Effects of Topically Delivered Benzamil and Amiloride on Nasal Potential Difference in Cystic Fibrosis

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The raised nasal transepithelial potential difference (PD) in cystic fibrosis (CF) reflects accelerated active transport of Na^+, and is inhibited by topical administration of the Na^+ channel blocker, amiloride. The aim of this study was to investigate the dose-effect and time course of topically administered Na^+ conductance inhibitors to inhibit nasal PD, including benzamil, an analog of amiloride. We measured the magnitude of drug inhibition of Na^+ transport [percent inhibition of baseline PD (ΔPD%)] and duration of inhibition of PD, defined as the time when drug inhibition of PD had recovered by 50% (effective time = ET_{50}). Amiloride [10^{-3} M (n = 16), 3×10^{-3} M (n = 9), 6×10^{-3} M (n = 7), 10^{-2} M (n = 3)] or benzamil [1.7×10^{-3} M (n = 7), and 7×10^{-3} M (n = 5)] were administered to the nasal surface via an aerosol generated by a jet nebulizer and a nasal mask. The concentration-dependent magnitude (ΔPD%) of inhibition was similar for amiloride and benzamil ($\sim$ 67–77%), whereas the duration of inhibition (ET_{50}) was about two-and-a-half times longer after benzamil administration as compared with equivalent concentrations of amiloride [1.6 $\pm$ 0.06 versus 4.5 $\pm$ 0.6 h (ET_{50} $\pm$ SEM), at 6–7 $\times$ 10^{-3} M]. *In vitro* studies of cultured normal nasal epithelia demonstrated directly that benzamil induced an approximately 2-fold more prolonged inhibition of active Na^+ transport than amiloride. These data suggest aerosolized benzamil is a candidate long-duration Na^+ channel blocker for CF. Hofmann T, Stutts MJ, Ziersch A, Rückes C, Weber WM, Knowles MR, Lindemann H, Boucher RC. Effects of topically delivered benzamil and amiloride on nasal potential difference in cystic fibrosis.

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Cystic fibrosis (CF) is a common, frequently fatal inherited disorder in the Caucasian population, affecting about one in 2,500 live births. The major cause of morbidity likely reflects ion transport abnormalities in airway epithelia, leading to mucostasis, bacterial colonization with chronic inflammation, and irreversible lung damage (1). The airway epithelia of patients with CF exhibit impaired cAMP-mediated chloride secretion (2, 3) and increased transcellular sodium absorption (4, 5). It is likely that these ion transport defects lead to abnormal surface liquid composition and abnormal mucus viscosity and clearance *in vivo* (6).

Because the respiratory epithelium under the inferior turbinate of the nose is functionally and morphologically similar to the ciliated epithelium of the lower airways, the nasal epithelium may serve as a model for studying ion transport in the lower airways (2, 7, 8). Studies of pharmacotherapy in CF are most easily initially explored in the nasal cavity because of the ease of access. Topical administration of epithelial Na^+ channel blockers, e.g., amiloride, to the nasal epithelium in CF decreases the basal potential difference (PD), a parameter which has been shown to directly correlate with the rate of basal sodium absorption in respiratory epithelial (4, 9).

The clinical value of amiloride aerosol therapy is currently under debate. There are data available which generally show short-term benefits of inhaled amiloride, particularly in younger subjects (6, 10–16). However, there are more conflicting results concerning the possible long-term benefits of amiloride (17, 18). Studies in normal subjects using a filter paper sampling technique showed that amiloride has a half-life of 35 to 40 min on airway surfaces (19), raising the speculation that the pharmacodynamics of the drug were not optimal for longer term therapy. Recent primary cell culture experiments found that benzamil, an analog of amiloride, is a more potent inhibitor of Na^+ transport in the human nasal epithelium *in vitro* (20, 21). These results, in agreement with published data from other epithelia, indicate that benzamil is more potent than amiloride in inhibiting epithelial sodium transport and, based on improved pharmacodynamic attributes, could be therapeutically more effective than amiloride (22). The aim of the present study was to measure the magnitude and duration of the effect of topically administered benzamil, as compared with amiloride, on sodium transport in the nasal epithelium of patients with CF *in vivo*. Aerosol application of drugs to the nasal or bronchial epithelium is known to lead to a 10- to 100-fold dilution of drug concentration (19) at the epithelial surface, and for this

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reason, we employed 10- to 100-fold higher sodium blocker concentrations in the nebulizer than previously used for the nasal superfusion technique. Parallel studies were performed in cultured nasal epithelial preparations, measuring the short circuit current which predominantly reflects active Na^+ transport (23).

METHODS

Study Subjects

We studied 41 CF patients (18 female, 23 male; age range 18 to 35 yr, 24.4 ± 0.7 yr, mean ± SEM). Six patients participated in both the amiloride and the benzamil protocols. The CF diagnoses were based on two elevated sweat Cl^- tests and clinical findings. All CF subjects attended the Pulmonary Department of the Justus Liebig Universität-Giessen, Pediatric Hospital. The clinical status of the patients played no role in the selection of patients for the drug/drug concentration tested. Each subject was without acute symptoms of nasal infection nor was there nasal polyposis at the time of the PD measurement. For 24 h prior to the study, no other drugs apart from the study drugs were applied to the nasal epithelium of the patients. The protocols were approved by the committee for human studies of the Justus Liebig Universität-Giessen, and written consent was obtained from the subjects.

Techniques

Potential difference measurement. The apparatus consisted of a high-impedance voltmeter (WTW PH530; Weilheim, Chapel Hill, NC; 10^{11} Ω), connected to a strip-chart recorder (Philips Two Line PM 8272; Philips, Netherlands). The voltmeter was connected via standard reference electrodes (Ingold type 373; Ingold, Steinbach, Germany) and saturated 3 M potassium chloride agar bridges to reference and recording electrodes (7, 9). Both were slowly (2 to 4 ml/h) perfused with Ringer's saline by use of infusion pumps ("perfused electrodes"). Bacterial contamination was avoided by use of polycarbonate filters (CareFil 0.2 μm; CareMed, Rothenburg, Germany).

Exploring electrode. Nasal PD measurements were made on the lateral wall and the inferior surface of the turbinate at 0.5 to 4 cm from the nasal meatus. We connected a nasal speculum to a cold light source to accomplish visualization. The recording electrode (umbilical vessel catheter, Argyle 3.5 Ch [= 1.17 mm] × 38 cm) was placed at the most distal (4-cm) site, and slowly withdrawn in direction of the nares. The highest (most negative) potential maintaining stability for at least 3 s was recorded in each nostril.

Reference electrode (24-gauge plastic catheter). An epicutaneous reference electrode was placed on the right forearm after a slight abrasion of the superficial stratum corneum, using fine sandpaper and applying a KCl-containing electrode gel (24).

Nasal aerosol administration. We administered 2 ml of each test solution to both nostrils via nebulization (∼ 10 min) using a jet nebulizer (Pari Master Model 84.0100; Pari GmbH, Starnberg, Germany, mass median aerodynamic diameter [MMAD]: 3.1 μm; output: 0.6 g/min) at a mean airflow rate of 5.2 L/min. Amiloride (Synopharm, Hamburg, Germany) and benzamil (RBI, Cologne, Germany) solutions were prepared using sterile filtration and were stored in sterile flasks. Instead of the usual mouthpiece, an adapter with a nasal mask (Laerdal Child No. 3, flow through) was used to assure nasal deposition of drugs. The patients were instructed to inhale through their nose, and exhale through their mouth.

Protocols

Before nasal aerosol application of amiloride or benzamil, the maximal nasal basal PD was measured in each CF patient by surveying (from 0.5 to 3 cm) the area under the turbinate. Occasionally, considerable differences in baseline PD between the two nostrils were found, reflecting possible differences in blood flow and/or epithelial lesions (25). Therefore, for all subjects, the mean of two measurements taken in the nostril with the highest ("most negative") value was defined as the patient's maximal PD (PD_{max}). The nostril with the highest initial mean PD_{max} was subsequently used to measure PD_{max} postaerosol administration. Following drug administration, PD was measured at the previously identified site of PD_{max} for up to 6 h, using anatomic landmarks (i.e., location of the inferior meatus and distance from the anterior tip of the turbinate). PD_{max} was measured as the highest sustained (> 3 s) PD in single measurements at each time interval after which the catheter was removed. This method of data reduction reflects a modification of the published method (7) but is more easily performed and less sensitive to unilateral epithelial variances.

Forty-one CF patients were studied, amiloride was administered to 35, and benzamil was administered to 12. Six CF patients received both benzamil and amiloride. Each CF patient in the amiloride group received only one dose of drug, and each CF patient in the benzamil group also received only one dose of drug.

After the baseline measurement, 10^{-3} M amiloride solution was aerosolized to the nasal epithelium of 16 CF patients (age 5 to 35 yr), 3×10^{-3} M amiloride to nine CF patients (13 to 32 yr), 6×10^{-3} M amiloride to seven CF patients (17 to 30 yr), and 10^{-2} M amiloride to three CF patients (22 to 28 yr). In the second group of patients (18 to 35 yr), benzamil 1.7×10^{-3} M was aerosolized to the nasal surface of seven CF patients and 7×10^{-3} M benzamil to five CF patients. All amiloride and benzamil solutions were prepared under sterile conditions using isotonic saline, except the 10^{-2} M amiloride and 7×10^{-3} M benzamil solutions, which were diluted in distilled water to avoid drug precipitation. The amiloride concentrations were chosen equal to those that had been clinically used as an aerosol for CF in our clinic. Both benzamil solutions were prepared with the rationale of achieving the longest possible duration of effect (i.e., nasal sodium transport inhibition) by aerosolizing the maximal achievable solution concentrations. For this reason, the benzamil solutions used in this study are not equimolar to the amiloride solutions: The 1.7×10^{-3} M benzamil solution is at the limit of solubility in isotonic saline, and 7×10^{-3} M benzamil is at the limit of solubility in water. The total doses delivered from the nebulizer (i.e., the volume, 2 ml, in the nebulizer, times concentration) for amiloride were 0.6 mg (10^{-3} M, n = 16), 1.8 mg (3×10^{-3} M, n = 9), 3.6 mg (6×10^{-3} M, n = 7), and 6 mg (10^{-2} M, n = 3), and for benzamil were 1.2 mg (1.7×10^{-3} M, n = 7) and 5 mg (7×10^{-3} M, n = 5).

The sodium transport inhibitory effects of amiloride and benzamil in the nasal respiratory epithelium were quantitated by calculation of the maximal decrease of nasal PD immediately after the aerosol administration of drug (as a percent of baseline, i.e., ΔPD%). The duration of the PD inhibition was monitored through repetitive (10-min to 1-h intervals) measurements of nasal PD_{max} until a return to ± 10% of the baseline value was detected (or until 6 h passed).

For patient convenience, the PD was measured over a time limited to 6 h after administration of drug. Based on the assumption that 50% PD inhibition is a rough threshold for a therapeutic effect, i.e., returns the twofold enhanced CF Na^+ transport to the normal range, the "effective time" (ET_{50}) was calculated as the time required for the PD after drug administration to return to 50% of the inhibited PD. ET_{50} was determined for each concentration of amiloride and benzamil tested.

In Vitro Studies

Airway epithelial cells were isolated from surgical specimens of normal subjects and cultured on permeable collagen supports as previously described (26). Prior studies established that the transepithelial PD and short circuit current (I_{sc}) generated by these preparations predominantly reflect amiloride-sensitive sodium absorption (4, 20). After baseline measurements of transepithelial PD and I_{sc} during continuous bilateral perfusion (1 ml/min) of Krebs bicarbonate Ringer (KBR), preparations were exposed on the luminal surface to amiloride (10^{-4} M), phenamil (10^{-4} M, another analog of amiloride, a generous gift from Dr. T. Kleyman, University of Pennsylvania), or benzamil (10^{-4} M) for 5 min, and the maximal inhibition of PD and I_{sc} were recorded. Following the 5-min drug perfusion, the lumenal superfusate was returned to KBR and the time required for I_{sc} and PD to return to basal values (maximum recovery) recorded.

Statistical Analysis

All measurements are expressed as mean ± SEM. The initial inhibition (ΔPD%, PD during drug/PD before drug × 100) and duration of effects on PD (ET_{50}) for amiloride and benzamil were tested using

PROC GLM, a general linear models procedure (27). Regression lines were estimated for both the amiloride and benzamil groups, and the hypothesis of equal slopes for amiloride versus benzamil was tested. In *in vitro* studies, the I_{sc} (% inhibition) and recovery (min) data for amiloride, phenamil, and benzamil were compared by unpaired Student's *t* test.

RESULTS

All nasal PDs were lumen negative with respect to the reference electrode. The magnitudes of the baseline PDs in CF patients (mean ± SEM), as referenced to the epicutaneous electrode, were in agreement with our own previous results (24) and those published elsewhere in the literature (7, 9, 28, 29). No differences in baseline PD (predrug) between the amiloride and benzamil groups were found: PDs were −49.3 ± 2.1 mV (mean ± SEM) for the CF group receiving amiloride (n = 35), and −47.9 ± 2.5 mV for the CF group receiving benzamil (n = 12).

Plots of the PD/time curves are shown for the lowest (10^{-3} M, n = 16) and for the highest concentration of amiloride (10^{-2} M, n = 3) tested (Figure 1A and B, respectively). ΔPD%s were 67.1 ± 2.9% for 10^{-3} M amiloride and 77.3 ± 4.3% for 10^{-2} M amiloride, values that were not significantly different. Figure 1A and B also illustrate the calculations of the average ET_{50}, which for 10^{-3} M amiloride is 0.7 ± 0.02 h, and for 10^{-2} M amiloride 2.2 ± 0.12 h.

Mean data for the high-dose benzamil concentration are shown in Figure 2. Note that the *x*-axis in Figure 2 is labeled in hours as compared with minutes in Figure 1A and B. The application of 7×10^{-3} M benzamil induces inhibition of PD of 76.2 ± 4.2%. The ET_{50} is estimated to 4.5 ± 0.6 h. The nasal PD was not monitored until return to the initial baseline value because of the time required (> 6 h) and patient compliance.

Figure 3 displays the dose–effect relationships for amiloride and benzamil with respect to effectiveness. No differences in ΔPD% between the six different treatment groups were found, indicating a maximal initial sodium inhibition by all applied concentrations of the two drugs (Figure 3A). The "effective times" (ET_{50}) were determined for each drug concentration (Figure 3B). ET_{50} was highly positively correlated with amiloride concentration ($r = 0.99$, $p < 0.001$). Although only two concentrations of benzamil were tested, it is evident that ET_{50} after administration of benzamil was at least twice as long at equivalent concentrations of amiloride. ET_{50} values in the high-dose benzamil group (7×10^{-3} M) were greater than for both the equivalent amiloride groups (6×10^{-3} M and 10^{-2} M). In the low-dose benzamil group (1.7×10^{-3} M), ET_{50} values were higher than in the 10^{-3} M amiloride, and the 3×10^{-3} M amiloride groups. When the durations of effect (ET_{50}) of amiloride and benzamil were compared using a general linear models procedure, the hypothesis of equal slopes was rejected ($p < 0.01$). Thus, it can be concluded that the durations of effect for the two drug treatment groups were significantly different. The complete analyses performed for all four concentrations of amiloride and the two concentrations of benzamil tested are shown in Table 1.

In vitro studies revealed an inhibition of I_{sc} by 10^{-4} M amiloride, phenamil, or benzamil that ranged from 61 to 77% of the basal value that was not statistically different among treatment groups (Figure 4A and Table 1). There was a similar degree of inhibition of PD *in vitro* (amiloride 73.4 ± 8.0%, phenamil 58.2 ± 3.0%, benzamil 64.8 ± 7.0%; n = 5) in each group. These data are consistent with reports of the maximally effective local concentration of each agent from previous studies of airway epithelia (21). The fact that no drug completely

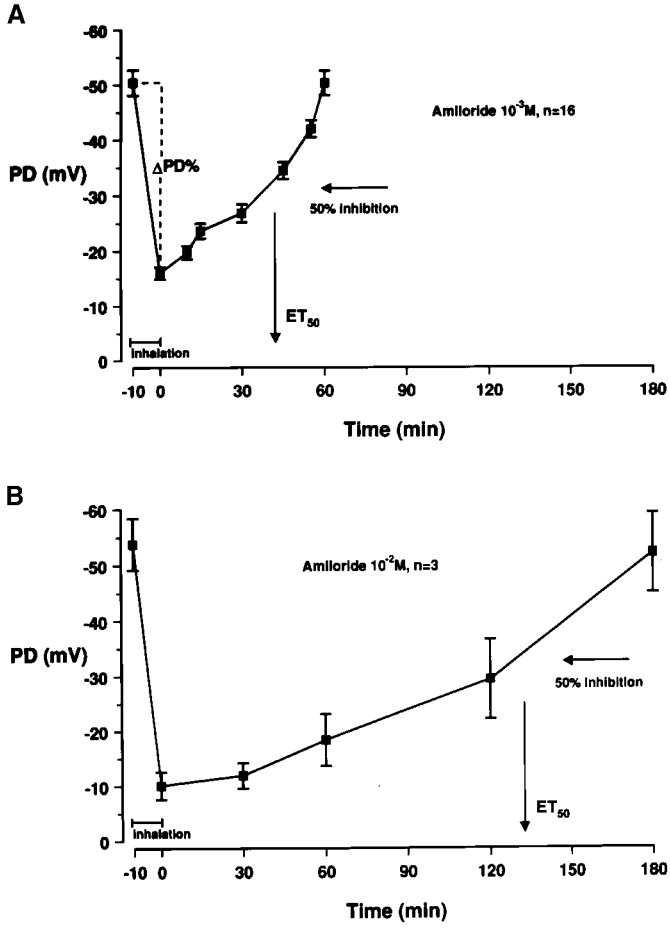

Figure 1. (*A*) Effect of 10^{-3} M amiloride (lowest dose) on nasal PD after nasal aerosol administration as a function of time. PD inhibition and return to baseline after 55 min are shown. ΔPD% is the initial PD inhibition in percent of the baseline (67.1 ± 2.9%), ET_{50} is the effective time of PD inhibition until return to the half-maximal inhibition (0.7 ± 0.02 h). (*B*) Effect of 10^{-2} M amiloride (highest dose) on nasal PD after nasal aerosol administration. PD inhibition and return to baseline (after ∼ 185 min) are shown. Mean ΔPD% is 77.3 ± 4.3%, and mean ET_{50} is 2.2 ± 0.12 h.

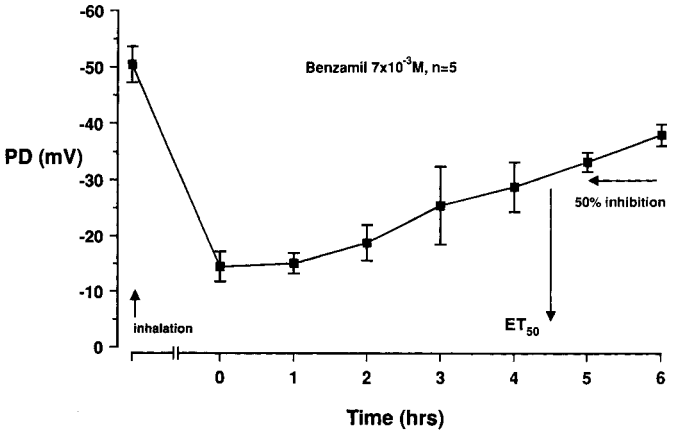

Figure 2. Effect of 7×10^{-3} benzamil on nasal PD after nasal aerosol application. PD inhibition (ΔPD%: 76.2 ± 4.2%) and return to baseline after more than 6 h are shown. ET_{50} is 4.5 ± 0.6 h.

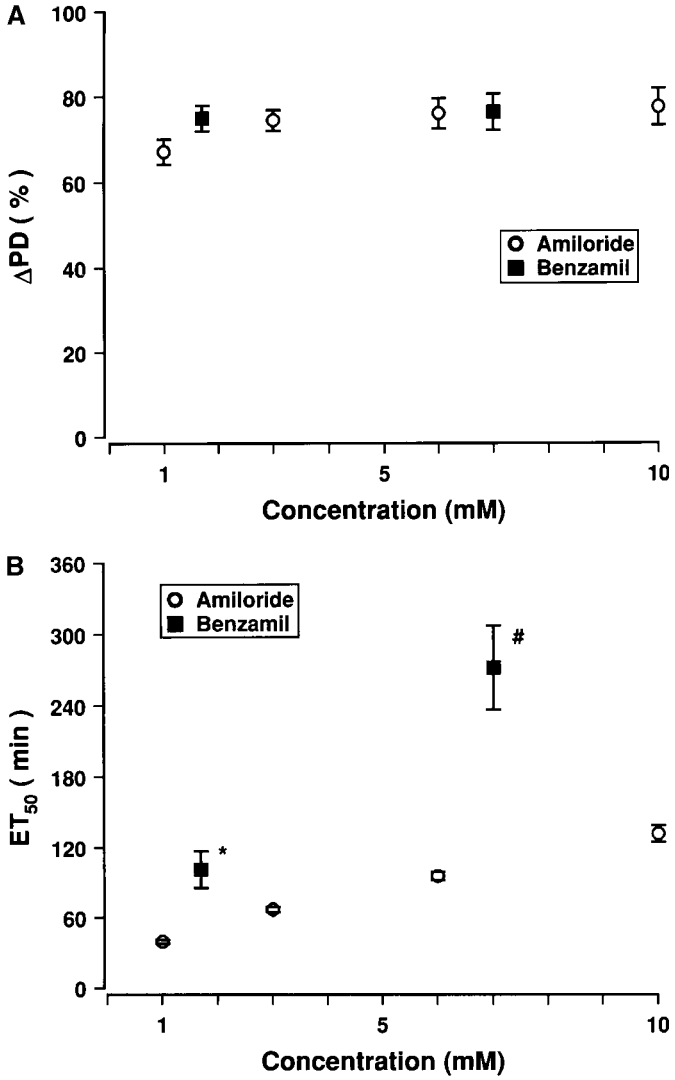

Figure 3. (*A*) Initial inhibition of nasal PD (ΔPD%) after nasal aerosol administration of different concentrations of amiloride [10^{-3} M (n = 16), 3×10^{-3} M (n = 9), 6×10^{-3} M (n = 7), 10^{-2} M (n = 3)] and benzamil [1.7×10^{-3} M (n = 7), and 7×10^{-3} M (n = 5)], expressed as percent of the baseline value (% ± SEM). No significant differences were detected between groups. (*B*) Duration of the PD inhibition after nasal aerosol administration of different concentrations of amiloride [10^{-3} M (n = 16), 3×10^{-3} M (n = 9), 6×10^{-3} M (n = 7), 10^{-2} M (n = 3)] and benzamil [1.7×10^{-3} M (n = 7), and 7×10^{-3} M (n = 5)] expressed as ET_{50}. When the durations of effect (ET_{50}) of amiloride and benzamil are compared using a general linear models procedure, the slopes are significantly different ($p < 0.01$).

abolished I_{sc} reflects the fact that chloride secretion is induced by Na^+ channel blockade in normal airway epithelia (4). Upon perfusion with drug-free solution, the tissues in each drug treatment group approached full recovery of pretreatment I_{sc} (and PD), suggesting that each drug is a reversible inhibitor. However, the time required to recover to the pretreatment values varied significantly (Figure 4B): The amiloride tested group returned to baseline I_{sc} most rapidly (32.6 ± 6.0 min; mean ± SEM, n = 5), followed by phenamil (55.0 ± 11.5 min, n = 5), whereas benzamil had the longest duration of effect (72.4 ± 7.8 min, n = 5, $p < 0.05$, different from amiloride and phenamil). Time of recovery of PD paralleled the time of recovery of I_{sc}.

TABLE 1

PHARMACODYNAMICS OF SODIUM BLOCKING AGENTS ON NASAL PD IN CYSTIC FIBROSIS NASAL EPITHELIUM BY FOUR DIFFERENT DOSES OF AMILORIDE, AND TWO DOSES OF BENZAMIL

Sodium Blocker	Concentration	n	ΔPD (%)	ET_{50} (*h*)	Time to Complete Return to Baseline (*min*)
Amiloride	10^{-3} M	16	67.1 ± 2.9	0.7 ± 0.02	~ 55
Amiloride	3×10^{-3} M	9	74.4 ± 2.4	1.1 ± 0.04	~ 80
Amiloride	6×10^{-3} M	7	75.9 ± 3.5	1.6 ± 0.06	~ 130
Amiloride	10^{-2} M	3	77.3 ± 4.3	2.2 ± 0.12	~ 185
Benzamil	1.7×10^{-3} M	7	74.9 ± 3.0	1.7 ± 0.3	~ 180
Benzamil	7×10^{-3} M	5	76.2 ± 4.2	4.5 ± 0.6	> 300

* No differences between groups.
† When the durations of effect of amiloride and benzamil were compared using a general linear models procedure, the slopes were significantly different ($p < 0.01$).

DISCUSSION

The overall goal of inhibiting sodium absorption by aerosolized amiloride therapy for CF lung disease is to normalize Na^+ transport for therapeutically relevant intervals. In previous clinical investigations, 10^{-3} M and 5×10^{-3} M amiloride solutions were tested as aerosol therapy in patients with CF lung disease (11, 17, 18, 30). Despite indications of short-term efficacy of amiloride employing surrogate markers of disease and certain clinical indices, long-term beneficial effects of amiloride have been difficult to detect. One possible reason for this failure to observe a large drug effect of amiloride in chronic studies is the short duration of amiloride action. The half-life of inhaled ^{14}C-amiloride on airway surfaces was measured and found to be short ($t_{1/2} \sim 35$ to 40 min) (19). Our findings are in agreement with these earlier estimates of the half-life of aerosolized amiloride on airway surfaces (Figure 1). Two options to enhance inhibition of sodium absorption for clinical use are available. These include a considerable increase of the daily dose of amiloride, or the use of substances with a higher affinity to the epithelial sodium channel. A potential benefit of the second option is reduction of the dosing frequency, which may improve patient compliance.

We used the nasal transepithelial potential difference as an index to compare the efficacy of a candidate high-affinity Na^+ channel blocker, benzamil, with amiloride, to inhibit the abnormally high rate of Na^+ transport in CF (9, 28). Certain features of the PD technique for this study differ from previously published methods. Previous PD studies have been performed using a subcutaneous reference electrode (9, 29). We modified this protocol by using an epicutaneous reference electrode to extend the time frame (> 5 h) over which drug effects can be easily monitored (31). Measuring nasal PD with an epicutaneous reference electrode yields slightly lower values than with a subcutaneous reference electrode (9, 29), whereas the variability in the measurement of PD is similar (31). We also used a different method to apply amiloride and benzamil to the nasal mucosa. Typically, drugs are administered by local superfusion with simultaneous PD measurement using a double-barreled catheter. In this procedure, the PD sensing electrode is located precisely at the site of constant perfusion of a known drug concentration (7). However, this methodology requires extensive experience and is not suitable for long duration studies. Application of drugs via nasal aerosol with periodic measurement of PD is a simple alternative that better mimics

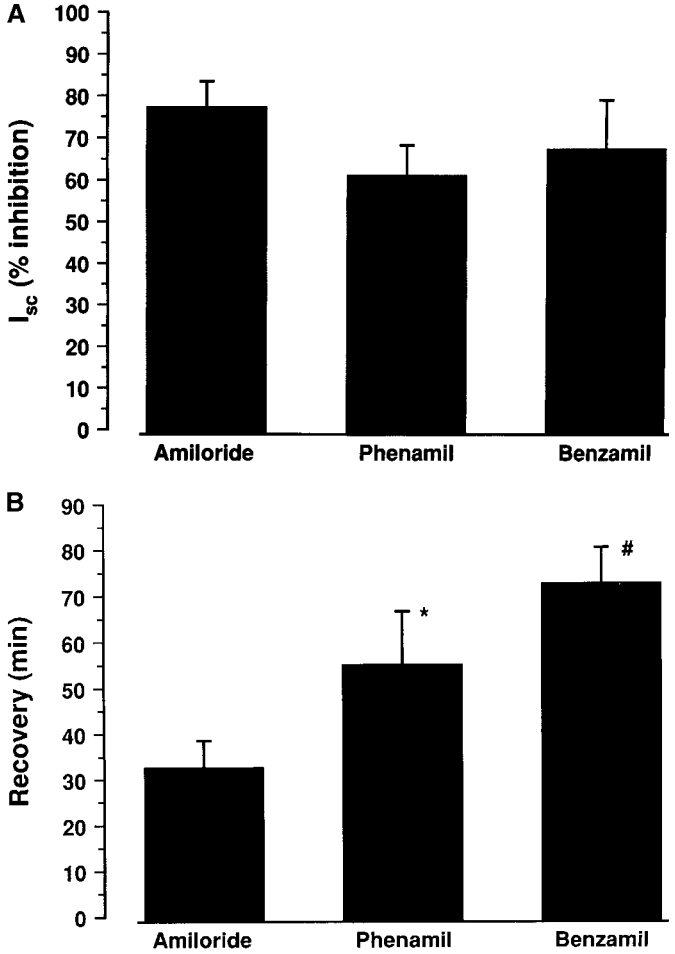

Figure 4. (*A*) Effect of amiloride and analogs, phenamil and benzamil (all 10^{-4} M), on inhibition of the short circuit current (% of original) of primary culture of normal human respiratory epithelium (n = 5 in each group), indicating efficacy of inhibiting sodium absorption. (*B*) Times to maximal recovery (I_{sc}, time after washout) from 5-min administration of amiloride, phenamil, and benzamil (all 10^{-4} M) to primary culture of normal human respiratory epithelium. Percent recoveries were amiloride 84.1 ± 7.1% (mean ± SEM), phenamil 80.8 ± 10.7%, and benzamil 79.4 ± 6.9%. *$p < 0.05$, different from amiloride. #$p < 0.05$, different from phenamil and amiloride.

the results of the lung aerosol deposition required for clinical administration. We also chose vehicles (distilled water or isotonic saline) that do not alter nasal PD when nebulized to the nasal epithelium of normal subjects (10). Repetitive topical administration of isotonic saline (0.25 ml every 15 min) does not alter the nasal PD over an 8-h interval (32). Our experience suggests that topical spray application is a reliable method that leads to a homogenous deposition of the drug to the nasal epithelium (10).

A maximal inhibition of basal PD was attained with all amiloride concentrations (ΔPD 67–77%, Table 1). Previous studies using the superfusion technique describe PD inhibition of 70–80% as the maximal inhibition noted *in vivo* in CF patients, indicating that our aerosol delivery system delivers maximally effective drug concentrations (9). It is not clear what ion transport processes (i.e., a residual non–cystic fibrosis transmembrane-conductance regulator [CFTR]-mediated Cl^- secretory current or amiloride-insensitive Na^+ absorption) support the remaining potential *in vivo* in CF patients. Benzamil at both concentrations tested yielded similar maximal ΔPD% as amiloride (Table 1, Figure 3A). Based on the observation that the ΔPD% values for the amiloride concentrations and the two benzamil concentrations tested were equal, we conclude that both drugs are capable of maximally blocking sodium transport.

The data clearly indicate that benzamil is an attractive alternative to amiloride (Figure 3A, B). As noted above, the efficacy of sodium transport inhibition after benzamil application *in vivo* is similar to amiloride, with a reduction of PD to values within the normal range (Table 1, Figure 3A). However, the time to return to baseline (ET_{50}) is significantly longer for benzamil than for amiloride (Figure 3B). The return of PD to basal values following drug administration suggests that the inhibition of PD induced by each drug, including benzamil, reflects blockade of the Na^+ channel and not damage to the epithelium. We speculate that the longer time for the PD to return to basal after benzamil reflects in part the higher affinity of the drug for the apical sodium channel.

The *in vitro* studies in cultured nasal epithelium complement the *in vivo* comparison of amiloride and benzamil. The similar magnitude of drug-induced inhibition of I_{sc} (and PD) *in vitro* indicates that maximal concentrations of each drug were studied, and the concordance of drug effects on I_{sc} and PD *in vitro* indicate that *in vivo* PD is an accurate assessment of actions on Na^+ transport (Figure 4A). The recovery of I_{sc} after removal of each drug application is consistent with a reversible inhibition of the Na^+ channel. The slower "washout" of benzamil inhibition is again similar to the *in vivo* results (Figure 4B). For the *in vitro* studies, drug concentration in the bulk solution was rapidly reduced to negligible levels by perfusion with drug-free solution. Therefore, the slower recovery of I_{sc} (and PD) after benzamil likely reflects a greater affinity of the agent for the sodium channel. However, slower washout of the more lipophilic benzamil from a tissue surface compartment contiguous with the Na^+ channel cannot be ruled out.

Previous binding studies using tritiated benzamil and phenamil, as well as other studies of rank order potency for sodium transport inhibition, are consistent with greater binding affinity and potency for benzamil relative to amiloride in renal epithelia (22). Very recently, Blank and colleagues compared the affinities of Na^+ channel blockers for the epithelial Na^+ channel in CF airway epithelia, including amiloride, and three of its analogs, benzamil, phenamil, and EIPA [5-(*N*-ethyl-*N*-isopropyl)-2′-4′-amiloride] (33). Their results showed that benzamil exhibits the highest affinity for these channels, indicated by the lowest half-maximal blocker concentration (in CF nasal tissue, 1,000-fold lower than amiloride). Phenamil, which had been described previously as having an irreversible effect on other Na^+ channels, caused a reversible blockade of the epithelial Na^+ channels. It was less potent than benzamil, but still 150-fold more potent than amiloride. Although benzamil is more potent than amiloride for Na^+ channel inhibition, benzamil has been reported to be less potent than amiloride for inhibition of the Na^+/H^+ exchanger (22). Consequently, benzamil may be the Na^+ channel blocker of choice for further *in vivo* clinical studies because of its specificity for the Na^+ channel. *In vitro* studies in sheep and rat tracheal epithelium (34, 35) did describe effects of very high concentrations of benzamil other than inhibition of apical Na^+ conductance, e.g., a decrease of the basolateral potassium conductance and blockade of cyclic guanosine monophosphate (cGMP)-gated Na^+ channels, but these effects are also seen with amiloride.

This is the first report on the sodium transport blocking effect of benzamil in the respiratory epithelium *in vivo*. The

greater target specificity of benzamil, greater potency, and its longer duration of sodium transport inhibition might lead to improved clinical outcomes, compared with amiloride, and reduce the frequency of drug administration required for CF patients. However, there are not yet sufficient safety data describing benzamil delivered systemically or via aerosol to estimate its therapeutic index. Compared with amiloride, benzamil contains an additional benzyl moiety instead of one of the hydrogen atoms of the terminal amino group and is, therefore, more lipophilic (22). Potentially increased toxicity compared with amiloride may be a consequence of this property of the analog. However, systemic central infusion of benzamil in Dahl S rats has been shown to prevent development of systemic hypertension after saline exposure, while not exhibiting any toxic effects (36). Complete safety studies will be mandatory prior to further clinical studies in the lung. However, in view of these results and the need for novel and effective drug treatments for CF patients, further studies of benzamil as therapy for CF lung disease appear warranted.

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