Arylaminobenzoate Block of the Cardiac Cyclic AMP-Dependent Chloride Current

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ABSTRACT

The cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channel has been identified in the cardiac muscle of a number of mammalian species, including humans. The goal of this study was to begin quantifying the structural requirements necessary for arylaminobenzoate block of the CFTR channel. The cardiac cAMP-dependent Cl− current (IcCl) was measured using the whole-cell arrangement of the patch-clamp technique in guinea pig ventricular myocytes during stimulation of protein kinase A with forskolin. At drug concentrations below the Ic50 value for channel block, reduction of IcCl by the arylaminobenzoates occurred in a strongly voltage-dependent manner with preferential inhibition of the inward currents. At higher drug concentrations, block of both the inward and outward IcCl was observed. Increasing the length of the carbon chain between the benzoate and phenyl rings of the arylaminobenzoates resulted in a marked increase in drug block of the channel, with Ic50 values of 47, 17, and 4 μM for 2-benzylamino-5-nitrobenzoic acid, 5-nitro-2-(2-phenylethylamino)-benzoic acid, and 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB), respectively. Increasing the carbon chain length further with the compound 5-nitro-2-(4-phenylbutylamino)-benzoic acid, caused no additional increase in the potency of drug block (Ic50 = 4 μM).

Inhibition of IcCl by the arylaminobenzoates was modulated by the pH of the external solution; increasing the pH from 7.4 to 10.0 greatly weakened NPPB block, whereas decreasing the pH to 6.4 enhanced block. In addition, block of IcCl was observed during intracellular dialysis of NPPB, and this action was not affected by raising the external pH.

Cystic fibrosis is an autosomal-recessive disease that results from mutations in the gene encoding the CFTR (Anderson et al., 1992; Riordan, 1994). In epithelial cells from a variety of tissues, CFTR functions as a PKA-activated Cl− channel (Kartner et al., 1991; Anderson et al., 1991b). In recent years, a cAMP-dependent Cl− channel also has been identified in guinea pig (Bahinski et al., 1989; Harvey and Hume, 1989a; Ehara and Ishihara, 1990; Matsuoka et al., 1990), rabbit (Harvey and Hume, 1989b), cat (Zhang et al., 1994), simian (Warth et al., 1996), and human (Warth et al., 1996) cardiac ventricular myocytes. The whole-cell and single-channel current properties of the cardiac channel display strong similarity to those of the epithelial CFTR channel (Anderson et al., 1992; Riordan, 1994). Furthermore, with the exception of a deletion in 30 amino acids coded by exon 5 of the CFTR gene, the rabbit cardiac gene shares >90% homology with CFTR (Horowitz et al., 1993; Hart et al., 1996). Expression of the cloned cardiac CFTR channel in Xenopus laevis oocytes results in the appearance of PKA-activated Cl− currents (Hart et al., 1996). Thus, cardiac tissue expresses an alternatively spliced variant of the CFTR channel.

Ion channel modulators, such as dihydropyridine Ca2+ channel antagonists and quaternary ammonium K+ channel blockers, have proved useful in elucidating channel gating mechanisms and mapping ion-permeation pathways. Although the CFTR Cl− channel has been widely studied in both human epithelial tissues and heterologous cells expressing the CFTR gene (Anderson et al., 1992; Riordan, 1994), little quantitative data are available concerning the pharmacology of this channel. Previous studies of CFTR have focused on the compound DPC, which blocks the CFTR channel when applied at relatively high concentrations (200 μM to 3 mM) (Anderson et al., 1991b, 1992; McCarty et al., 1993). Greger and colleagues modified the structure of DPC to produce a group of arylaminobenzoate compounds that varied in the phenyl-to-benzoate group, carbon chain length (Wangemann et al., 1986). One of these arylaminobenzoates, NPPB, was identified as a potent blocker of Cl− channels in the TAL of the kidney (Wangemann et al., 1986; Tilmann et al., 1991). However, the effects of NPPB and other arylaminobenzoates

ABBREVIATIONS: CFTR, cystic fibrosis transmembrane conductance regulator; IcCl, cAMP-dependent Cl− current; BNBA, 2-benzylamino-5-nitrobenzoic acid; NPEB, 5-nitro-2-(2-phenylethylamino)-benzoic acid; NPPB, 5-nitro-2-(3-phenylpropylamino)-benzoic acid; NPBA, 5-nitro-2-(4-phenylbutylamino)-benzoic acid; DMSO, dimethylsulfoxide; DPC, diphenylamine-2-carboxylate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
have not been studied in detail on the CFTR Cl⁻ channel. Thus, the goal of the current study was to quantify the blocking action of arylaminobenzoates on the cardiac cAMP-dependent Cl⁻ current.

Materials and Methods

Isolation of cardiac myocytes. An enzymatic dissociation procedure based on that of Mitra and Morad (1985) was used to isolate the myocytes. Briefly, hearts were removed from adult guinea pigs (200–300 g), mounted on a Langendorf-type column, and perfused for 10 min with a Ca²⁺-free Tyrode’s solution containing collagenase (0.25–0.32 units/ml) (type B; Boehringer-Mannheim Biochemicals, Mannheim, Germany) and protease (0.2 mg/ml) (type 14 or 25, Sigma Chemical, St. Louis, MO). After 10 min of perfusion with 0.2 mM Ca²⁺-containing Tyrode’s, the heart was dissected into small pieces and single cells obtained by gentle agitation. Cells were stored at room temperature (22–25°C) in normal Tyrode’s solution (see below) and used 1–10 hr after isolation.

Recording procedure. The patch-clamp method (Hamill et al., 1981) was used to record whole-cell ventricular currents using PC-501 (Warner Instrument, Hamden, CT), L/M EPC-7 (Adams and List, Westminster, NY), and Axopatch 200 (Axon Instruments, Foster City, CA) amplifiers. Pipettes were made from Gold Seal Accu-fill 90 Micropets (Clay Adams, Parsippany, NJ) and had resistances of 1–3 MΩ when filled with internal solution. Series resistance was determined by measuring the time constant of the capacity current and the membrane capacitance. For a typical set of experiments, series resistance ranged from 5.2 to 7.9 MΩ in the guinea pig ventricular myocytes with a mean ± standard error of 6.2 ± 0.2 MΩ (18 cells). Typically, >50% of the series resistance could be compensated electronically. Membrane currents were measured with 12-bit analog/digital converters (Scientific Associates and Axon Instruments). Data were sampled at 10 KHz, filtered at 2–3 KHz with a low-pass Bessel filter (Frequency Devices, Haverhill, MA), and stored using computer programs (Northgate (Edn Prairie, MN) and Dell (Austin, TX)).

A reference electrode made from an Ag/AgCl pellet was connected to the bath using an agar salt bridge saturated with Tyrode’s solution. Data were adjusted for liquid junction potentials that arose between both the pipette solution and bath solution and between the reference electrode and the bath (Walsh and Long, 1994). Liquid junction potential values were measured at the start and end of experiments and were between 0 and +5 mV.

Measurement of the cardiac IC₇. Isolated cells were initially placed in a normal Tyrode’s solution consisting of 132 mM NaCl, 5 mM KCl, 2 mM MgCl₂, 1 mM CaCl₂, 5 mM dextrose, and 5 mM HEPES, pH 7.4. After establishment of the whole-cell voltage-clamp, the solution was changed to a K⁺–free solution containing 140 mM NaCl, 1 mM MgCl₂, 1 mM CaCl₂, 5 mM dextrose, 5 mM HEPES, and 1 mM BaCl₂, pH 7.4 (total [Cl⁻] = 146 mM (osmolarity: 280 mOsm)). IC₇ was eliminated by the addition of 200–500 mM nisoldipine (Miles Laboratories, West Haven, CT) to the external solution. IC₇ was eliminated by holding the membrane potential at −40 mV and adding 10 µM tetrodotoxin to the bath. Patch electrodes were filled with a pipette solution consisting of 70 mM CsCl, 40 mM Ca-aspartate, 2 mM MgCl₂, 1 mM CaCl₂, 11 mM EGTA, 5 mM ATP (K⁺ salt), and 10 mM HEPES, pH 7.3 (total [Cl⁻] = 76 mM) (osmolarity: 280 mOsm). The ratio of EGTA/CaCl₂ in these solutions sets the free intracellular Ca²⁺ concentration to ~10 nM (Fabiatio, 1988).

The cardiac IC₇ was recorded during voltage steps applied to various potentials from a holding potential of −40 mV. A small recording chamber (0.5-ml volume) was used to facilitate solution changes. IC₇ was activated by the addition of 2 µM forskolin to the external solution, and drug block was quantified after 5 min of arylaminobenzoate exposure. This represents a time point at which block of IC₇ had saturated (see Fig. 4). To determine possible current rundown during these experiments, control measurements were performed over this time period. At +60 mV, there was no rundown in IC₇ observed during the first 5–6 min of experimentation. In contrast, at −90 mV, there was a decrease of 8 ± 5% (10 cells) in IC₇. Because of the variability in current amplitude from one cell to another, we did not correct for this decrease in the drug experiments.

Expression of CFTR in X. laevis oocytes. The cDNA for the human epithelial CFTR channel was generously supplied by Dr. Alan Smith (Genzyme, Cambridge, MA), and CFTR transcripts were prepared using the Message Machine kit (Ambion, Austin, TX). Stage V and VI oocytes were injected with 50 nl of cRNA (0.1–0.2 mg/ml) using a microinjector (Drummond Scientific, Broomall, PA). CFTR Cl⁻ currents were measured 1–3 days after injection using a TEV 200 two microelectrode voltage clamp (Dagan, Minneapolis, MN). The oocyte bathing solution consisted of 96 mM NaCl, 2 mM KCl, 1 mM MgCl₂, and 5 mM HEPES, pH 7.4. CFTR currents were activated by application of a cAMP cocktail containing forskolin (10 µM), 8-chlorophenylthiou cAMP (500 µM) and 3-isobutyl-1-methylxanthine (1 mM).

Preparation and use of the arylaminobenzoate compounds. The structure of the drugs BNBA, NPEB, NPBB, and NPBA are shown in Fig. 1. These arylaminobenzoates were generously supplied by Dr. Rainer Greger (Albert-Ludwigs University, Freiburg, Germany). The drugs differ in the length of the carbon chain between the benzoate (with carboxyl group) and phenyl rings. The pKa of NPBB was determined titrimetrically to be ~4.5. This is consistent with a previous reported calculation (Wangemann et al., 1986). The percentage of charged drug (C) was calculated using the equation:

\[ C = \frac{K_a}{K_a + [H^+]} \]

where Kₐ is the acid dissociation constant for the drug molecule. Stock solutions of the drugs were prepared in 100% DMSO and diluted into the external solution so the final volume of DMSO was ≤0.1%.

The voltage dependence of drug block was determined by fitting the relationship between the K(V) of the compound \([K_a(V) = [drug] * I_d/I_o - I_o, with I_o and I_d representing the IC₇ amplitudes measured before and after the addition of the drug, respectively] and the voltage with the equation:

\[ K_a(V) = K_a(0) * \exp \left( \frac{zFV}{RT} \right) \]

where Kₐ(0) is the Kₐ value at 0 mV, θ is the electrical distance sensed by the blocker, F is Faraday’s constant, R is the gas constant, and T is the temperature. In all the calculations, it was assumed the valence (z) was ~1 and there was a single binding site for the arylaminobenzoates.

All experiments were conducted at room temperature (22–25°C). Averaged values presented are mean ± standard error. Where appropriate, the Student’s t-test was used to compare the significance of the differences between groups. A p-value of <0.05 was considered significant. All experiments were conducted at room temperature (22–25°C). Averaged values presented are mean ± standard error. Where appropriate, the Student’s t-test was used to compare the significance of the differences between groups. A p-value of <0.05 was considered significant. All experiments were conducted at room temperature (22–25°C). Averaged values presented are mean ± standard error. Where appropriate, the Student’s t-test was used to compare the significance of the differences between groups. A p-value of <0.05 was considered significant. All experiments were conducted at room temperature (22–25°C). Averaged values presented are mean ± standard error. Where appropriate, the Student’s t-test was used to compare the significance of the differences between groups. A p-value of <0.05 was considered significant. All experiments were conducted at room temperature (22–25°C). Averaged values presented are mean ± standard error. Where appropriate, the Student’s t-test was used to compare the significance of the differences between groups. A p-value of <0.05 was considered significant. All experiments were conducted at room temperature (22–25°C). Averaged values presented are mean ± standard error. Where appropriate, the Student’s t-test was used to compare the significance of the differences between groups. A p-value of <0.05 was considered significant.
propriate, statistical significance was estimated using Student’s t

**Results**

**Arylaminobenzoate block of the cardiac cAMP-dependent Cl⁻ channel.** Fig. 2 (top) displays whole-cell background currents recorded from a cardiac ventricular cell during 100-msec voltage steps applied to various potentials. The external application of 2 µM forskolin caused the activation of a time-independent, outward-rectifying Cl⁻ current when recorded with a Cl⁻ concentration of 76 mM in the pipette and 146 mM in the bath (E_{Cl} = −16 mV). The physiological properties of this cardiac I_{Cl} have been described previously (Bahinski et al., 1989; Harvey and Hume, 1989a; Matsuoka et al., 1990; Walsh and Long, 1992). As shown in Fig. 2, the addition of a 20 µM concentration of NPPB caused a strong reduction in both inward and outward I_{Cl}. Overall in six myocytes examined, 20 µM NPPB decreased the inward (at −90 mV) and outward (at +60 mV) I_{Cl} by 94 ± 4% and 91 ± 4%, respectively. Partial recovery from NPPB block could be observed during long periods of drug washout (5–10 min). In three washout experiments, I_{Cl} recovered to within 16 ± 10% of the initial amplitude measured in the absence of the drug. A more rapid and complete recovery from drug block was obtained in experiments with lower concentrations of NPPB (see Fig. 9).

**Voltage-dependence of arylaminobenzoate block.** The expressed epithelial CFTR Cl⁻ channel is blocked by the arylaminobenzoate DPC (200 µM) in a strongly voltage-dependent manner (McCarty et al., 1993; McDonough et al., 1994). Because voltage-dependent block of the cardiac channel was not evident in the initial experiments with 20 µM NPPB (Fig. 2), it was determined whether voltage-dependent block might be observed with lower concentrations of the drug. The application of 2 µM NPPB produced a clear voltage-dependent block of the channels with >30% reduction in the inward current and no decrease in the outward current (Fig. 3). The electrical distance sensed by the NPPB molecule, θ, was determined using Woodhull analysis (Woodhull, 1973) as described in Materials and Methods. An average θ of 32 ± 3% (four myocytes) was calculated with the orientation from the inside membrane. A similar value (θ = 28 ± 5%) was measured during internal dialysis of NPPB. Relatively low concentrations of other arylaminobenzoates also blocked I_{Cl} in a voltage-dependent manner. For example, the drug BNBA (25 µM) blocked the inward current (at −90 mV) by 34 ± 7% but inhibited the outward current (at +60 mV) by only 4 ± 4%. In four experiments, an average θ of 31 ± 2% was determined with this drug concentration.

Voltage-dependent block of the cardiac I_{Cl} also was apparent immediately after the application of high concentrations of the drugs. Fig. 4 plots the time course of NPPB (20 µM) block of I_{Cl} measured during voltage steps to either −90 and +60 mV. As shown in Fig. 4 (left), there was a strong block of the inward I_{Cl} during the initial 30 sec after NPPB addition to the chamber but no block of the outward I_{Cl} during this time. However, both inward and outward currents were reduced to a similar extent after 4–5 min of drug exposure. In three time course experiments, the inward current and outward currents were reduced by 32 ± 13% and 0 ± 2%, respectively, after a 30-sec exposure to NPPB.

**Effect of increasing carbon chain length on arylaminobenzoate potency.** Previous studies have shown that increasing the carbon chain length between the benzoate and phenyl rings of the arylaminobenzoates increases the potency for Cl⁻ current inhibition in the thick ascending limb of the kidney (Wangemann et al., 1986). To determine whether a similar order of potency exists for block of the cardiac cAMP-dependent channel, we tested the effect on I_{Cl} of the drugs shown in Fig. 1. Fig. 5 displays concentration-versus-inhibition curves for the drugs BNBA, NPEB, NPB, and NPPA. Because block of I_{Cl} by the arylaminobenzoates was voltage dependent, especially at low drug concentrations (see Fig. 3), all the data displayed in Fig. 5 were recorded at −90 mV. Increasing the carbon chain length produced a clear increase in the potency of arylaminobenzoate block of the cardiac cAMP-dependent channel, with IC_{50} values of 47 µM, 17 µM, and 4 µM for BNBA (one carbon atom), NPEB (two carbon atoms), and NPPB (three carbon atoms), respectively. Increasing the carbon chain further with NPPA (four carbon atoms) caused no additional increase in drug potency over that of NPPB (IC_{50} for NPPA = 4 µM).

**pH dependence of arylaminobenzoate block.** All of the drugs shown in Fig. 1 contain a negatively charged carboxyl group that causes the pK_{a} of the compounds to be < 5. The pK_{a} of NPPB was determined to be close to 4.5, and thus

![Fig. 2. Block of the cardiac I_{Cl} by NPPB. Top, whole-cell background currents recorded during 100-msec voltage steps applied from a holding potential of −40 mV to −80 mV through +60 mV in 20-mV increments. Currents were measured under basal conditions (CON) and after the addition of 2 µM forskolin (FORS). The forskolin-activated I_{Cl} was inhibited by 100% and 96%, respectively, at −80 and +60 mV by a 20 µM concentration of NPPB. Cell AB4–368. Bottom, current-voltage relationship for forskolin-sensitive I_{Cl} obtained in the presence and absence of 20 µM NPPB. Values are mean ± standard error for six experiments. Values for NPPB were obtained at 5 min after addition of the drug to the bath; this represents a time period when block of I_{Cl} by NPPB had saturated (see Fig. 4).]
according to eq. 1, the drug molecules are predominately charged (>99%) in external solution at pH 7.4. Increasing the pH of the solution to 10.0 should not significantly alter the amount of charged to neutral form of the drug (100% of NPPB molecules are ionized at pH 10.0). It was surprising to find, therefore, that block of the cardiac I\textsubscript{Cl} by NPPB was almost completely abolished when the pH of the external solution was increased to 10.0 (Fig. 6). Even at an NPPB concentration of 50 μM, which produces >95% block at pH 7.4, there was only a small and nonsignificant decrease in the current at pH 10.0 (10 ± 6%, four cells, p > 0.2). However, a strong and rapid block of the current could be induced at this time by replacing the external solution with a drug-containing external solution buffered to pH 7.4 (results not shown), as
expected from the previous experiments (see Fig. 2). Increasing the internal solution pH also decreased the block of the I_{Cl} by NPPB. At a concentration of 20 μM, NPPB caused a 94 ± 4% (n = 4) decrease in the current at pH 7.3 and 61 ± 6% (five cells) decrease at pH 8.4. This difference was statistically significant (p < 0.05).

Because raising the pH of the external solution to 10.0 greatly weakened the block of the cardiac I_{Cl} by NPPB, it was determined whether lowering the external pH also might influence drug action. According to eq. 1, ~1.2% of the drug molecules are neutral at pH 6.4. As shown in Fig. 7, decreasing the pH of the external solution to 6.4 resulted in an enhanced block of I_{Cl} by NPPB with the IC_{50} value decreasing from 4 μM at 7.4 to 0.6 μM at 6.4. Under these conditions, a substantial block of I_{Cl} (35 ± 11%, three cells) could be obtained with a NPPB concentration as low as 500 nM. As was the case at pH 7.4, block of I_{Cl} occurred in a voltage-dependent manner.

**Internal block by NPPB.** The results displayed in Figs. 2–7 indicate that arylaminobenzoate block of the cardiac cAMP-dependent Cl⁻ channel occurs preferentially at negative membrane potentials and is enhanced by lowering the pH of the external solution. One explanation for these results would be that the drugs act primarily through a membrane-accessible pathway. Decreasing the external pH might enhance the movement of the drugs into the membrane by altering charged groups on the drug. Once inside the cell, block by the charged drug molecules (at the internal pH 7.3) would be favored at negative membrane potentials. To test this model, NPPB was added to the patch pipettes and dialyzed into the myocytes.

Fig. 8 summarizes the results of experiments in which 10 μM NPPB was dialyzed into the ventricular cells. For the data displayed in Fig. 8 (left), the cells were bathed in external solution buffered to pH 7.4. The results (Fig. 8, right) were obtained in external solution at pH 10.0. In each experiment, NPPB was allowed to dialyze into the myocyte for 10–12 min before the application of forskolin to activate I_{Cl}. Based on previous dialysis experiments and the parameters of the current study (i.e., myocyte size, electrode resistance, and so on) (Pusch and Neher, 1988; Walsh and Long, 1994), it was predicted that NPPB should equilibrate between the pipette and interior of the cell with a time constant of 3–4 min. Internal NPPB produced a strong block of both the inward and outward Cl⁻ currents compared with DMSO-dialyzed, control myocytes (Fig. 8, FORS). In five experiments, NPPB reduced the average current measured at −90 mV by 89%. This is similar to that obtained with external NPPB (85 ± 10%). Most importantly, during internal dialy-
sis, increasing the external pH to 10.0 did not prevent NPPB block of either the inward or outward currents (Fig. 8, right).

Drug recovery experiments also were performed to gain further information on the mechanism of NPPB block. For these experiments, I_cl was first blocked by NPPB at pH 7.4. The drug then was washed out of the chamber with external solution at either pH 7.4 or 10.0. If NPPB binds to a pH-regulated site on the external side of the cell membrane, the rate of recovery from drug block at pH 10.0 should be significantly faster than that obtained at pH 7.4. In contrast, if NPPB acts through a membrane-accessible pathway, screened from the external solution, the recovery at the two pH values should not be significantly different. As shown in Fig. 9, the time course of recovery from NPPB (5 \mu M) block at −90 mV was similar at both pH values. In three experiments each, the time constant for recovery was 3.5 ± 0.4 min at pH 7.4 and 2.9 ± 0.3 min at pH 10.0 (p > 0.05).

**NPPB Block of the Epithelial CFTR Channel.** NPPB was found to block the cardiac I_cl in a voltage- and pH-dependent manner (Figs. 3, 6, and 7). Because the cardiac I_cl is believed to represent an alternatively spliced variant of the CFTR Cl− channel (Horowitz et al., 1993; Hart et al., 1996), it was important to determine whether NPPB has similar actions on the epithelial form of the channel. Fig. 10 displays the results of experiments in which the effect of NPPB was determined on the human epithelial CFTR channel expressed in X. laevis oocytes. At a concentration of 50 \mu M, externally applied NPPB caused a voltage-dependent block of the CFTR current (Fig. 10, left) with a 25 ± 2% decrease measured at −90 mV, and a 3 ± 2% change at +60 mV (four oocytes). As was the case with the cardiac I_cl, block of the CFTR current could be abolished completely when the pH of the external solution was increased to pH 10 (Fig. 10, right) (percentage change at −90 and +60 mV = 1 ± 3% and 6 ± 2%, respectively; five oocytes).

**Discussion**

**Arylaminobenzoate block of the cardiac cAMP-dependent channel.** The goal of the current study was to begin quantifying the structural requirements necessary for drug block of the cardiac cAMP-dependent Cl− channel. This was accomplished by studying the effect of a group of arylaminobenzoate compounds that varied in the phenyl-to- benzoate ring, carbon chain length. Our approach was based on previous findings that the arylaminobenzoate compound DPC blocks both the epithelial (Anderson et al., 1992; McCarthy et al., 1993) and cardiac (Walsh and Wang, 1996) isoforms of the CFTR channel. McCarthy et al. (1993) found that DPC and the structurally related chemical flufenamic acid (at 200 \mu M concentrations) produce a ∼40% block of the CFTR Cl− current in X. laevis oocytes expressing the human epithelial channel. Single-channel analysis suggested that DPC is an open channel blocker of CFTR that permeates through the membrane to reach its binding site (McCarthy et al., 1993). In guinea pig ventricular myocytes, DPC reduces the whole-cell I_cl with an IC_{50} value of 270 \mu M (Walsh and Wang, 1996).
The most significant finding of this study was that at least up to the compound NPBA, there was no exclusion of any of the arylaminobenzoates from the cardiac Cl− channel binding site. In fact, there was an overall tendency for increased potency with increased drug size (see Fig. 5). The incorporation of an extra carbon chain between the amino group and the phenyl ring of DPC, to produce BNBA, resulted in a 6-fold increase in the potency of IC50 for BNBA over that for DPC (Walsh and Wang, 1996). Increasing the carbon chain length beyond that of BNBA in the drugs NPEB and NPPB caused a sequential decrease in the IC50 for current block (IC50 = 17 and 4 μM for NPEB and NPPB, respectively). These results display a striking similarity to the block of squid axon (Armstrong, 1971) and Drosophila Shaker (Choi et al., 1993) K+ channels by tetra-n-alkylammonium ions, in which increasing the size of the alkyl chain increases drug potency. Thus, as is the case for K+ channels, block of the cardiac Cl− channel seems to involve interactions of the lipophilic alkyl chain of the arylaminobenzoates with hydrophobic moieties either on the channel or within the lipid membrane.

Voltage and pH dependence of arylaminobenzoate block. Arylaminobenzoate block of the cardiac cAMP-dependent current occurred in both a voltage- and pH-dependent manner. Voltage-dependent block of the epithelial CFTR channel has been reported for DPC and flufenamic acid (McDonough et al., 1994). The presence of an externally modulated drug binding site is consistent with the results demonstrating that block by internally applied NPPB is in a predominately charged form (99%) at pH 7.4, whereas block by externally applied NPPB is regulated by increasing the internal pH.

Changes in the external pH also could have direct actions on the channel protein that modulate drug binding. Increasing the external pH might eliminate NPPB block by titrating the neutral form of the drug (1.2% neutral drug) enhances drug block (Fig. 7). This model is consistent with the results demonstrating that block by internally applied NPPB is not affected by the external pH (Fig. 8), whereas block by externally applied NPPB is regulated by increasing the internal pH.

Changes in the external pH also could have direct actions on the channel protein that modulate drug binding. Increasing the external pH might eliminate NPPB block by titrating positively charged amino acid residues in the Cl− channel, which interact with the carboxyl group of the drugs. Both a lysine at position 335 and an arginine at position 347 are found in the sixth membrane spanning segment of CFTR and contribute positive charge to the pore of the channel (Anderson et al., 1991a; McDonough et al., 1994). However, this model fails to explain why decreasing the external pH increases drug potency or why increasing the external pH has no effect on IC50 block by internal NPPB (Fig. 8). Furthermore, the presence of an externally modulated drug binding site is not supported by the drug recovery experiments (Fig. 9), in which drug recovery should have been enhanced under alkaline conditions. Therefore, pH-induced change in the charge...
of the drug molecules remains the most reasonable explanation for the observed modulatory actions.

Relevance of the study to cardiac pharmacology. A β-adrenergic-activated Cl− channel has been identified in guinea pig (Bahinski et al., 1989; Harvey and Hume, 1989a; Ehara and Ishihara, 1990; Matsuoka et al., 1990), rabbit (Harvey and Hume, 1989b), cat (Zhang et al., 1994), and simian (Warth et al., 1996) cardiac ventricular cells. Molecular analysis strongly suggests that these cells express an alternatively spliced variant of the CFTR channel (Horowitz et al., 1993; Hart et al., 1996). Although an initial report suggested that CFTR is expressed in human cardiac tissue (Levesque et al., 1992), other studies have failed to identify a cAMP-dependent Cl− current in human cardiac myocytes (Oz and Sorota, 1995; Sakai et al., 1995). If present in the human heart, the CFTR channel could play an important role in controlling the action potential duration. Due to the properties of the cardiac CFTR current-voltage relationship, activation of this channel during β-adrenergic stimulation will cause a decrease in the duration of the cardiac action potential and shorten the QT interval of the electrocardiogram. Block of the ICl, by the Cl− channel blocker anthracene-9-carboxylate increases the duration of the action potential in guinea pig ventricular myocytes during exposure to isoproterenol (Levesque et al., 1993). By lengthening the cardiac action potential duration, future aryaminobenzoate derivatives might represent a new and unique group of class III antiarrhythmic agents that would be effective during sympathetic stimulation. The action of other putative CFTR channel blockers, such as sulfonylureas (Sheppard and Welsh, 1992; Venglark et al., 1996) and clofibrate acid analogues (Walsh and Wang, 1996), also will require careful attention.

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References


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