isoproterenol (*left* panel). The *right* panel represents cells incubated in the absence of epinephrine ("basal"), with epinephrine in the presence and absence of propranolol, or with propranolol alone. The data points shown represent means of triplicate determinations ± SEM and are expressed as picomoles of cAMP per well (of a multiwell culture plate) per 5 min. Data in both panels were obtained using the same cell preparation.

cAMP accumulation, and this increase was completely blocked by 1 μM propranolol (Fig. 11, *right*). Propranolol alone had no effect on cAMP levels. Isoproterenol increased cAMP levels to a similar extent in several clonal cell lines. The increase in cAMP content over baseline levels in response to 10^{-5} M isoproterenol was 978, 1,030, and 1,077 pmoles cAMP/10^7 cells/10 min for clones D, L, and N, respectively. These results show that enhanced cAMP accumulation was promoted by agonist occupancy of β-adrenergic receptors in MDCK cells.

DISCUSSION

In this study we have shown that MDCK renal epithelial cells have α_1- and β_2-adrenergic receptor subtypes, that both receptor types can be expressed by a single cell, that the receptors show different sensitivity to guanine nucleotides, and that α_1- and β_2-receptors mediate distinct intracellular responses. The radioligand binding experiments showed that α- and β-receptors of MDCK can be classified as classic α_1- and β_2-receptors and that there are no detectable α_2- or β_1-receptors. In contrast, studies of membranes prepared from kidney cortex have indicated that the number of α_2- and β_1-receptors is two to three times greater than that of α_1- and β_2-receptors (41–43), suggesting that MDCK cells represent only a minor portion of the adrenergic receptor-bearing cells in the kidney cortex. Our findings suggest that growth of MDCK in vitro does not alter the recognition properties of these receptors, inasmuch as the receptors show binding specificities characteristic of classic α_1- and β_2-adrenergic receptor subtypes. In this regard, it is interesting that the affinity of the endogenous agonists norepinephrine and epinephrine for β_2-receptors is much higher than that for α_1-receptors of MDCK cells.

The findings further our understanding of the nature of adrenergic receptors in renal tubules. The functions of renal α_1-receptors are poorly understood, and these receptors have been thought to be located primarily on the renal vasculature (20, 41). However, recent studies indicate that renal α_1-receptors mediate gluconeogenesis (27), which probably occurs in

tubule cells (17, 26). Other work suggests that renal tubule α-receptors may regulate ion and water reabsorption by the nephron (6, 20, 32, 35). The results of our experiments with MDCK cells provide direct evidence that α_1-receptors are located on renal tubule epithelium. Further, although data indicating that β-adrenergic receptors are present on tubule epithelium have been presented (7, 20, 31), we show that tubule cells can possess a "pure" population of β_2-adrenergic receptors. Our findings imply that the administration of drugs acting at α_1- and β_2-adrenergic receptors may perturb tubule cell function. Because α_1- and β_2-receptor subtypes are present on several clonal isolates from parent MDCK cells, at least some (but probably not all) tubule cells are able to express both receptor subtypes. Additional work will be required to determine exactly which tubular cells in vivo possess one or both types of adrenergic receptors.

Previous work has indicated that α- and β-adrenergic receptors are regulated quite differently by guanine nucleotides and other factors in target cells. However, few studies have been performed with cells of which one could be confident that both classes of receptors are simultaneously expressed (18, 34). As observed in other systems (44), Gpp(NH)p shifted the high-affinity component of agonist binding to β-adrenergic receptors of MDCK cell membranes to a lower affinity, such that only low-affinity sites were detected. In contrast, epinephrine bound to a single class of α_1-receptor sites on MDCK membranes, and the affinity of these sites for agonist was not altered by guanine nucleotide. α_1-Receptors are not generally thought to be coupled to adenylate cyclase or to a guanine nucleotide regulatory protein. However, in other α_1-receptor systems guanine nucleotides have been shown to decrease agonist affinity (14, 22). α_1-Receptors of MDCK cells appear to be in a homogeneous (pseudo-Hill slope for epinephrine = 0.87 ± 0.05 [$n = 3$]), low-affinity state in the absence of guanine nucleotide, and this may account for the lack of guanine nucleotide effect. This lack of regulation of α_1-receptors by guanine nucleotides may reflect properties of the native receptors or may be secondary to the membrane preparation or incubation conditions.

In addition to differences in receptor regulation by guanine nucleotides, our findings show important differences in the linkage of α_1- and β_2-receptors to second-messenger systems. Activation of adenylate cyclase by catecholamines in renal membranes and in MDCK cells has been correlated with changes in transepithelial transport (20, 35). We have confirmed that adenylate cyclase activation is mediated by β-receptors (in MDCK, by β_2-receptors). α_1-Adrenergic stimulation, in a variety of systems, results in calcium mobilization and alterations in membrane phospholipid metabolism (11). The stimulation of PtdIns synthesis by α_1-agonists in MDCK may be a key step in the events resulting from α_1-receptor occupation. α-Adrenergic stimulation of PtdIns synthesis is thought to result from initial stimulation of PtdIns hydrolysis (11), which may result in the release of arachidonic acid, which may in turn result in increased prostaglandin synthesis. α-Adrenergic-induced changes in prostaglandin synthesis have been reported for MDCK cells (23). Our data suggest that alterations in PtdIns metabolism may be an initial step in this response.

In spite of differences in receptor recognition properties and in linkage to second-messenger systems, it has been suggested that α- and β-adrenergic receptors may be located at separate sites on the same protein or may be interconvertible entities (21). Most recently, receptor interconversion has been suggested as a mechanism to account for temperature-induced changes in adrenergic effects on renin release by the kidney (5). Thus far, we have found no evidence for receptor interconversion in clonal MDCK cells. MDCK cells can express both α_1- and β_2-receptors or only β_2-receptors. The receptors behave as distinct macromolecules in terms of their modulation by guanine nucleotide, apparently because they are coupled to different regulatory proteins. Each receptor mediates discrete biochemical responses: α_1-agonists increase PtdIns turnover, and β_2-agonists increase cAMP levels, indicating that the receptors likely are discrete entities. In additional studies we have obtained preliminary data on receptors solubilized from clone D, indicating structural differences in α_1- and β_2-receptors from these cells (45).

In this study we also obtained information regarding the heterogeneity of the MDCK cell line showing that MDCK clones differ in morphology and in adrenergic receptor expression. Expression of α_1-receptors was observed only in motile clones but was not correlated with the ability of clones to form domes. Clonal morphologies and receptor expression were stable phenotypic features, which suggests that the various cell types were present in the parent cell line and did not arise by spontaneous mutation. Morphologic differences between clonal MDCK lines have been observed by others (22, 47). Differences in ion transport (37) and arachidonic acid metabolism (24) between different nonclonal MDCK sublines have been noted. Variations between nonclonal MDCK sublines may have arisen by selection for one cell type over another during maintenance in culture. Inasmuch as MDCK was derived from a mince of kidney cortex (12), cell heterogeneity is not unexpected. MDCK cells appear to be derived mainly from distal tubule/collecting duct; however, heterogeneity of cell types exists even within these segments. Valentich (47) has suggested that two MDCK cell types may be derived from the principal and intercalated cells of the collecting duct. Our data confirm his conclusions regarding the stability of morphologically distinct clones, but additional studies will be required to draw further analogies between our clonal lines and the cell types identified by Valentich. In addition, we have not yet determined the cellular factor(s) that result in the expression of the motile phenotype. MDCK cells synthesize basal lamina when grown on collagen (47) and also release biomatrix components when grown on plastic (4). Clonal variations in the production of such molecules may affect cell attachment and motility, but this hypothesis will require further study. We believe that cloned MDCK cells will serve as unique model systems for further studies of the cellular regulation of renal adrenergic receptors and for evaluating other cellular properties of this cultured renal cell line.

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