J. Membrane Biol. 178, 225–233 (2000)
DOI: 10.1007/s002320010030

The Journal of
Membrane
Biology

Characterization of Lonidamine and AF2785 Blockade of the Cyclic AMP-Activated Chloride Current in Rat Epididymal Cells

X.D. Gong, P.Y.D. Wong
Department of Physiology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

Received: 8 February 2000/Revised: 25 September 2000

Abstract. It has been shown previously that the antifertility agents Lonidamine and its analogue AF2785, [1-(2,4-dichlorobenzyl)-indazole-3-acrylic acid] are potent inhibitors of the cAMP-activated chloride channel (CFTR) in rat epididymal cells. In this study, we further characterized the blocking actions of these two compounds and compared them with the known chloride channel blocker diphenylamine-2-carboxylate (DPC). Results show that the order of potency in blocking the cAMP-activated current is AF2785 > Lonidamine > DPC. All three compounds shared similar blocking characteristics. Firstly, their blockade of the current exhibited voltage dependence; all three agents blocked the current more markedly at negative than at positive membrane potentials. Secondly, they blocked the channels from the outside of the cell. Thirdly, their blocking efficacies were maximal at low extracellular pH. Lastly, the time course of the block by AF2785 and DPC appeared to be more rapid than that of Lonidamine. It is hoped that further studies with other indazole compounds will add knowledge to the physiology and pharmacology of CFTR in the epididymis. Such information will be of great importance to our quest for novel male contraceptives.

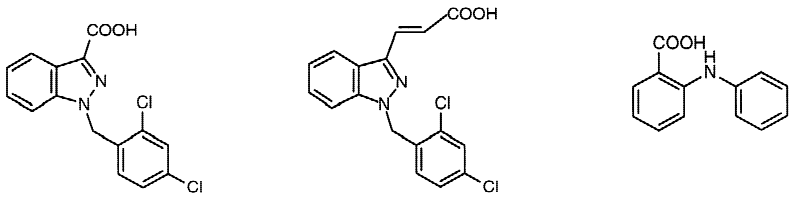
Key words: Lonidamine — AF2785 — CFTR — Epididymis

Introduction

Cystic fibrosis is an autosomal-recessive disease that results from mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR) (Quinton, 1999). In epithelial cells from a variety of tissues, CFTR functions as the PKA-activated or cAMP-dependent Cl^- channels. The epididymis is known to be among the organs that express the cystic fibrosis transmembrane conductance regulator (Pollard et al., 1991; Wong et al., 1992; Trezise et al., 1993; Wong, 1998; Leung et al., 1996). It is believed that CFTR plays an important role in the formation of the epididymal microenvironment (Wong, 1990; 1992). As with other transport proteins, CFTR is amenable to pharmacological intervention. Genistein has been shown to increase chloride secretion in the epididymis by activating CFTR, an effect claimed to offer therapeutic benefit to CF men (Leung & Wong, 2000). Conversely, blockers of CFTR could be used to disrupt the epididymal microenvironment by blocking fluid secretion (Wong, 1988*a*). By virtue of their inhibition of the formation of the epididymal microenvironment, new blockers of epididymal CFTR can evolve into novel male contraceptives.

Although the CFTR Cl^- channel has been widely studied in both human epithelial tissues and heterologous cells expressing the CFTR gene, little quantitative data are available concerning the pharmacology of this channel. There are putative blockers of CFTR at present, but they are neither selective nor potent (Schultz et al., 1999). Most studies of CFTR in this area have focused mainly on two classes of compounds: arylaminobenzoates such as diphenylamine-2-carboxylate (DPC), which blocks the CFTR channel when applied at a relatively high concentration (200 μM–3 mM); or sulfonylureas (ATP-sensitive K^+ channel blockers) such as tolbutamide and glibenclamide. Recently, we found the antifertility agents Lonidamine and its analogue AF2785, [1-(2,4-dichlorobenzyl)-indazole-3-acrylic acid] inhibited the cAMP-induced short-circuit current and whole-cell current in epididymal cells (Gong et al., 2000). In the present study, we further characterized the blocking effects of Lonidamine and AF2785 on the cAMP-activated whole cell chloride current in epididymal cells under a whole-cell patch-clamp condition.

Correspondence to: P.Y.D. Wong



Lonidamine AF2785 DPC

Fig. 1. Chemical structures of Lonidamine, AF2785 and DPC.

Materials and Methods

Culture of Rat Epididymal Epithelial Cells

Rat cauda epididymal cells were cultured as previously described (Wong, 1988*b;* Wong et al., 1999; Leung & Wong, 2000). Briefly, immature male Sprague-Dawley rats weighing 150 g were used as a source of the epididymal tissue. Rats were killed by CO_2 inhalation. The epididymides were dissected out and immersed in sterile Hank's balanced salt solution (HBSS). The tissue was cut into small pieces and treated successively with 0.25% trypsin (w:v) and 0.1% collagenase (w:v). The disaggregated cells were suspended in Eagle's minimum essential medium (EMEM) containing nonessential amino acids (0.1 mM), sodium pyruvate (1 mM), glutamine (4 mM), 5α-dihydrotestosterone (1 nM), 10% fetal bovine serum, penicillin (100 IU/ml), and streptomycin (100 μg/ml), and seeded at a cell concentration 10^5 cells/ml into a 35 mm dish for whole-cell patch-clamp study.

Whole-Cell Recordings

After 5 to 8 days in culture, subconfluent cell monolayers were incubated in Ca^{2+}-free Hank's balanced salt solution containing 1 mM EGTA for 20 min to separate the cells. Recordings were performed at room temperature using an Axopatch-1D amplifier and DigiData 1200 series Interface (Axon Instruments, Foster City, CA). Ionic current was recorded using a whole-cell patch-clamp technique (Huang et al., 1993; Gong et al., 2000). The cell membrane potential was held at −70 mV. Signals were filtered at 1 kHz, then digitized with Digidata 1200 (Axon Instruments). Sampling rate was set at 500 μsec. The pClamp 8 program was used for data recording and analysis. Patch pipettes (2–5 μΩ) were pulled from 1.0 mm o.d., 0.5 mm i.d. borosilicate glass pipettes (Sutter Instrument) using a horizontal puller (Sutter Instrument). They were polished before use. Normally, the pipettes were filled with a solution containing 120 mM CsCl, 20 mM TEA-Cl and 10 μM cAMP, pH adjusted to 7.4 with CsOH. The bath solution was Krebs-Henseleit solution (K-H solution) which contained (in mM): NaCl, 4.7 KCl, 2.56 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_4, 24.8 $NaHCO_3$, 11.1 glucose, pH adjusted to 7.4 with 0.3 N NaOH.

Lonidamine, its analogue AF2785, and DPC were applied by the use of a linear array of eight tubings connected to solution reservoirs with appropriate mixtures of K-H solution and antagonists. Solution flow through each pipe was individually controlled by a perfusion system (Scientific Instruments, New York) and the pClampex 8 manually. The solution flow rate was 0.5 ml per min. Once whole-cell recording was achieved, the epididymal cells under study were continuously superfused by a stream of solution from flow pipe, only one barrel was used for perfusion at a given time. To achieve fast solution changes, a barrel with small dead-volume (10 μl) was used.

Data Analysis

Whole cell current analysis was performed using pClamp8 software (Axon Instruments). The concentration-inhibition data were fit with equation:

$$\frac{I}{I_{control}} = \frac{1}{1+\left\{\frac{[drug]}{IC_{50}}\right\} n},$$

where I is the current after drug addition, $I_{control}$ is the current before drug addition, IC_{50} is the concentration of drug that causes 50% inhibition of the current and n is the slope factor of the inhibition curve. Results are expressed as mean ± SEM.

In analysis of the kinetics of inhibition, datapoints were taken from the time course of current inhibition between the initial application of the drug and the maximal inhibition after drug application at time interval of 0.25 sec. The kinetics was analyzed by using the equations:

$$k = \frac{1}{t} ln \frac{[I_{initial}]}{[I_{maxi}]},$$

$$t_{1/2} = \frac{ln\, 2}{k}$$

where k is the time constant. $I_{initial}$ is the activated chloride current before drug was applied. I_{maxi} is the maximally inhibited current after drug application. t is the period from $I_{initial}$ to I_{maxi}. $t_{1/2}$ is half-inhibition time constant.

Chemicals

EMEM, fetal bovine serum and nonessential amino acids were purchased from Gibco Laboratories (New York). Penicillin/streptomycin, HBSS, sodium pyruvate, 5α-dihydrotestosterone, trypsin, collagenase I, and cAMP were from Sigma (St. Louis, MO). Lonidamine and AF2785, [1-(2,4-dichlorobenzyl)-indazole-3-acrylic acid] were obtained from Professor B. Silvestrini of Rome (Gong et al., 2000). They were synthesized by the method previously described (Corsi et al., 1976). Diphenylamine-2-carboxylate (DPC) was obtained from Riedel-de-Haën, Germany. Lonidamine, AF2785 and DPC were dissolved in DMSO. These solvents alone were found not to affect the whole-cell current. The chemical structures of the chloride channel blockers used in the study are shown in Fig. 1.

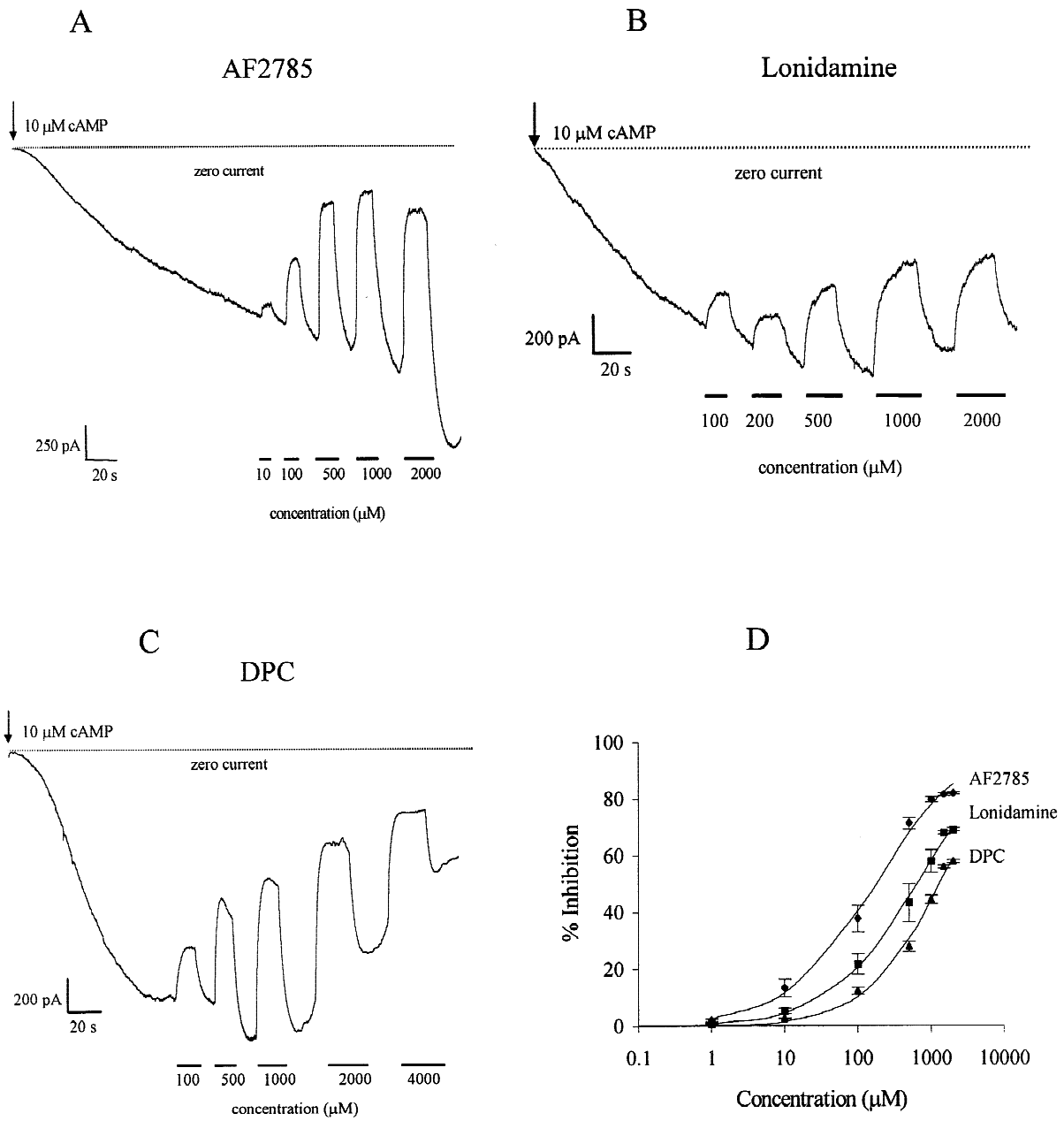


Fig. 2. Concentration dependence of the blockade of the rat epididymal cAMP-activated chloride current by AF2785 (*A*), Lonidamine (*B*), and DPC (*C*). 10 μM cAMP was added to the pipette solution to activate the chloride channels. AF2785, Lonidamine or DPC was applied to the bath solution for 10 sec as indicated by the bars. The membrane potential was held at −70 mV. The concentration-inhibition curves are shown in (*D*). Each point represents the mean ± SEM of 3 to 6 cells. Similar results were obtained when addition of different concentrations of the drugs was made in reverse order.

Results

Blockage of cAMP-Activated Cl^- Channel by Lonidamine, AF2785 and DPC

The sensitivity of the cAMP-activated Cl^- current in the rat epididymal cells to Lonidamine, AF2785, and the known Cl^- channel blocker DPC was studied. It has been established that under the whole-cell patch-clamp condition adopted in the present study, cAMP evoked a whole-cell current which has properties of a cAMP-activated chloride current (CFTR) (Huang et al., 1993; Gong et al., 2000).

Figure 2 shows chloride current activation by cyclic

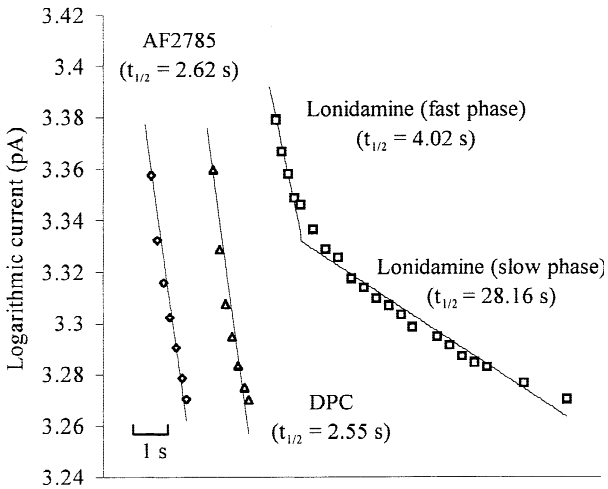


Fig. 3. Kinetics of AF2785, Lonidamine and DPC inhibition of the rat epididymal cAMP-activated chloride current. The figure was constructed by logarithmic plot of the current inhibition obtained from Fig. 2 at the concentrations of 500 μM, 500 μM and 1000 μM for AF2785, Lonidamine and DPC, respectively. Datapoints were taken between the initial application of the drugs to the maximal inhibition of the activated currents at time intervals of 0.25 sec. A single exponential was resolved for AF2785 and DPC. For Lonidamine, two exponential components (a fast phase and a slow phase) were seen.

AMP and subsequent inhibition by AF2785, Lonidamine and DPC applied extracellularly at various concentrations. CsCl (120 mM) and TEA-Cl (20 mM) were used as the internal solution to block the potassium channels. No currents were detected when the internal solution contained only 120 mM CsCl and 20 mM TEA-Cl (*data not shown*). Addition of 10 μM cAMP into the internal solution resulted in a slow activation of a current which demonstrated an inward current. The current reached a maximal value after about 5 min. Different concentrations of AF2785, Lonidamine, or DPC were added to the external K-H solution superfusing the cell. The blocking effects of Lonidamine, AF2785 and DPC were rapid and readily reversible upon washout (Fig. 2*A–C*). We have studied the concentration-inhibition relationship for the three chloride channel blockers on the cAMP-activated Cl^- current. Figure 2*D* summarizes the results. The apparent IC_{50} values for AF2785, Lonidamine and DPC were calculated to be 170.6, 631.5 and 1294 μM, respectively. Their order of potency was therefore AF2785 > Lonidamine > DPC. There is a difference in the time course of inhibition and recovery produced by the agents. AF2785 and DPC blocked the current more rapidly than Lonidamine (Fig. 2). The kinetics of the block followed first order kinetics. A single exponential was found for AF2785 and DPC with $t_{1/2}$ of 2.62 sec and 2.55 sec, respectively. For Lonidamine two exponentials were resolved. They have $t_{1/2}$ of 4.02 sec and 28.16 sec for the fast and slow component, respectively (Fig. 3).

Voltage-Dependence of Lonidamine, AF2785 and DPC Blockade of the cAMP-Activated Current

To test whether Lonidamine and AF2785 blockade of the cAMP activated Cl^- channels was voltage dependent, two voltage protocols were used. The first protocol consisted of a stepwise depolarization, in steps of 20 mV, from −100 to +100 mV with a holding potential of −70 mV (Fig. 4*A*); the second protocol used a 'ramp' method whereby the holding potential changed continuously from −100 to +100 mV within 180 msec (Fig. 4*E*).

Figure 4 shows the blocking characteristics of DPC on the cAMP-evoked chloride currents in rat epididymal cells. Figure 4*B* shows the *I–V* relationship of the whole cell current recorded under basal condition. Figure 4*C* shows activation of the current by intracellular cAMP (10 μM). The current was reduced by addition of DPC (500 μM) into the bath solution (Fig. 4*D*). It is apparent that greater inhibition was seen at negative than at positive holding potentials. Similar results were obtained when voltage change was achieved by the 'ramp' protocol (Fig. 4*F*).

To assess the voltage dependence of Lonidamine and AF2785 blockade of the cAMP-activated Cl^- channels in rat epididymal cells, the same procedures were applied. Figure 5*A* and *B* show the effects of AF2785 (100 μM) on c-AMP activated whole-cell current and the corresponding *I/V* relationships. As with DPC, AF2785 blocked the current at both positive and negative holding potentials but the inhibition was more marked at negative potentials. Identical results were obtained when voltage change was elicited by the 'ramp' protocol (Fig. 5*C*). A similar voltage-dependent block of the cAMP-activated Cl^- conductance was also produced by Lonidamine (Fig. 5, *D* to *F*).

pH-Dependence of Lonidamine, AF2785 and DPC Block

As a carboxylic acid function group is present in Lonidamine, AF2785 and DPC (Fig. 1), it is of interest to know whether the inhibition of the cAMP-activated current by the three agents was pH-dependent. Figure 6*A, B* and *C* shows respectively, the inhibitions produced by 500 μM DPC, 100 μM Lonidamine and 100 μM AF2785 at three different pHs. Figure 6*E* summarizes the results. Reducing the pH from 7.4 to 6.5 produced about a 2-fold increase in all three blockers' potency, whereas increasing the pH from 7.4 to 8.3 produced a significant decrease in Lonidamine and DPC blockade, without a significant effect on AF2785. To determine whether the effects of pH were due to changes in the cAMP-activated Cl^- current per se, we examined the pH effect on the cAMP-activated current in the absence of the blockers. Figure 6*D* shows the cAMP-activated Cl^- current was

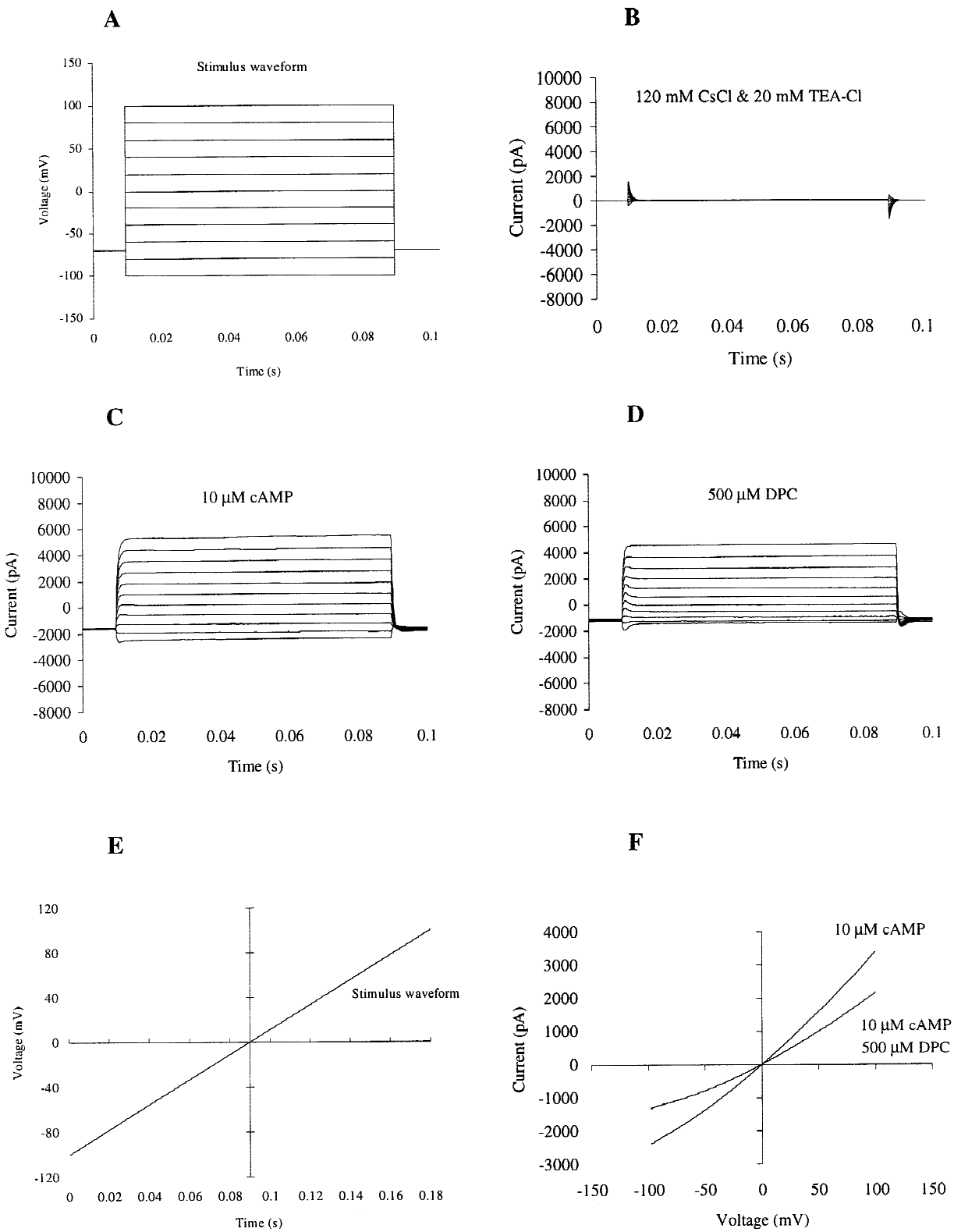


Fig. 4. The blocking characteristics of DPC on the cAMP-evoked chloride channels in rat epididymal cells. (*A–D*) Voltage was changed in a stepwise manner, in steps of 20 mV, from −100 to +100 mV with a holding potential of −70 mV. The stimulus waveform is shown in (*A*). (*B*) *I–V* relationship of the whole cell current under basal condition. (*C*) *I–V* relationship after activation by cAMP (10 μM), and (*D*) after subsequent exposure to DPC (100 μM). (*E* and *F*) Voltage change was elicited continuously from −100 to +100 mV within 180 msec using a 'ramp' protocol. The stimulus waveform shown in (*E*). (*F*) *I/V* relationship of cAMP-activated current before and after addition of extracellular DPC (500 μM).

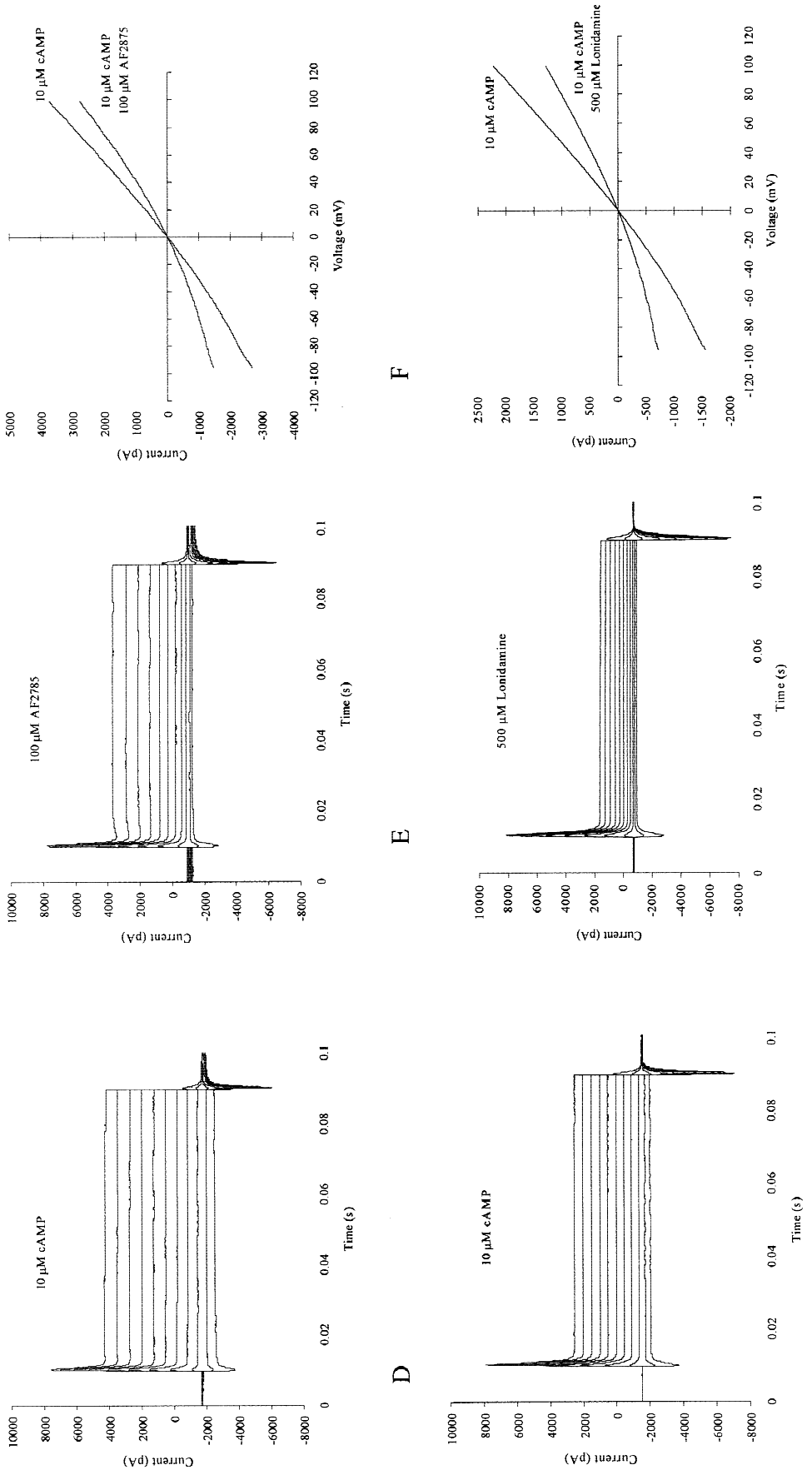

A
10 μM cAMP
Current (pA)
Time (s)
B
100 μM AF2785
Current (pA)
Time (s)
C
10 μM cAMP
10 μM cAMP
100 μM AF2875
Current (pA)
Voltage (mV)
D
10 μM cAMP
Current (pA)
Time (s)
E
500 μM Lonidamine
Current (pA)
Time (s)
F
10 μM cAMP
10 μM cAMP
500 μM Lonidamine
Current (pA)
Voltage (mV)

Fig. 5.

Fig. 5. The blocking characteristics of AF 2785 and Lonidamine on the cAMP-evoked chloride current in rat epididymal cells (*A, B, E,* and *F*) Voltage was changed in a stepwise manner, in steps of 20 mV, from −100 to +100 mV with a holding potential of −70 mV. (*A* and *D*) *I-V* relationship of cAMP-activated current. (*B* and *C*) *I/V* relationship after exposure to extracellular AF2785 (100 μM) (*B*), and Lonidamine (500 μM) (*E*). (*C* and *F*) *I/V* relationship of cAMP-activated current before and after addition of AF2785 (*C*) and Lonidamine (*F*) obtained using the 'ramp' protocol of voltage change from −100 to +100 mV within 180 msec.

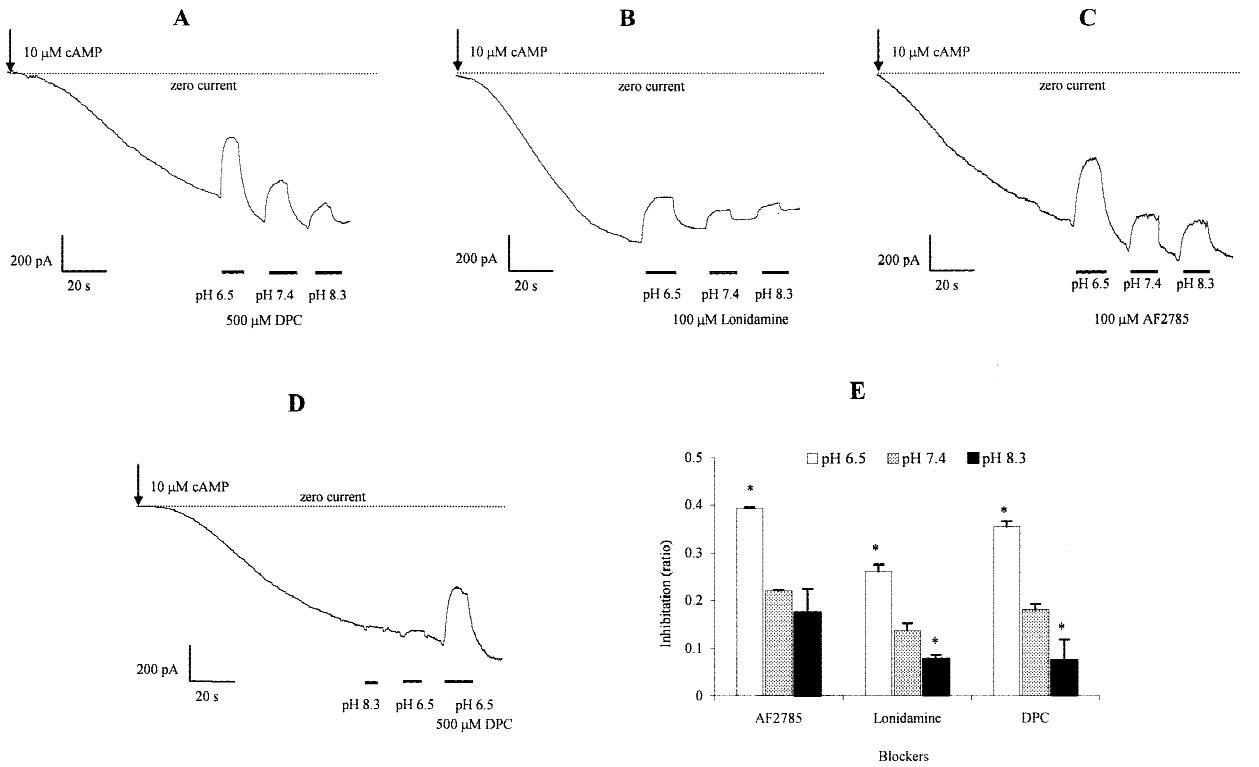


Fig. 6. pH-dependence of AF2785, Lonidamine and DPC blockade of the cAMP-evoked chloride current in rat epididymal cells. *A, B* and *C* respectively, show inhibition by DPC (500 μM), Lonidamine (100 μM) and AF2785 (100 μM) at three indicated pHs. (*D*) Effect of pH on cAMP-activated chloride current. (*E*) Summary of the results from 5 experiments. Each column shows the mean ± SEM. Asterisks indicate significant difference from the respective inhibition at pH 7.4 at $P < 0.01$. Similar results were obtained when changes in pH were made in reverse order.

not affected by extracellular pH between the pH range from 6.5 to 8.3.

Effects of Lonidamine, AF2785 and DPC Applied Intracellularly on the cAMP-Activated Current

Since Lonidamine and AF2785 appeared to be open channel blockers of the cAMP-activated Cl^- channels as evidenced from the voltage-dependence shown above, it is interesting to know whether they could also block the channels when added to the inside of the cells. A high concentration (500 μM) of Lonidamine, AF2785 or DPC was added to the internal pipette solution. It was found that co-application of any of the drugs with cAMP did not affect the current activation by cAMP (Fig. 7*A* to *D*), nor did it affect the subsequent inhibition of the current by the blockers added extracellularly at their usual effective concentrations (Fig. 7*A* to *D*). Furthermore, the presence of high concentrations (500 μM) of AF2785, Lonidamine or DPC in the pipette solution did not affect the *I/V* relationship of the current activated by cAMP (*results not shown*).

Discussion

Cystic fibrosis transmembrane Conductance Regulator (CFTR) functions as a small conductance cAMP-activated chloride channel. In epithelial tissues, the channel is an integral part of a mechanism which leads to secretion of chloride and secondarily fluid. The epididymis is among the epithelia in the body that express the CFTR protein (Pollard et al., 1991; Wong et al., 1992;

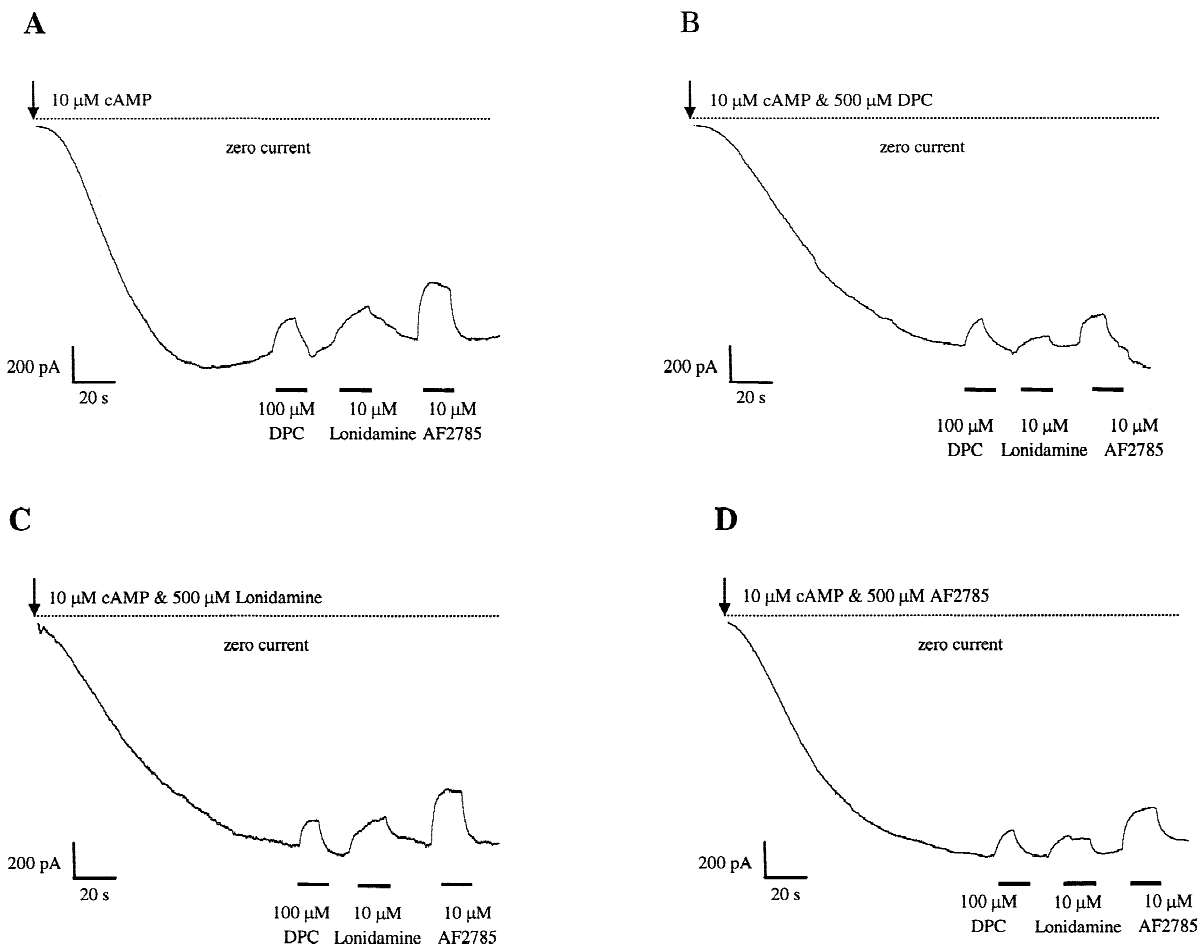


Fig. 7. Effects of co-application of (*B*) DPC (500 μM), (*C*) Lonidamine (500 μM) or (*D*) AF2785 (500 μM) with cAMP (10 μM) on whole-cell current and subsequent responses to DPC (100 μM), Lonidamine (10 μM) and AF2785 (10 μM) added extracellularly. Control responses to cAMP and inhibitors are shown in (*A*). Membrane potential was held at −70 mV.

Trezise et al., 1993; Leung et al., 1996; Wong, 1998). This protein is located in the apical membrane of the principal cells where it gates the movement of anions (and secondarily water) into the lumen of the duct (Wong, 1990; Wong et al., 1999; Bertog et al., 2000). Evidence is accumulating that mutations of the CFTR gene are responsible for various forms of male infertility ranging from complete agenesis of the epididymis/vas, through blockage of the epididymis, to mere loss of sperm quality (*see* Wong, 1998). Studies of the physiological role of CFTR and the consequences of pharmacological intervention of its functions will have applications to reproductive medicine.

In a previous paper, we reported indazole compounds, *viz* Lonidamine and AF2785 inhibited chloride secretion when added to the apical side of the epithelium. This effect is likely due to blockage of CFTR as these agents inhibited the cAMP-activated chloride current (a hallmark of CFTR activity) in rat epididymal cells under whole-cell patch-clamp condition (Gong et al., 2000). There is a close relationship between inhibition of chloride secretion and inhibition of the cAMP-activated current by the inhibitors (Gong et al., 2000). Judging from the IC_{50} values (concentrations required to block 50% of the cAMP-activated whole-cell current), these agents are more potent than the conventional chloride channel blockers such as DPC (Fig. 2), glibenclamide, and IAA (Gong et al., 2000). Lonidamine and AF2785 belong to the indazole group of compounds (Fig. 1) which are chemically distinct from the conventional chloride channel blockers (Schultz et al., 1999). However, they all possess a carboxylic acid group which may be a functional entity interacting with the channel protein. For all three agents, a higher pH than 7.4 (pH 8.3) reduced but a lower pH than 7.4 (pH 6.5) enhanced the blocking efficacy (Fig. 6). This may suggest effective blockage of the channels requires the carboxylic acid in un-ionized form. The indazole nucleus may confer a higher efficacy and this may explain why AF2785 and Lonidamine are more potent than the conventional chloride channel

blockers. Between the two indazole derivatives, AF2785 was more potent than Lonidamine and this may reflect a difference in the nature of the hydrocarbon chain of the carboxylic acid linked to the indazole nucleus. The indazole compounds represent a completely new class of compounds with chloride channel-blocking activities.

Several features of the block of CFTR by the new indazoles have emerged from this study. The inhibition by Lonidamine, AF2785 and DPC are voltage-dependent, suggesting they are all open channel blockers. Secondly, all three were unable to block the cAMP-activated Cl^- channels if they were applied to the inside of the cells (Fig. 7), suggesting they can approach the CFTR pore only from the outside. Although Lonidamine produced an inhibition with a magnitude only slightly less than that produced by AF2785, the time course of onset and offset appeared to occur more slowly than the other two agents. The time course of the block follows first order kinetics. AF2785 and Lonidamine blocked the current with a single rate constant. However, Lonidamine blockade could be resolved with two rate constants. These characteristics may suggest that AF2785 and DPC interact with a single binding site in the CFTR molecule, whereas Lonidamine interacts with two binding sites with different kinetics.

In conclusion, this paper has described an entirely new class of CFTR inhibitors based on the indazole nucleus. In general the indazole compounds are more potent than the conventional chloride channel blockers in blocking the cAMP-activated chloride channels (CFTR) in the epididymis. However, they all act in a remarkably similar way. Further studies employing single channel recording technique to delineate the kinetics of the block produced by a range of indazole derivatives (based on AF2785 and Lonidamine) will lead to formulation of a model of drug interaction with CFTR in the epididymis. In the long term, these pieces of information will be instrumental to the development of new contraceptive strategies aiming at interfering with the formation of the epididymal fluid microenvironment.

This work was supported by a grant from the Rockefeller Foundation/ Ernst Schering Research Foundation to PYDW. We are grateful to Professor B. Silvestrini and Dr. Y. Cheng for their kind supply of Lonidamine and AF2785.

References

Bertog, M., Smith, D.J., Bielfeld-Ackermann, A., Bassett, J., Ferguson, D.J.P., Korbmacher, C., Harris, A. 2000. Ovine male genital duct epithelial cells differentiate in vitro and express functional CFTR and EnaC. *Am. J. Physiol.* **278:**c885–c894

Corsi, G., Palazzo, G., Germani, C., Barcellona, P.S., Silvestrini, B. 1976. 1-Halobenzyl-1H-indazole-3-carboxylic acids. A new class of antispermatogenic agents. *J. Med. Chem.* **19:**778–783

Gong, X.D., Wong, Y.L., Leung, G.P.H., Cheng, C.Y., Silvestrini, B., Wong, P.Y.D. 2000. Lonidamine and analogue AF2785 block the cyclic AMP-activated chloride current and chloride secretion in the rat epididymis. *Biol. Reprod.* **63:**833–838

Huang, S.J., Fu, W.O., Chung, Y.W., Zhou, T.S., Wong, P.Y.D. 1993. Properties of cAMP-dependent and Ca^{2+}-dependent whole-cell Cl^- conductances in the rat epididymal cells. *Am. J. Physiol.* **33:**C794–802

Leung, A.Y.H., Wong, P.Y.D., Yankaskas, J.R., Boucher, R.C. 1996. CAMP- but not Ca^{2+}-regulated Cl^- conductance is lacking in cystic fibrosis mice epididymides and seminal vesicles. *Am. J. Physiol.* **271:**C188–C193

Leung, G.P.H, Dun, S.L., Dun, N.J., Wong, P.Y.D. 1999. Serotonin via 5-HT_{1B} and 5-HT_{2B} receptors stimulates anion secretion in the rat epididymal epithelium. *J. Physiol.* **519:**657–667

Leung, G.P.H., Wong, P.Y.D. 2000. Activation of cystic fibrosis transmembrane conductance regulator in rat epididymal epithelium by genistein. *Biol. Reprod.* **62:**143–149

Pollard, C.E., Harris, A., Coleman, L., Argent, B.E. 1991. Chloride channels on epithelial cells from cultured human fetal epididymis. *J. Membrane Biol.* **124:**275–284

Quinton, P.M. 1999. Physiological basis of cystic fibrosis: a historical perspective. *Physiol. Rev.* **79:**S3–22

Schultz, B.D., Singh, A.K., Devor, D.C., Bridges, R.J. 1999. Pharmacology of CFTR chloride channel activity. *Physiol. Rev.* **79:**S109–144

Trezise, A.E.O., Linder, C.C., Grieger, D., Thompson, E.W., Meunier, H., Griswold, M.D., Buchwald, M. 1993. *CFTR* expression is regulated during both the cycle of the seminiferous epithelium and the oestrous cycle of rodents. *Nat. Genet.* **3:**157–164

Wong, P.Y.D. 1988*a*. Inhibition by chloride channel blockers of anion secretion in cultured epididymal epithelium and intact epididymis of rats. *Br. J. Pharmacol.* **94:**155–163

Wong, P.Y.D. 1988*b*. Mechanism of adrenergic stimulation of anion secretion in cultured rat epididymal epithelium. *Am. J. Physiol.* **254:**121–133

Wong, P.Y.D. 1998. *CFTR* gene and male fertility. *Mol. Hum. Reprod.* **4:**107–110

Wong, P.Y.D. 1990. Electrolyte and fluid transport in the epididymis. *In:* Epithelial Secretion of Water and Electrolytes. J.A. Young and P.Y.D. Wong, editors. pp. 333–348. Springer-Verlag, Heidelberg

Wong, P.Y.D., Chan, H.C., Leung, P.S., Chung, Y.W., Wong, Y.L., Lee, W.M., Ng, V., Dun, N.J. 1999. Regulation of anion secretion by cyclo-oxygenase and prostanoids in cultured epididymal epithelia from the rat. *J. Physiol.* **514:**809–820

Wong, P.Y.D., Huang, S.J., Leung, A.Y.H., Fu, W.O., Chung, Y.W., Zhou, T.S., Yip, W.W.K., Chan, W.K.L. 1992. Physiology and pathophysiology of electrolyte transport in the epididymis. *In:* Spermatogenesis-Fertilization-Contraception: Molecular, Cellular and Endocrine Events in Male Reproduction. E. Nieschlag and U.-F. Habenicht, editors. pp. 319–344. Springer-Verlag, Heidelberg