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Angiotensin II Receptor Type I-Regulated Anion Secretion in Cystic Fibrosis Pancreatic Duct Cells

H.C. Chan[1], S.H. Law[1], P.S. Leung[1], L.X.M. Fu[2], P.Y.D. Wong[1]

[1]Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong
[2]Goteborg University, Wallenberg Laboratory, Sahlgrens Hospital, Gothenburg, Sweden

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Abstract. The β-adrenergic (cAMP-dependent) regulation of Cl^- conductance is defective in cystic fibrosis (CF). The present study explored alternative regulation of anion secretion in CF pancreatic ductal cells (CFPAC-1) by angiotensin II (AII) using the short-circuit current (I_{SC}) technique. An increase in I_{SC} could be induced in CFPAC-1 cells by basolateral or apical application of AII in a concentration-dependent manner (EC_{50} at 3 μM and 100 nM, respectively). Angiotensin receptor subtypes were identified using specific antagonists, losartan and PD123177, for AT_1 and AT_2 receptors, respectively. It was found that losartan (1 μM) could completely inhibit the AII-induced I_{SC}, whereas, PD123177 exerted insignificant effect on the I_{SC}, indicating predominant involvement of AT_1 receptors. The presence of AT_1 receptors in CFPAC-1 cells was also demonstrated by immunohistochemical studies using specific antibodies against AT_1 receptors. Confocal microscopic study demonstrated a rise in intracellular Ca^{2+} upon stimulation by AII indicating a role of intracellular Ca^{2+} in mediating the AII response. Depletion of intracellular but not extracellular pool of Ca^{2+} diminished the AII-induced I_{SC}. Treatment of the monolayers with a Cl^- channel blocker, DIDS, markedly reduced the I_{SC}, indicating that a large portion of the AII-activated I_{SC} was Cl^--dependent. AII-induced I_{SC} was also observed in monolayers whose basolateral membranes had been permeabilized by nystatin, suggesting that the I_{SC} was mediated by apical Cl^- channels. Our study indicates an AT_1-mediated Ca^{2+}-dependent regulatory mechanism for anion secretion in CF pancreatic duct cells which may be important for the physiology and pathophysiology of the pancreas.

Correspondence to: Dr. H. Chang Chan

Key words: Angiotensin II — CFPAC-1 cells — Cl^- secretion — AT_1-receptor — Ca^{2+}

Introduction

It has been suggested that the cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-dependent Cl^- channel which plays a pivotal role in pancreatic ductal bicarbonate secretion by recirculating the Cl^- imported into duct cells through the Cl^--HCO_3^- exchanger [2]. Mutations in CFTR result in defective β-adrenergic (cAMP-dependent) regulation of the Cl^- channel leading to impaired HCO_3^- secretion in the pancreatic duct. Alternative Cl^- channel activation pathways have been sought for circumventing the defect in CF. A CF pancreatic duct cell line, CFPAC-1, has been developed to serve as a continuous cell line that displays the CF defect [19]. Previous studies on this cell line have demonstrated the activation of Cl^- conductance by ATP [12]. The action of ATP on anion secretion across cultured polarized monolayers of these CF cells has been recently demonstrated to be mediated by a P_{2U}-linked Ca^{2+}-dependent regulatory mechanism [5]. In addition to the previously observed Ca^{2+}-activated single Cl^- channel activity [19], Ca^{2+} and CaMKII-dependent Cl^- secretion has also been demonstrated recently in these cells [7]. Taken together, these results suggest a Ca^{2+}-dependent Cl^- channel activation pathway which may have therapeutic potential. We undertook this study to explore regulation of anion secretion across the CF pancreatic ductal epithelium by angiotensin II (AII) since AII is known to be a Ca^{2+} mobilizing agent [3, 4, 17] and shown to be present in the pancreas [9]. Activation of Cl^- channels or stimulation of Cl^- secretion by AII has also been reported in a number of normal secretory epithelia including the airway [18, 20], intestinal [11] and

the epididymal epithelia [25]. The present study is the first to demonstrate an effect of AII on pancreatic anion secretion via AT_1 receptors, which involves activation of apical Cl^- channels by AII-induced Ca^{2+} mobilization.

Materials and Methods

MATERIALS

Iscove's modified Dulbecco's medium, Hank's balanced salt solution (HBSS), trypsin, Angiotensin II (AII), ATP, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), Dulbecco's phosphate buffer saline (DPBS) and nystatin were purchased from Sigma (St. Louis, MO). Fetal bovine serum was from Gibco Laboratories (New York). Amiloride hydrochloride was obtained from Merck Sharp & Dohme Research Lab. (Rahway, NJ). Thapsigargin was from Research Biochemicals International (Natick, MA). Sylgard resin (184 silicone slastemer kit) and silicone rubber (3140 RTV), for making the permeable supports, were purchased from Dow Corning Corp. (Midland, Mich). 0.45 μm Millipore filters were obtained from Millipore Corp. (Bedford, MA). Losartan and PD123177 were a generous gift from the DuPont Merck Pharmaceutical Company.

SOLUTIONS

Krebs-Henseleit (K-H) solution had the following composition (mM): NaCl, 117; KCl, 4.5; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 24.8; KH_2PO_4, 1.2; glucose, 11.1. The solution was gassed with 95% O_2, 5% CO_2 at 37°C and the pH of the solution was 7.4. In permeabilization studies, a gradient of 40:120 mM (apical:basolateral) Cl^- was applied across the epithelium by substituting a portion of Cl^- with gluconate in the apical solution.

CELL CULTURE

The culture procedure for cystic fibrosis pancreatic duct (CFPAC-1) cells was similar to that described previously [5]. The cells were grown in Iscove's modified Dulbeccos' medium supplemented with 10% fetal bovine serum. The cells (1.2×10^6 cells/ml) were plated onto each floating permeable support which was made of Millipore filter with a silicone rubber ring attached on top of it for confining the cells (with an area of 0.45 cm^2). Before the cells were plated the permeable supports were placed carefully on to culture medium so that they could float on top of the surface. Cultures were incubated at 37°C in 5% CO_2/95% air atmosphere, and reached confluence in 3–4 days.

IMMUNOHISTOCHEMICAL STUDIES

The CF pancreatic duct cells were plated onto Millipore filters or coverslips for immunohistochemistry. After three days in culture cells were rinsed with DPBS, pH 7.4 for 15 min. Cells grown on filters were frozen in embedding medium using isopentane. Cryostat sections (8 μm) from cells grown on filters were cut on Cryotome (Shandon AS 620 Cryotome). Sections were transferred onto gelatin-coated glass slides and air-dried for 30 min. Sections on filters or cells on coverslips which were permeabilized in DPBS containing 0.5% Triton X-100 for 10 min, were immediately fixed with cold acetone (–20°C) for 10 min. Remaining acetone was removed by drying followed by washing with DPBS for 15 min. Unspecific binding was blocked by 0.1% (w/v) bovine serum albumin (BSA) in DPBS for 1 hr at room temperature, followed with DPBS washing for 10 min. Samples were then processed for indirect immunofluorescence staining technique. Samples were first incubated overnight at 4°C with affinity-purified anti-peptide antibodies, namely angiotensin II receptor subtypes AT_1 and AT_2 (1:200) diluted in DPBS containing 0.1% BSA and 0.5% Triton X-100. After washing briefly with DPBS several times, samples were incubated with anti-rabbit IgG-fluorescein conjugated secondary antibody (Boehringer Mannheim, working dilution: 40 μg/ml) for 60 min at room temperature. Samples were again washed with DPBS several times and embedded in mounting medium (Vectashield, Vector). Samples were then examined by confocal laser scanning microscopy (Bio-Rad MRC-1000).

The following control experiments were employed: (a) substitution of primary antibody with buffer; (b) incubation of primary antibody with rabbit non-immune serum; (c) preadsorption of primary antibody with excess peptide antigen (1 mg/ml).

SHORT-CIRCUIT CURRENT MEASUREMENT

The measurement of I_{SC} has been described previously [22, 24]. Monolayers grown on permeable supports were clamped vertically between two halves of the Ussing chamber. Monolayers were bathed in both sides with K-H solution which was maintained at 37°C by a water jacket enclosing the reservoir. The solution was bubbled with 95% O_2 and 5% CO_2 such that the pH of the solution was maintained at 7.4. Drugs could be added directly to the apical or basolateral side of the epithelium. Usually, the epithelium exhibited a basal transepithelial PD which was measured by the Ag/AgCl reference electrodes (Metrohm, Switzerland) connected to a preamplifier that in turn connected to a voltage-clamp amplifier (World Precision Instruction, DVC-1000). In most of the experiments, the change in I_{SC} was defined as the maximal rise in I_{SC} upon agonist stimulation and it could be normalized by the unit area of the epithelial monolayer. In some experiments, a transepithelial PD of 0.1 mV was applied. The change in current in response to the applied potential was used to calculate the transepithelial resistance of the monolayer using the Ohmic relationship.

CONFOCAL SCANNING MICROSCOPY

CFPAC-1 cells grown on glass coverslips were loaded with the Ca^{2+}-sensitive fluorescence dye Fluo 3-AM (3 μM) from a 1 mM stock in dry DMSO to the culture medium without serum. The cells were then incubated at 37°C for 45 min in dark. After loading, the cells were washed three times with medium and placed in the incubation chamber with 0.4 ml physiological saline solution containing (in mM):130 NaCl, 2 $CaCl_2$, 5 KCl, 1 $MgCl_2$, 10 glucose, and 20 HEPES, pH 7.4.

Fluorescence images were captured by a laser scanning confocal microscope (MRC 1000UV confocal imaging system, Bio-Rad) equipped with an Argon-ion UV laser and connected to an inverted microscope (Nikon Diaphot, Japan) fitted with a 100 × 1.4 NA objective. Fluo-3 was excited at 488 nM and Ca^{2+}-dependent fluorescence was captured at 520 nm.

STATISTICAL ANALYSIS

Results are expressed as mean ± SEM. Comparisons between groups of data were made by Student's unpaired *t*-test. A '*P*' value of less than 0.05 was considered statistically significant.

Results

AII-ACTIVATED I_{SC}

The cultured CFPAC-1 monolayers responded to AII, both apical and basolateral addition, with a rapid tran-

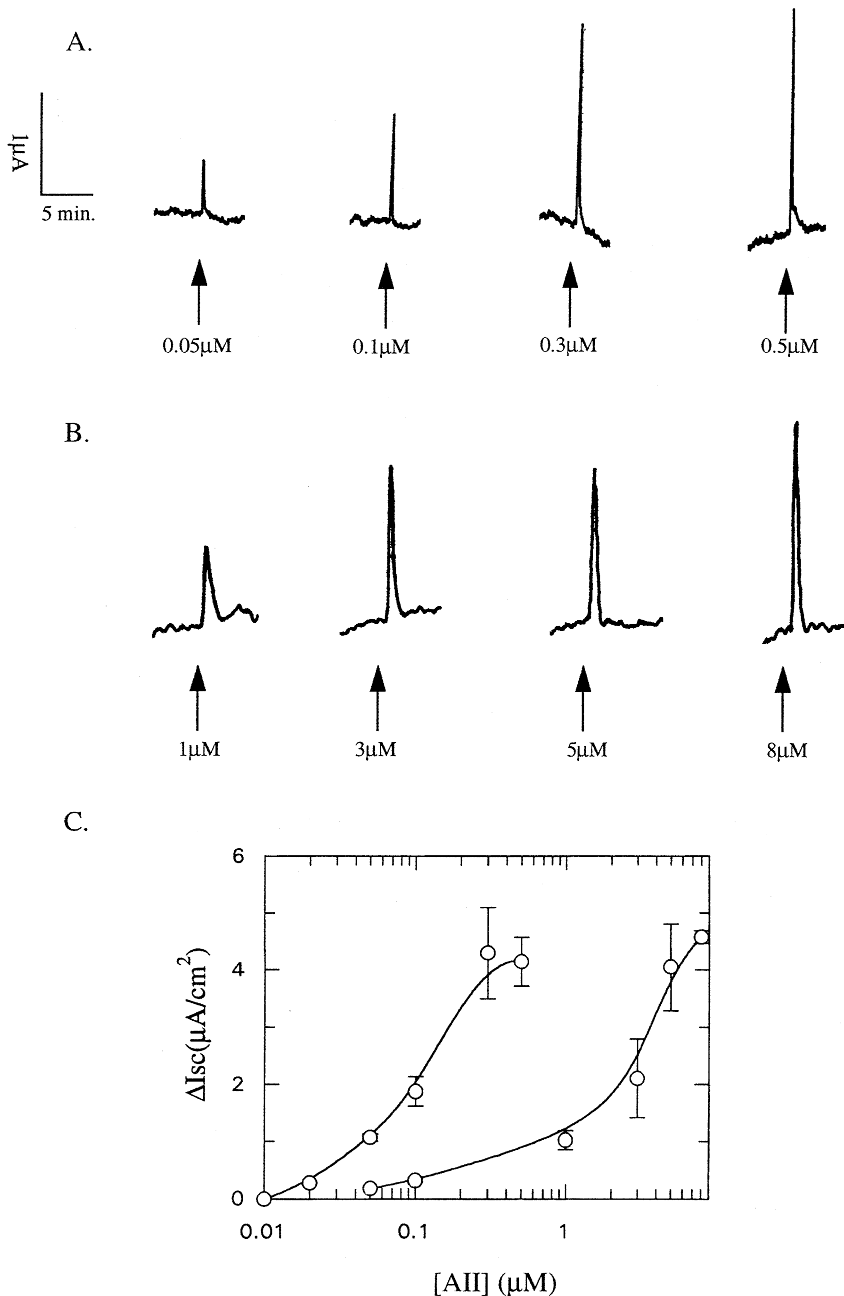

Fig. 1. Responses of I_{SC} to apical and basolateral addition of AII. I_{SC} recordings obtained from separate monolayers in response to different concentrations of AII added to either apical (*A*) or basolateral (*B*) solutions. Arrows indicate the time of drug addition. Each recording is representative of at least 3 experiments. (*C*) Concentration-response curves of apical and basolateral addition of AII with EC_{50} values of 100 nM and 3 μM, respectively. Value for each point is mean ± SEM.

sient increase in the I_{SC} (Fig. 1*A* and *B*). Both apical and basolateral responses were concentration-dependent as shown by the concentration-response curves (Fig. 1*C*). The responses were measured at the peak of the I_{SC} after a challenge of AII (usually <5 seconds for apical response and 10–20 sec for basolateral response). The apical response differed from the basolateral response in the time course of response (*see above*) and the value of EC_{50}, 100 nM and 3 μM, respectively. The duration of the apical response also appeared to be more transient as compared to that of basolateral response (Fig. 1*A* and *B*).

We also tested whether the apparent differential responses of apical and basolateral membranes were genuine or due to leakage of AII across the epithelium. Monolayers were challenged with AII either twice on the same membrane or the second time on the other membrane, and the responses were compared. As shown in Fig. 2*A* and *B*, when monolayers were challenged with AII twice on the same membrane, either apical or basolateral, the second response was diminished, presumably due to the desensitization of receptors. However, if monolayers were challenged with AII a second time on the other membrane, another distinct response could be observed (Fig. 2*C*), suggesting the presence of receptors in both apical and basolateral membranes of CFPAC-1 cells.

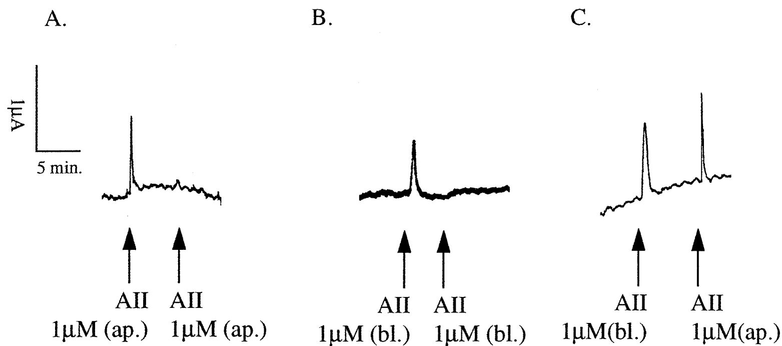

Fig. 2. Effect of repeated stimulation with AII. I_{SC} recordings showing repeated stimulation with AII (1 μM) on the same membrane, apical (*A*) or basolateral (*B*). Note that the second response is diminished. (*C*) I_{SC} recording showing second stimulation with AII (1 μM) on the opposite membrane.

Involvement of AT_1 Receptors

To examine whether the effect of AII on the I_{SC} was mediated by a specific receptor, experiments were carried out in which the monolayers were pretreated with specific antagonist for AT_1 or AT_2 receptors, losartan or PD123177, respectively [*see review,* 23]. Addition of losartan (1 μM) to either apical ($n = 3$, Fig. 3*A* and *B*) or basolateral ($n = 3$, *not shown*) membrane could completely (100%) block the respective AII-induced I_{SC}, but had no effect on subsequent ATP-induced response, suggesting that losartan specifically blocked AT_1-mediated response. On the other hand, PD123177 (1 μM) did not exert significant effect on the subsequent AII-induced I_{SC} as shown in Fig. 3*C*. After treatment with PD123177, basolateral and apical addition of AII (1 μM) stimulated increases in the I_{SC}, 1.5 ± 0.2 ($n = 3$) and 1.7 ± 0.1 ($n = 3$) μA/cm^2, respectively, which were not significantly different from the corresponding control values of 1.5 ± 0.2 ($n = 4$) and 1.8 ± 0.4 ($n = 4$) μA/cm^2, excluding the involvement of AT_2 receptors.

Immunohistochemical Demonstration of AT_1 Receptors

The presence of AT_1 receptors in CFPAC-1 cells was further confirmed by immunohistochemical studies using specific antibody against AT_1 receptor. Cells grown on coverslips showed strong immunoreactivity with antibody against AT_1 receptor (Fig. 4*A*), indicating the presence of AT_1 receptor in CFPAC-1 cells. Receptors expressed in polarized monolayers formed on permeable supports (Millipore filters) were also examined. While in some monlayers more intense immunostaining was associated with the apical membrane than that with the basolateral region (Fig. 4*B*), immunoreactivity in both apical and basolateral regions was found in others (Fig. 4*C*). The specificity of the immunostaining was demonstrated by negative staining observed in control experiments in which specific antibody was either omitted or preadsorbed with specific antigen (Fig. 4*D*).

Ca^{2+}-dependence of the AII Response

To see whether Ca^{2+} was involved in the AII-stimulated I_{SC} response, the effect of Ca^{2+} depletion on the AII-induced I_{SC} was examined. When the extracellular free Ca^{2+} was chelated by addition of 2 mM EGTA, the apical AII-induced I_{SC}, as well as the ATP-induced I_{SC}, was not affected ($n = 3$, Fig. 5*A* and *B*). The basolateral AII-induced I_{SC} was not affected by the addition of EGTA ($n = 3$, *data not shown*). However, when the intracellular Ca^{2+} store was emptied by treatment with a microsomal Ca^{2+}-ATPase, thapsigargin [21], the AII-induced I_{SC} was largely reduced (65%), from 1.8 ± 0.2 μA/cm^2 ($n = 4$) to 0.6 ± 0.2 μA/cm^2 ($n = 4$) as shown in Fig. 5*C*, indicating an intracellular Ca^{2+} dependence of the AII-induced response. The ATP-induced I_{SC}, which had been previously shown to be mediated by intracellular Ca^{2+} [5], was also reduced by the above treatments, from 11.6 ± 2.1 to 2.2 ± 0.3 μA/cm^2, confirming that thapsigargin treatment significantly reduced Ca^{2+}-activated I_{SC}.

A transient rise in intracellular Ca^{2+} in response to AII (1-100 μM) was also demonstrated by confocal laser scanning microscopy (Fig. 6). The response time varied from less than 5 sec up to 20 sec and the maximum increase in fluorescent intensity also varied from some 10% up to 4-fold. Occasionally, oscillation of Ca^{2+} fluorescent intensity upon stimulation with AII was also observed.

The possibility of involvement of eicosanoids in mediating the AII-induced response was tested by pretreatment of the monolayers with piroxicam (5 μM), an inhibitor of prostaglandin synthesis. The basolateral AII-induced I_{SC} ($n = 4$, *data not shown*), as well as the apical AII-induced I_{SC} ($n = 3$, Fig. 7), was found to be insensitive to piroxicam.

Involvement of Apical Cl^- Channels

The AII-induced I_{SC} (either apical or basolateral addition of AII) could be inhibited by treatment with the Cl^- channel blocker, DIDS (apical), prior to addition of AII

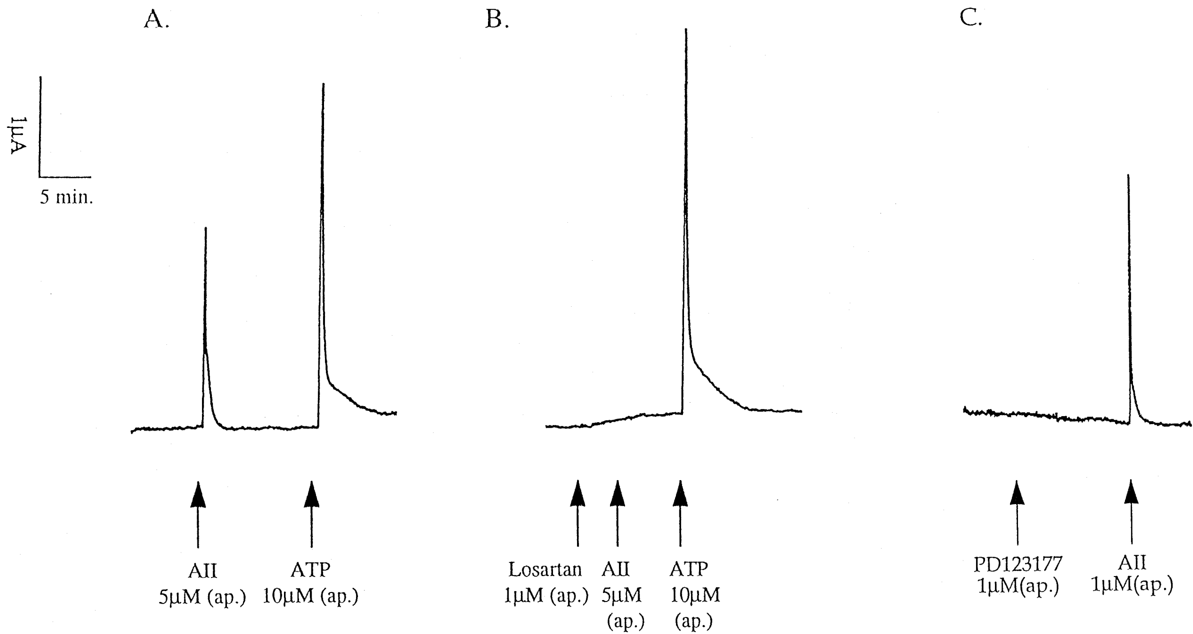

Fig. 3. Effect of AT_1 and AT_2 receptor antagonists, losartan and PD123177, on the AII-activate I_{SC}. (*A*) AII-induced and ATP-induced I_{SC} responses in an untreated monolayer (control, $n = 4$). (*B*) I_{SC} recording obtained from the monolayer ($n = 3$) pretreated with losartan (1 μM, ap) shows inhibition of AII-induced response but not the subsequent ATP-induced response. (*C*) I_{SC} recording obtained from the monolayer ($n = 3$) pretreated with PD123177 (1 μM, ap) exhibits no inhibition on the AII-induced response.

(Fig. 8), suggesting that the AII-activated I_{SC} response could be mediated by apical Cl^- channels. The effect of DIDS on the AII-activated I_{SC} was concentration-dependent with $IC_{50} = 20$ μM (Fig. 8*C*). No effect on the AII-activated I_{SC} was observed for basolateral addition of DIDS ($n = 3$). Addition of amiloride (10 μM) to the apical aspect had no effect on the AII-induced I_{SC} ($n = 4$, *data not shown*), excluding the participation of Na^+ reabsorption.

To investigate further whether the AII-activated I_{SC} response was mediated by apical Cl^- channels, experiments were carried out in which the basolateral membrane was permeabilized by an antibiotic, nystatin (500 μM), which produced pores on the membrane allowing permeation of monovalent ions. Precipitation of nystatin was observed once it was added to the basolateral compartment; therefore, it would be expected that the effective concentration of nystatin was much less than the added concentration. Solutions with Cl^- gradient (40:120 mM, apical to basolateral) were applied across the epithelium since active transport mechanism would be disabled by permeabilization of the basolateral membrane. After addition of nystatin, the transepithelial resistance gradually decreased, as shown in Fig. 9, due to the permeabilization of the basolateral membrane by nystatin. The transepithelial resistance could be calculated from the transient current pulses that resulted from an intermittently applied voltage of 0.1 mV. An increase in the transient current pulses indicates a decrease in resistance, from 220.7 ± 24.0 to 149.1 ± 29.8 Ωcm^2 ($n = 3$), before and after nystatin, respectively. Basal current was also increased with time after treatment with nystatin (Fig. 9), indicating that nystatin was effective. After treatment with nystatin, Ba^{2+} was also added to the basolateral solution to exclude any possible involvement of basolateral K^+ channels (Fig. 9). Under this condition, the basolaterally permeabilized monolayers responded to AII with an increase in I_{SC} (Fig. 9), indicating the involvement of apical Cl^- channels.

Discussion

The present study is the first to demonstrate an effect of AII on electrogenic ion transport, and the presence of AT_1 receptors in pancreatic duct cells of human origin. It has recently been demonstrated that several key components of the renin angiotensin system (RAS) exist in canine pancreas [9], and that high affinity AII binding sites are found in dog [8] and rat pancreas including exocrine pancreas [13]. However, the function of RAS in the pancreas, especially the exocrine pancreas, is not clearly understood. The present study has provided the first evidence that AII could stimulate Cl^- secretion in CFPAC-1 cells, suggesting that AII may be important for normal exocrine function of the pancreas, particularly, the regulation of pancreatic ductal HCO_3^- secretion via activation of apical Cl^- channels which play a pivotal

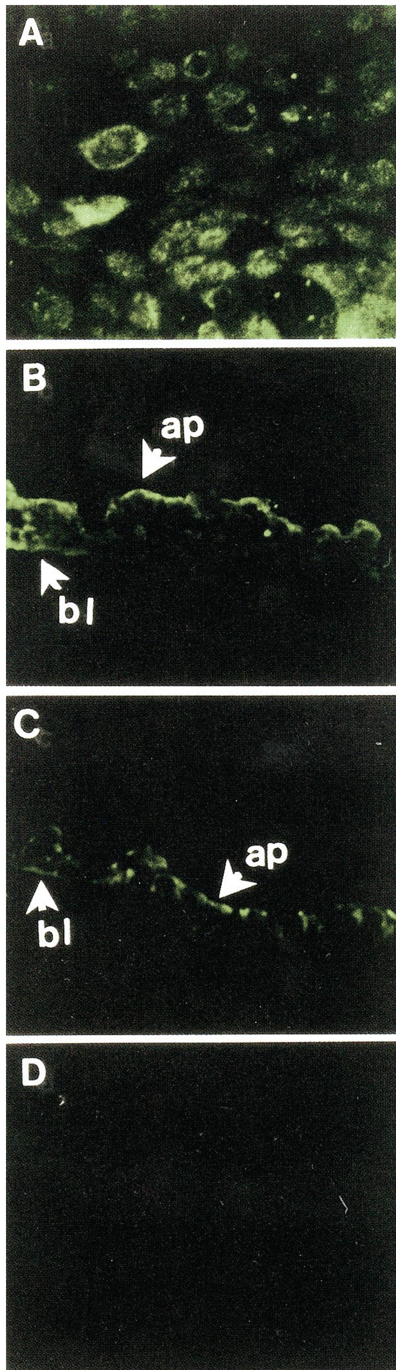

Fig. 4. Demonstration of AT_1 receptors in CFPAC-1 cells by immunohistochemistry. Confocal laser scanning micrographs showing immunoreactivity of AT_1 receptor antiserum obtained from cells grown on coverslip (*A*), monolayers grown on Millipore filters (*B* and *C*). (*D*) Negative immunostaining obtained by omitting the specific antibody. Experiments were repeated for at least 3 times.

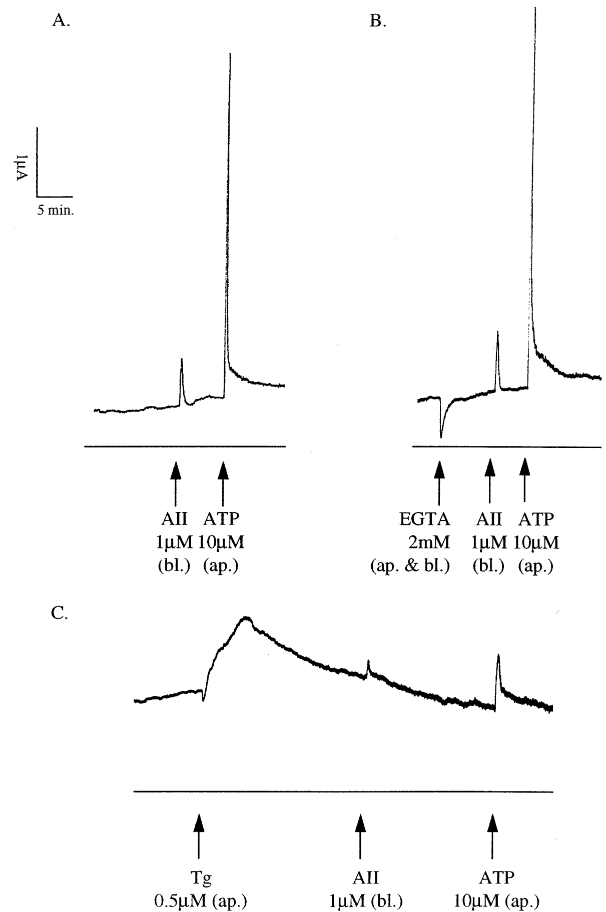

Fig. 5. Effect of Ca^{2+} depletion on the AII-activated I_{SC}. (*A*) AII-induced and ATP-induced I_{SC} responses in an untreated monolayer (control, $n = 4$). (*B*) I_{SC} recording obtained from the monolayer ($n = 3$) pretreated with EGTA (2 mM in both apical and basolateral solutions) shows no effect of extracellular depletion of Ca^{2+} on the I_{SC}. (*C*) I_{SC} recording obtained from the monolayer ($n = 4$) pretreated with thapsigargin (Tg, 0.5 μM, ap) shows inhibition of AII-induced response and subsequent ATP-induced response.

role in recirculating the Cl^- imported into duct cells through the Cl^--HCO_3^- exchanger. It could be argued that the AII-induced response observed in CFPAC-1 cells was due to culture conditions or to some property of the transformed cells. However, the effect of AII on electrogenic ion transport has also been observed in a number of epithelia including those from the trachea [18, 20], intestine [11] and epididymis [25]. The fact that the AII-induced response has also been observed in various primary cultures of epithelia make it unlikely that AII-induced response observed in CFPAC-1 cells is an artifact.

The effect of AII on Cl^- secretion across CFPAC-1 monolayers appears to be mediated by AT_1 receptors based on the observed inhibitory effect of a specific AT_1 antagonist, losartan, on the AII-induced I_{SC}. The fact that CFPAC-1 monolayers respond to both apical and

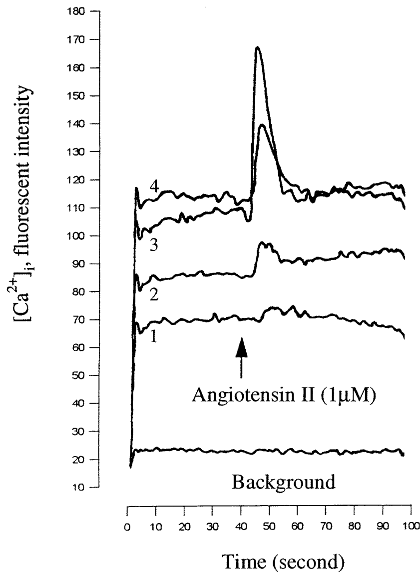

Fig. 6. Demonstration of AII-induced changes in intracellular Ca^{2+} by confocal laser scanning microscopy. Fluorimetric intensity in individual cells (1–4) from the same experiment (representative of 9) is plotted against time with the arrow indicating the time of AII (1 μM) addition. CFPAC-1 cells grown on glass coverslips were loaded with the Ca^{2+}-sensitive fluorescence dye Fluo 3-AM (3 μM) 45 min. Fluo-3 was excited at 488 nM and Ca^{2+}-dependent fluorescence was captured at 520 nm.

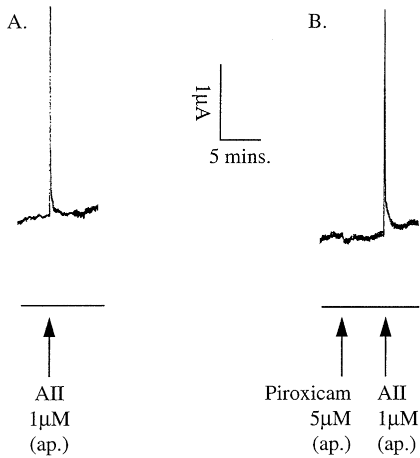

Fig. 7. Effect of piroxicam on the AII-activated I_{SC}. Recordings (representative of 3) of AII-induced I_{SC} (apical addition of 1 μM AII) obtained from control (*A*) and the monolayer pretreated with piroxicam (5 μM, ap), an inhibitor of cyclooxygenase (*B*). No inhibition of AII-induced response was observed.

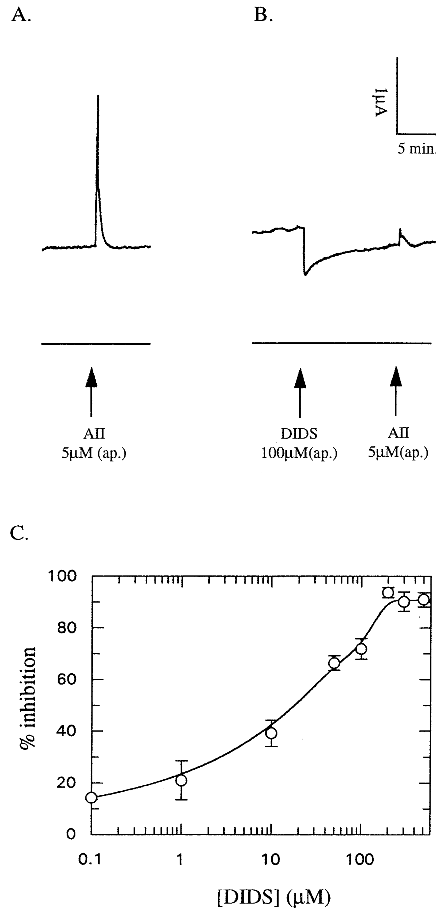

Fig. 8. Effect of the Cl^- channel blocker, DIDS, on the AII-activated I_{SC}. Recordings of AII-induced I_{SC} (apical addition of 5 μM AII) obtained from control (*A*) and the monolayer (n = 3) pretreated with the Cl^- channel blocker (*B*), DIDS (100 μM, ap). (*C*) Effect of DIDS on the AII-activated I_{SC} (basolateral addition of 5 μM AII). Note that IC_{50} is about 20 μM. Data were collected from 3–9 separate monolayers for each concentration of DIDS.

basolateral challenge of AII with apparently different EC_{50} values and time course for current activation suggests that AT_1 receptors are present in both membranes. Immunohistochemical studies have also revealed the presence of AT_1 receptors in CFPAC-1 cells, consistent with the pharmacological studies. While both canine tracheal and rat intestinal epithelia have been reported to respond to basolateral additon of AII only [11, 18], the rat epididymal epithelium responds to both apical and basolateral additon of AII [25], similar to that observed in CFPAC-1 cells. Although it is possible that the expression of receptors in both membranes is influenced by the culture condition, our immunohistochemical observation in intact mouse pancreas (*unpublished data*) also indicates differential expression of AT_1 receptors in apical and basolateral membranes of the mouse pancreatic duct in vivo. While mainly the AT_2 receptors have been reported to be expressed in the canine pancreas [8], the present study suggests that AT_1 receptors predominate in the pancreatic duct cells of human origin. Although this difference may reflect species difference in receptor expresion, it should be borne in mind that the observed expression of receptors under culture conditions may not

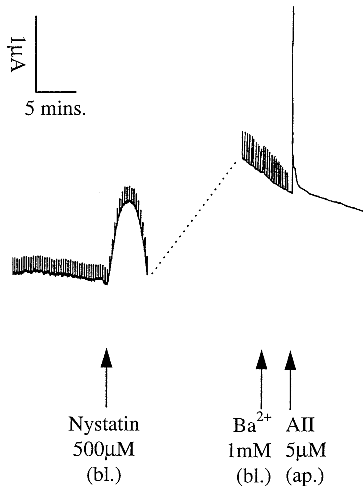

Fig. 9. AII-induced I_{SC} response obtained in basolaterally-permeabilized epithelium. AII-induced I_{SC} recording obtained in a monolayer (representative of 4) treated with nystatin (500 µM, basolateral) for at least 1 hr and then Ba^{2+} (1 mM, basolateral) 4 min prior to addition of AII (5 µM, apical). The time course of the recording is compressed (*dashed line*) so that the waiting period after addition of nystatin is not shown. The effect of nystatin was demonstrated by reduced transepithelial resistance and elevated basal current. The transepithelial resistance could be calculated from the transient current pulses resulted from an intermittently applied voltage of 0.1 mV. An increase in the transient current pulses indicates a decrease in resistance.

necessarily represent receptors expressed in the human pancreas in vivo.

Present evidence from both electrophysiological and confocal microscopic studies suggests that the effect of AII on CFPAC-1 cells is mediated by intracellular Ca^{2+}. While confocal microscopy demonstrated a rise in intracellular Ca^{2+} in response to AII, the I_{SC} measurement indicated an intracellular Ca^{2+} dependence of the AII-activated I_{SC} since it could be abolished by depletion of intracellular but not extracellular Ca^{2+}. AII is known to belong to the family of Ca^{2+}-mobilizing hormones and its effect on inducing Ca^{2+} mobilization and Ca^{2+} influx in a variety of cells is well documented [3, 4, 17]. The observed dependence of the AII-activated I_{SC} on intracellular Ca^{2+} suggests that the AII-induced change in intracellular Ca^{2+} is the key event leading to stimulation of electrogenic ion transport in CFPAC-1 cells. Previous studies of AII effect on other epithelia indicate the involvement of eicosanoids in mediating the AII effect on electrogenic ion transport [11, 18, 25]. A mechanism involving Ca^{2+}-dependent increase in intracellular cAMP via formation of prostaglandins through cyclooxygenase pathway has been proposed to explain the stimulatory effect of AII on serosa to mucosa Cl^- flux observed in canine tracheal epithelium [18]. However, the present study did not find any effect of piroxicam, a cyclooxygenase inhibitor, on the AII-induced I_{SC}, excluding the involvement of eicosanoids in mediating the AII effect in CFPAC-1 cells. The observed insensitivity of the AII-induced I_{SC} to piroxicam in CFPAC-1 cells could be due to defective cAMP-dependent activation of Cl^- channels, and does not rule out the possible involvement of cAMP via Ca^{2+}-activated prostaglandin formation in AII-induced Cl^- secretion in normal pancreatic duct cells. The present results indeed demonstrate a cAMP-independent stimulatory pathway for Cl^- secretion in CF cells, further supporting the previous finding in various epithelia that separate Cl^- conductances could be activated by either cAMP or Ca^{2+} independently [1, 5, 10, 15]. Distinct CFTR (cAMP-activated) and Ca^{2+}-activated Cl^- currents have also been found in pancreatic duct cells of a transgenic CF mouse [14] and a human pancreatic duct cell line [16].

Although Ca^{2+}-dependent Cl^- conductance has been previously observed in CFPAC-1 cells [12, 19], it has not been determined to which membrane, apical or basolateral, the Cl^- conductance is located. For a Cl^- conductance to govern Cl^- secretion, it must be located in the apical membrane. In other words, the therapeutic potential of the Ca^{2+}-activated Cl^- conductance is crucially determined by its localization in the apical membrane. The present study suggests that AII stimulates Cl^- secretion in CFPAC-1 cells by activating an apical Cl^- channel. First, the AII-activated I_{SC} was blocked by the Cl^- channel blocker, DIDS, applied to the apical but not the basolateral membrane. Second, our results obtained from nystatin-treated monolayers, whose basolateral membranes were permeabilized, also indicate that the effect of AII converges on apically located Cl^- channels. Under basolaterally-permeabilized condition, the observed AII-activated I_{SC} in the presence of a Cl^- gradient could only be explained by the presence of apical Cl^- channels whose activation seems to depend on intracellular Ca^{2+}. Previous patch-clamp study on CFPAC-1 cells has demonstrated that single Cl^- channel activity was stimulated by Ca^{2+} ionophores but not by forskolin or cAMP analogues [19]. It seems likely that the stimulation of Cl^- secretion by AII in CFPAC-1 cells is mediated by AT_1-linked Ca^{2+} mobilization which in turn activates apical Cl^- channels, presumably, the Ca^{2+}-dependent Cl^- channels described previously [19]. Similar observation that the Ca^{2+}-activated Cl^- channels appear to be located in the apical membrane has also been made recently in CFPAC-1 cells [5]. In that study, ATP was also found to activate an apical Cl^- channel via a Ca^{2+}-dependent pathway. It is interesting to note that the DIDS sensitivity of the ATP-activated I_{SC} observed earlier [5] is similar to that presently observed for the AII-activated I_{SC}, suggesting that the same Cl^- channel, e.g., Ca^{2+}-activated, may be activated by AII and ATP.

The present study is the first to demonstrate an effect of AII on anion secretion by pancreatic duct cells. The

present finding, together with previous observed effects of AII on a number of epithelia, suggests that AII may play a significant role in regulation of exocrine anion secretion. The present results obtained from CFPAC-1 cells indicate that the effect of AII is mediated by a Ca^{2+}-dependent and prostaglandins formation (or cAMP)-independent signaling pathway which appears to be different from that observed in other normal epithelia (*see above*). It remains to be elucidated whether the same signaling pathway is involved in normal pancreatic duct cells or this pathway is upregulated in CFPAC-1 cells. The AII-induced Ca^{2+}-dependent Cl^- secretion observed in CFPAC-1 cells may have therapeutic implications since the Ca^{2+}-dependent pathway has been suggested to be an alternate pathway for circumventing defective cAMP-dependent Cl^- secretion in the pancreatic duct cells of a transgenic CF mouse [14]. It remains to be investigated whether the AII-induced transient response is able to maintain sufficient secretion in the pancreatic duct or its response could be augmented by other Ca^{2+} mobilizing agents.

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