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Biochimica et Biophysica Acta 1329 (1997) 26–38

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

Characterization of the effects of amphotericin B on ion channels in MDCK cells using the patch-clamp technique [1]

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Received 13 January 1997; accepted 25 February 1997

Abstract

Cultured Madin–Darby Canine Kidney cells were used as a model to study the mechanism of nephrotoxicity of amphotericin B using the patch-clamp technique. At the whole-cell level, amphotericin B altered potassium conductances in two types of these cells categorized on the basis of whole-cell potassium currents. The first cell type, classified as Type I, exhibited no significant whole-cell potassium currents. The second type, Type II, exhibited depolarization-induced outward potassium currents that rundown over time. In both of these subpopulations, exposure to amphotericin B at a concentration of 68 nM for a prolonged period of time (~ 30–45 min) led to an increased whole-cell potassium conductance. In Type I cells, it increased by a factor of 16 and in Type II cells, by a factor of 3.5. Furthermore, the potassium currents observed in Type I cells following amphotericin B treatment bore no resemblance to currents through pores formed by amphotericin B in artificial membranes. At the single-channel level, incubation with amphotericin B led to a significantly higher potassium channel activity in both inside-out and outside-out patches. Kinetic studies in inside-out patches revealed that the increases in channel activity were associated with a decrease in the mean closed time and an overall increase in the mean open time. In summary, our data suggest that the direct toxicity of amphotericin B is primarily related to its ability to disturb normal ion channel functioning rather than to formation of pores in cell membranes. © 1997 Elsevier Science B.V.

Keywords: Amphotericin B; Nephrotoxicity; Ion channel; Patch clamp

1. Introduction

Amphotericin B has a potent antifungal action [1]. After more than thirty years of clinical use in the treatment of most systemic fungal infections, amphotericin B remains the drug of first choice despite the introduction of several newer antifungal agents such as Miconazole and Ketoconazole [2–4]. This may be because amphotericin B has caused few types of resistance. Nevertheless, its nephrotoxicity is of primary concern in patient treatment and is manifested as disturbances in glomerular and tubular function [3]. Patients treated with amphotericin B commonly exhibit reduced glomerular filtration rate (GFR), azotemia, renal tubular acidosis, impaired renal concentrating ability, and electrolyte abnormalities, especially those involving potassium and sodium ions.

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[1] Supported by grant # 5R01 GM40410-03 of the National Institutes of Health.

PII S0005-2736(97)00055-2

Mechanisms of amphotericin B-induced nephrotoxicity are poorly understood and may result from both direct and indirect effects [5]. The direct effects are believed to involve direct interactions between amphotericin B and the plasma membranes of renal tubular cells whereas the indirect effects are thought to lead to nephrotoxicity deriving from decreases in renal blood flow and GFR. While mechanisms underlying these indirect effects are still in dispute, the direct membrane effects have been extensively studied in various model systems. The present investigation is focused on the direct action of amphotericin B on plasma membranes.

One explanation for the direct effect is the sterol hypothesis [6], according to which amphotericin B binds to sterols such as cholesterol in the lumenal membrane of renal tubular cells, leading to an increase in the ion permeability of the lumenal membrane. This results in a loss of electrolytes from the cell. Andreoli [7,8] has suggested that amphotericin B interacts with membrane sterols, forming aqueous pores approximately 40–105 nm diameter. Several lines of evidence support the formation of such pores. For example, in sterol-rich planar lipid bilayers, treatment on both sides with (5×10^{-8} to 2×10^{-7} M) amphotericin B was reported to decrease electrical resistance across these membranes by a factor of one million. This decrease was believed to be due to the formation of aqueous pores for the following two reasons: (1) the correlation between aqueous salt concentration and electrical resistance in the system studied approached that in bulk water [6,9]; and (2) chloride fluxes across black lipid membranes treated on both sides with amphotericin B followed the Goldman–Hodgkin–Katz field equation [10]. Both observations suggest the presence of aqueous channels in these membranes treated with amphotericin B. Demonstrated stepwise changes in the electrical conductance of these membranes further indicate that this fungicide can induce the formation of aqueous pores across lipid bilayers [11,12].

However, the idea that sterol molecules are required to form amphotericin B pores has been challenged based on the fact that increases in both the binding of amphotericin B with phospholipids and also the ion permeability have been noted in both sterol-free small unilamellar and sterol-free multilamellar vesicles. Doubts have also been raised concerning the possibility of pore formation in vivo since aqueous pores have only been detected in black lipid membranes treated with amphotericin B on both sides of these membranes. Furthermore, the anion selectivity of these channels cannot explain the loss of cations, especially K^+, from cells and liposomes when treated with amphotericin B [13–17].

The purpose of the present study was to investigate the direct action of amphotericin B in renal tubular cells in culture. Mardin–Darby Canine Kidney cells (MDCK cells) were used as a cell model for distal renal tubular cells because they resemble distal tubular cells in many respects [18–21]. Using the patch-clamp technique [22], our study revealed that amphotericin B increased the opening probability of the K^+ channels present in the MDCK cells through a mechanism that was independent of pore formation.

2. Materials and methods

2.1. Cell culture

Cloned MDCK, MDCK C-7, cells were kindly contributed by Dr. Cereijido (Department of Physiology, Biophysics and Neuroscience, Center of Investigation, Mexico). The passages used were numbers 101 to 120. Serial cultures were maintained at 37°C in disposable plastic culture flasks (Costar 3375, Cambridge, MA) with an air–5% CO_2 atmosphere and Dulbecco's Modified Eagle's Medium (Gibco 430-1600EB, Grand Island, NY) supplemented with 0.08 U/ml insulin (Gibco 890-8125IG, Grand Island, NY), and 10% fetal bovine serum (Gibco 210-6510AJ, Grand Island, NY). Upon reaching confluency, the cells were harvested with 0.1% trypsin (Sigma T0646, St. Louis, MO) prepared in reconstituted Dulbecco's phosphate buffered saline (Sigma D-5652, St. Louis, MO), and plated on 35×10 mm culture dishes (Corning Sterile 25000, Corning, NY) for patch-clamp recording.

Prior to recording, the monolayer of cells in culture dishes were washed 3–5 times with a high-potassium, low-calcium bathing solution. The monolayers were then allowed to stand at room temperature in the bathing solution for approximately 30 min. This led to disruption of the tight junctions between cells

and resulted in partial rounding of the flat monolayer cells.

2.2. Electrolyte solutions and solutions containing amphotericin B

The pipette solution bathing the cytoplasmic side of the membrane used in both outside-out and whole-cell recordings contained (in mM): NaOH, 10; KOH, 141; methanesulfonic acid, 154; $Ca(OH)_2$, 1.54; $Mg(OH)_2$, 1; glucose, 10; and Hepes, 10; EGTA, 2.3. The solution bathing the extracellular side of the membrane, also used in both outside-out and whole-cell recordings, contained (in mM): NaOH, 146; KOH, 5; methanesulfonic acid, 154; $Ca(OH)_2$, 2; $Mg(OH)_2$, 1; glucose, 10; and Hepes, 10. 10 mM of TEA or CsCl were added where indicated to block potassium currents. The solution used to investigate the effects of amphotericin B on channel kinetics in inside-out membrane patches was a symmetric high-potassium solution containing (in mM): potassium gluconate, 150; glucose, 10; Hepes, 10; $CaCl_2$, 1; and EGTA, 1.03. The rest of the inside-out patch-clamp study used a symmetric high-potassium solution containing low levels of Ca^{2+} (approximately 130 nM), and (in mM): NaOH, 10; KOH, 141; methanesulfonic acid, 154; $Ca(OH)_2$, 2; $Mg(OH)_2$, 1; glucose, 10; Hepes, 10; and EGTA, 10. The final pH values of all solutions used were adjusted to 7.3 (± 0.1) using KOH.

Saturated solutions of amphotericin B were prepared in 10-ml aliquots by adding 4 mg of amphotericin B (Sigma, St. Louis, MO). Sonication was used to facilitate the dissolution of amphotericin B since it was only slightly soluble in aqueous solution lacking organic cosolvents. Using a UV spectrometer, we estimated that the concentrations of amphotericin B in the electrolyte solutions used were approximately 6.8×10^{-8} M.

For the patch-clamp studies, a time series of control currents was first obtained prior to the addition of amphotericin B after achieving the desired patch-clamp configuration (see [22]). These currents served as the basis for evaluating the effects of amphotericin B. The bathing solution for the cell or the membrane patch under recording was then replaced by the solution containing amphotericin B. The stability of our recordings enabled experiments to last up to 2 h.

2.3. Patch-clamp recording

An EPC-7 patch-clamp amplifier (List Electronic, Darmstadt, F.R.G.) interfaced through a MCDAS board (Axon Instrument, U.S.A.) were used in conjunction with an 80386-based computer to control voltage as well as to record currents using Pclamp software (5.5.1, Axon Instruments, U.S.A.). The same software was used to analyze the single-channel kinetics. The patch-clamp pipettes were made by pulling glass capillaries (OD 1.5 mm, ID 0.75 mm, Sutter Instruments, Navato, CA) using a Flaming Brown micropipette puller (Model P-87, Shutter Instrument, Navato, CA). Prior to use, the pipettes were coated with Sylgard® 184 (ET 030497, Dow Corning, Midland, MI), and then fire polished. The inner pipette diameters were approximately 1–1.5 μm and exhibited < 10-MΩ resistance when filled with various pipette solutions. All patch-clamp studies were performed at room temperature.

3. Results and discussion

Three different recording configurations were used to examine the effects of amphotericin B on ionic conductance across MDCK cell membranes. These included inside-out and outside-out patches and whole cells. As described in Section 2.2, control currents were obtained from individual patches and cells before the initial bathing medium was replaced with the amphotericin B solution. Membrane currents were measured under voltage clamping to determine the effects of exposure to amphotericin B.

3.1. Effects of amphotericin B on channels in outside-out patches

The results of the amphotericin B studies using outside-out membrane patches fall into three categories. First, for a number of outside-out patches exhibiting no channel activity at any voltage level under control conditions, incubation with amphotericin B for up to 2 h did not alter even the DC level of baseline current across these patches ($n = 10$). We inferred from this that no ion channels were present in these patches. We tentatively infer that amphotericin B, at the concentrations employed, does not affect the conductance of patches lacking ion channels.

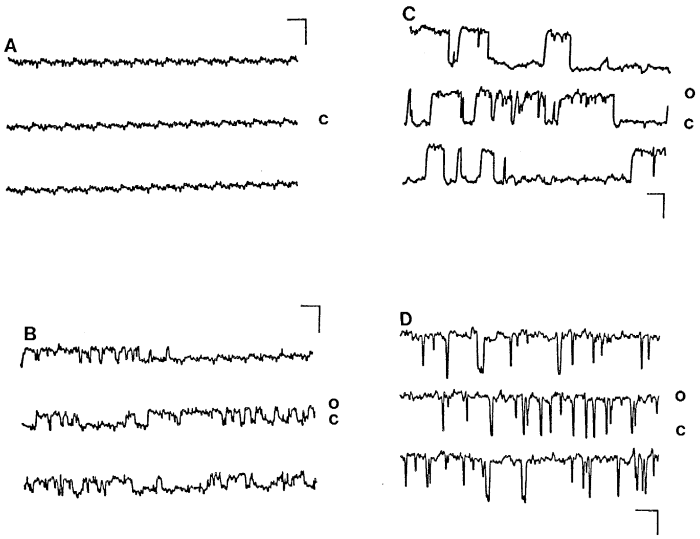

Fig. 1. Effects of amphotericin B on outside-out membrane patches. Small 'o' indicates that the channel is open and small 'c' indicates that the channel is closed. The current calibration bar is 5 pA for (A) and (B), and 10 pA for (C) and (D). The time calibration bar is 10 ms. (A) and (B) are current traces obtained from a patch that originally showed no channel activity at a holding potential of 80 mV. (C) and (D) are current traces from another patch containing a channel whose opening probability was ~30% at 75 mV prior to addition of amphotericin B.

A. Single-channel current recording showed no channel opening prior to exposure to a saturated solution of amphotericin B.

B. In the same membrane patch, single-channel currents appeared at ~45 min after addition of amphotericin B.

C. In another outside-out membrane patch, the opening probability prior to addition of amphotericin B was ~30%.

D. In the same membrane patch as in (C), the opening probability at the same holding voltage, +75 mV, doubled after addition of amphotericin B to the bathing solution.

In the second category, there was no channel activity before treatment with amphotericin B, but channel events were detected after treatment (Fig. 1A and B, $n = 7$). Since we monitored channel activity prior to treatment for at least half an hour, these channel events appear to have been caused by amphotericin B.

In the third category, channel opening was observed before treatment with amphotericin B, and the opening probability increased further after treatment ($n = 6$). In this category, we noted that amphotericin B increased the opening of K^+ channels regardless of their specific types. Table 1 lists two examples of single-channel activity before and after the addition of amphotericin B to illustrate the non-specific effect of amphotericin B on various K^+ channels. One typical change in channel activity is shown in Fig. 1C and D.

Table 1
Single-channel parameters in outside-out membrane patches before and after the addition of amphotericin B to the extracellular side of the membrane patch

	Open probability (%)		Single-channel current amplitude (pA)		Open-time constant (ms)		Closed-time constant (ms)	
	before	after	before	after	before	after	before	after
Membrane held at 69 mV	~0	35.3	–	–	–	1.14 6.63	–	1.00 11.4
Membrane held at 75 mV	33	83.8	13.1	17.8	2.65 16.1	1.79 8.32	1.64 23.4	0.66 2.49

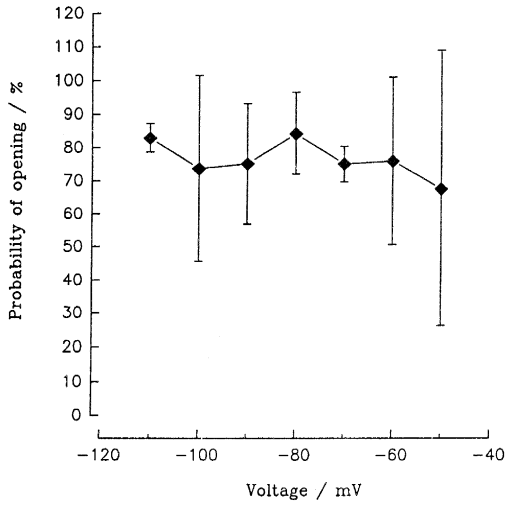

Fig. 2. Effects of amphotericin B on inside-out membrane patches. The opening probabilities at any voltage level were zero prior to addition of amphotericin B. After addition of amphotericin B, the opening probabilities eventually increased to ~80% (for four different patches) at hyperpolarization membrane potentials. These channels appeared to be inwardly rectified because no opening events were observed at depolarization membrane potentials.

In general, increases in channel activity in the latter two categories occurred within 20–45 min of incubation with amphotericin B. However, this increase in channel activity was not accomplished through an increase in the number of ion channels present in the patch since we had never observed simultaneous opening of multiple channels increase after exposure to amphotericin B.

3.2. Effects of amphotericin B on inside-out patches

Experiments with inside-out patches produced findings similar to those from outside-out patches. Exposure to amphotericin B increased K^+ channel activity. This increase occurred at all depolarization voltages examined (−70 to +100 mV). As observed

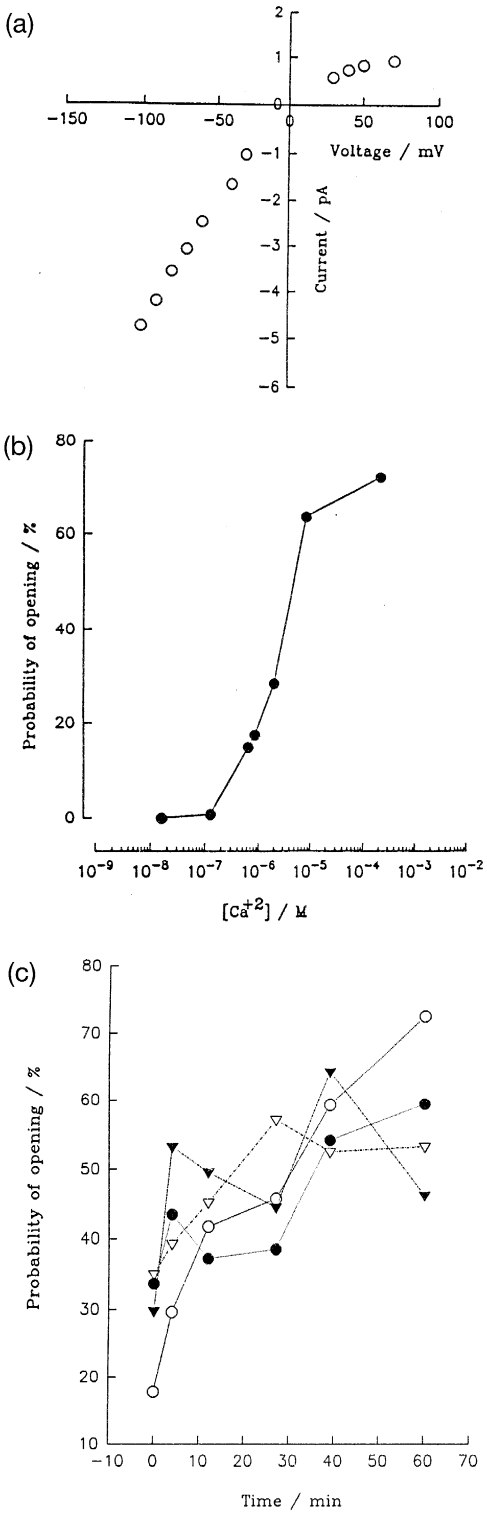

Fig. 3. An inwardly-rectified 52 pS calcium-dependent K^+ channel in one inside-out patch was used to study the effects of amphotericin B on the kinetic properties of a single channel. A. Conductance plot of this channel. B. This 52 pS channel was identified as a calcium-dependent cation channel. C. The opening probability changed over time at all four voltage levels, membrane potential = −40 mV (○), membrane potential = −60 mV (●), membrane voltage = −80 mV (▽), and membrane voltage = −100 mV (▼), studied after the addition of amphotericin B. The time zero data were obtained just before the addition of amphotericin B.

in outside-out patches, the increases did not occur immediately after amphotericin B treatment, but developed after more than 0.5 h. Fig. 2 shows the average increases in channel activity at various voltages. In all of the patches at all voltages ($n = 4$), the opening probability was zero prior to amphotericin B treatment.

To investigate the mechanism by which amphotericin B altered the activity of an ion channel, we examined an additional inside-out patch in greater detail. We first identified the channel in the patch as a 52-pS inwardly-rectifying calcium-activated potassium channel (Fig. 3A and B). We then analyzed its single-channel kinetics prior to the application of amphotericin B. Because it was calcium-dependent, we recorded the single-channel events in the presence of a moderate level of free Ca^{2+} (approximately 2 μM). This high Ca^{+2} concentration enhanced the channel activity prior to amphotericin B treatment, and thus allowed meaningful kinetic parameters to be determined prior to incubation with amphotericin B. After addition of amphotericin B, as indicated in Fig. 3C, the open probability increased over time. The most dramatic effects of amphotericin B appear to be

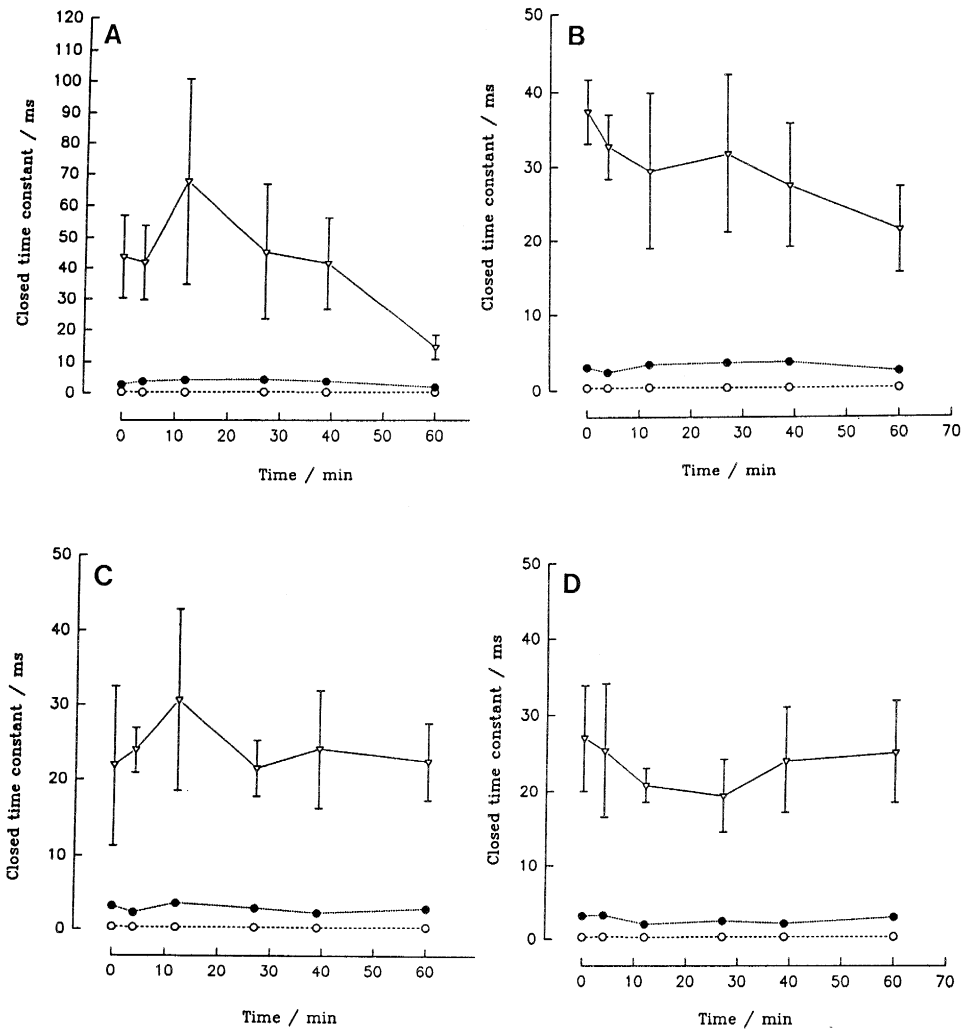

Fig. 4. The effects of amphotericin B on the three closed-time constants at the four different holding potentials [(A) −40 mV, (B) −60 mV, (C) −80 mV and (D) −100 mV] studied. The largest changes occurred after the channel was exposed to amphotericin B for ~1 h, and was associated with the closed state with the longest mean resident time (▽), especially at −40 mV. The mean resident time of the other two closed states (●, ○) appeared not to be affected by amphotericin B.

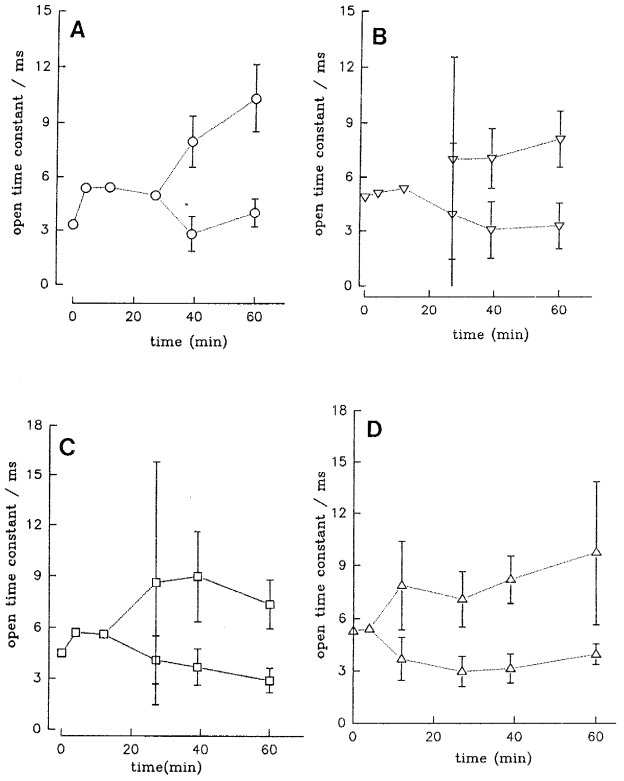

Fig. 5. The effects of amphotericin B on open-time constants at the four different holding potentials [(A) −40 mV, (B) −60 mV, (C) −80 mV and (D) −100 mV] studied. Prior to exposure to amphotericin B, the channel in the patch had only one open state (hence, one time constant); after exposure to amphotericin B, two open states were required to describe the opening events of the channel. The overall open probability increased at all four voltage levels partly because more than 50% of the channel open events had longer open durations after amphotericin B treatment. At −40, −60, −80, and −100 mV, 61.3%, 71.2%, 55.4%, and 60%, respectively, of the open events were primarily attributable to the open state with the longer mean open resident time.

associated with channel gating at the lowest depolarization applied (−40 mV). Because the channel opened more frequently prior to amphotericin B treatment at more positive voltages, the reverse of this trend after amphotericin B treatment indicated that amphotericin B could abolish the voltage dependence of this channel. This led us to conclude that amphotericin B may alter the gating properties of a channel. Further analysis of the single-channel kinetics revealed that the effects of amphotericin B were partly related to a change in the slowest time constant of the closed-time distribution. The two shorter closed-time constants remained unchanged throughout the experiment at all voltages studied, but the longest closed-time constant changed dramatically at lower voltages (Fig. 4). Since the weights for the three closed-time constants did not vary significantly (not shown), we inferred that the decrease over time of the longest time constant caused by amphotericin B was a significant factor contributing to the channel's increased opening probability.

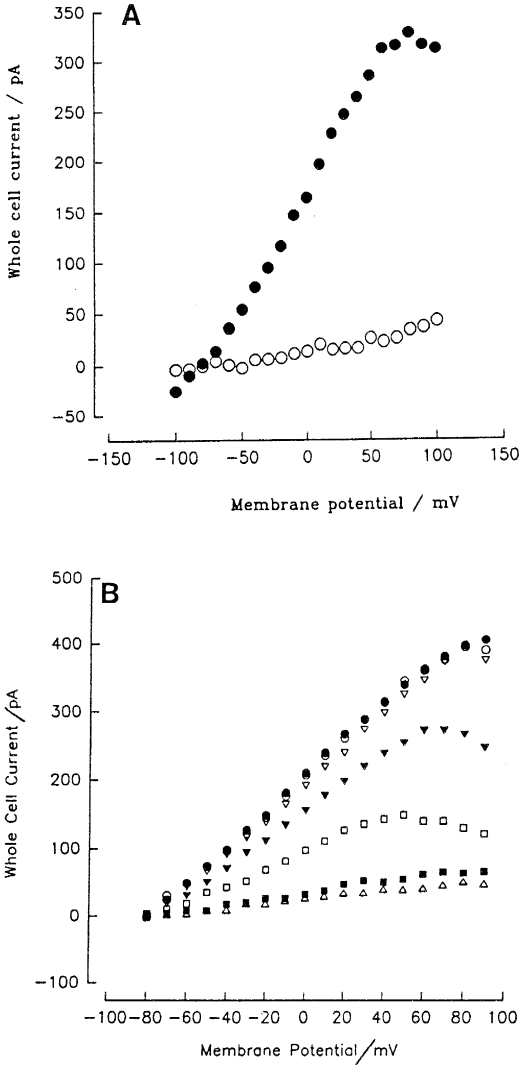

Fig. 6. Two types of whole-cell conductances were found in cloned MDCK cells. A. The Type I cells showed no significant conductances at membrane voltages ranging from −100 to +100 mV (○), whereas the Type II cells had conductances larger than a few nS (●). B. The conductances of the Type II cells decreased over time, time = 0 min (○), 2 min (●), 9.8 min (▽), 15.5 min (▼), 20.3 min (□), 25 min (■), 27.5 min (△). See text for the voltage paradigm used.

In addition to shortening the lifetime of one closed state, incubation with amphotericin B led to configurational changes in open states. We found that prior to amphotericin B, the open-time distribution could be described by a single exponential function. However, a sum of two exponentials was required after the addition of amphotericin B (Fig. 5). Since at all voltages studied, larger pre-exponential weights were associated with a longer lifetime for the additional open state, we concluded that an overall increase in the lifetime of channel opening resulted (see Fig. 5 legend). We believe that the decrease in the lifetime of the closed state and the increase in the mean opening duration, taken together, account for the overall increase in the channel activity observed.

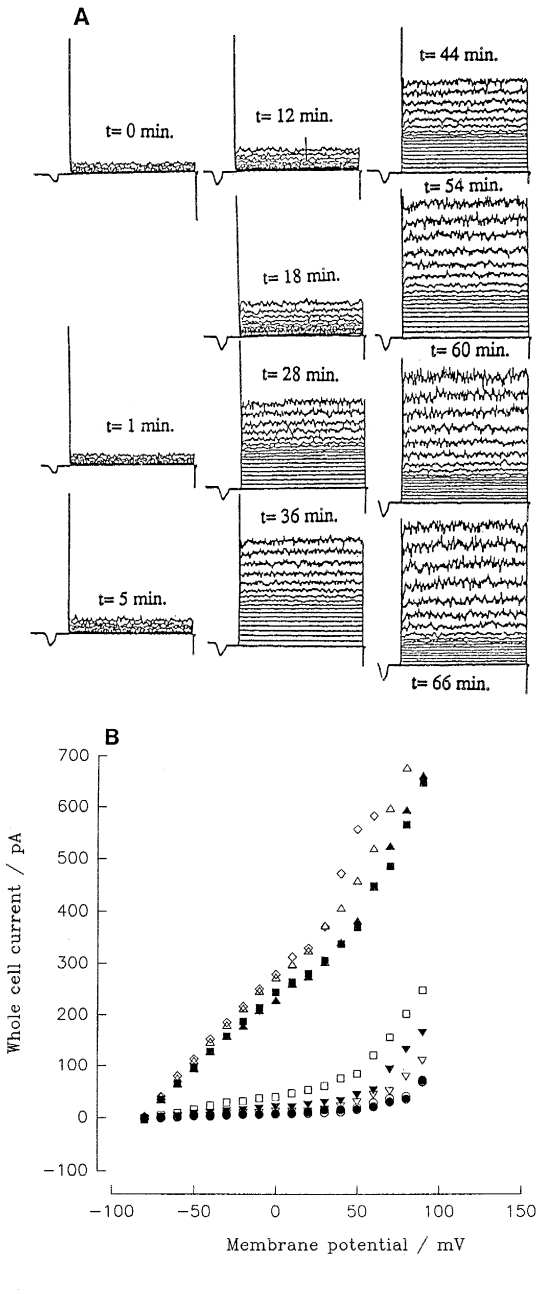

Fig. 7. Effects of amphotericin B on Type I whole-cell currents over time. A. Before adding amphotericin B to the solution bathing the whole cell, at times zero and 1 min, whole-cell currents were recorded, and whole-cell conductances were found to be insignificant (Type I). After 4.5 min, amphotericin B was added to the solution bathing the cells. The increase in conductance became apparent at 12 min. The increase in conductance continued for approximately another hour. In this cell, there were at least two distinct conductive pathways for K^+ to translocate across the whole-cell membrane. Judging from the individual current traces at various time points and voltage levels, amphotericin B appeared to have distinct effects on these two pathways. For example, compare the current records at 36 and 66 min; over time, amphotericin B caused larger increases in currents responding to higher clamped voltage levels than those clamped at lower voltage levels. B. The associated whole-cell conductance plots before and after exposure to amphotericin B, control at time = 0 min (○), and 1 min (●), and after exposure to amphotericin B at time = 5 min (▽), 12 min (▼), 18 min (m), 28 min (○), 36 min (△), 44 min (▲), 54 min (◇). The voltage paradigm used is as described in Fig. 6.

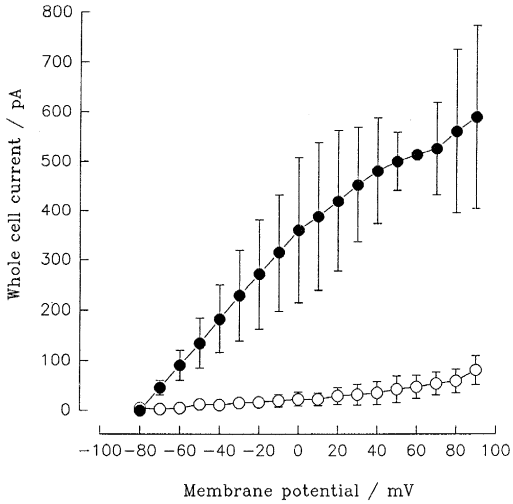

Fig. 8. Amphotericin B caused the mean whole-cell conductance to increase from 0.301 ± 0.213 nS (○) to 4.80 ± 1.57 nS (●) in Type I cells, corresponding to a mean net increase of 1590%. The mean $\pm$ S.D. are given. All conductances referred here are slope conductances.

These results at the single-channel level demonstrate that amphotericin B is capable of modifying the activity of ion channels. The fact that amphotericin B increased channel activity regardless of the membrane side to which it was added, and that this increase would only take place after a prolonged period of incubation, strongly suggests that its actions are not mediated through specific binding, nor through a second messenger system, since either a membrane-delimited second messenger system or one acting through specific binding would be expected to act much more quickly. The additional fact that amphotericin B was able to increase the opening of all the channels examined suggests that this effect may be mediated through a change in the lipid environment. To investigate this possibility, we studied the effect of amphotericin using the whole-cell configuration.

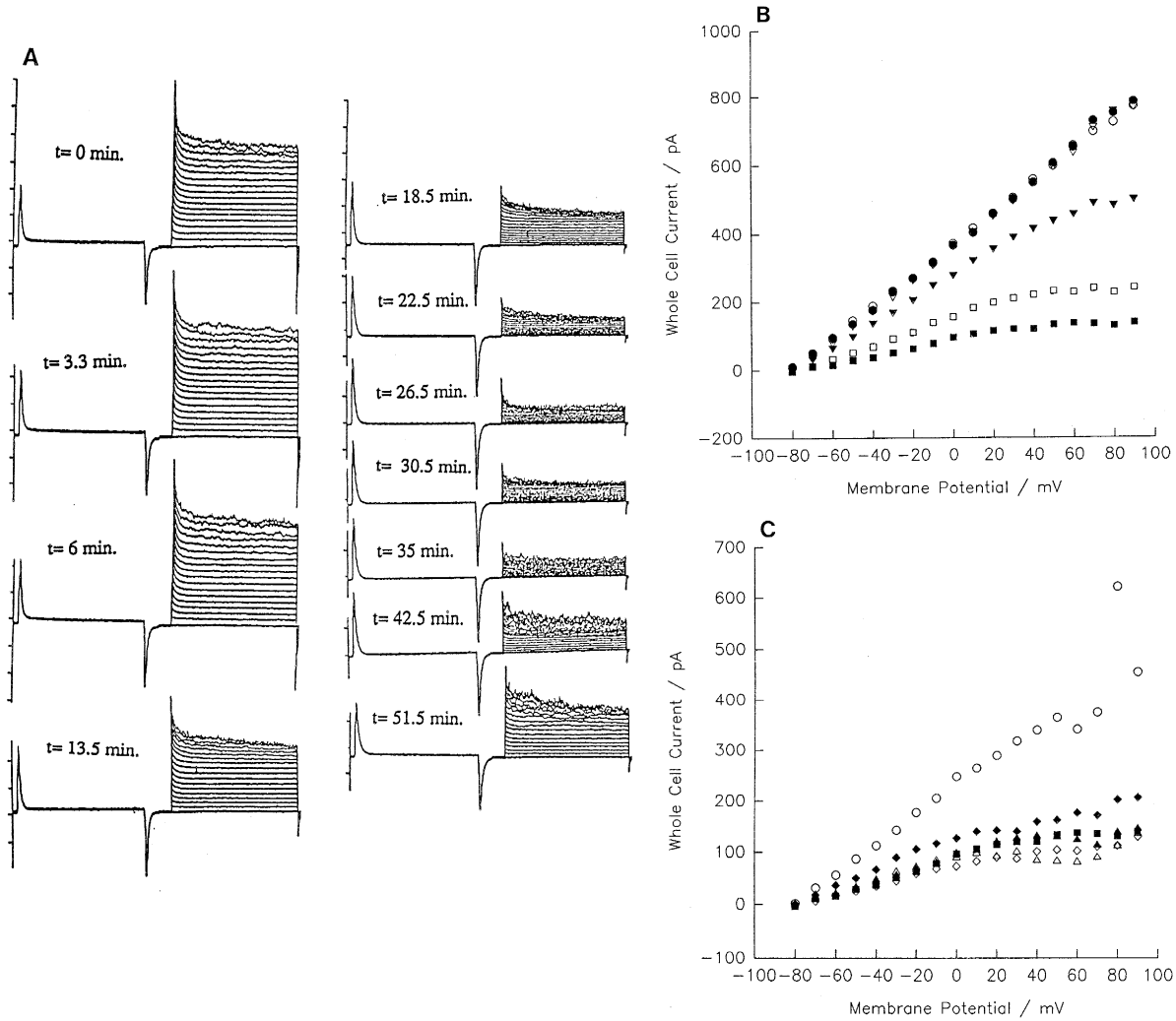

Fig. 9. Effects of amphotericin B on Type II whole-cell currents. A. Prior to exposure to amphotericin B, the whole-cell currents decreased over time, a phenomenon often referred to as rundown. Twenty-two minutes later, amphotericin B was introduced to bathe the whole cell, and increases of whole-cell currents became apparent ~20 min later. B and C. The associated plots show whole-cell conductance before (B) and after (C) the cell was exposed to amphotericin B, control at time = 0 min (○), 3.3 min (●), 6 min (▽), 13.5 min (▼), 18.5 min (□), and after exposure to amphotericin B at time = 22.5 min (■), 26.5 min (△), 30.5 min (▲), 35 min (◇), 42.5 min (◆) and 51.5 min (○). The same voltage paradigm as described in Fig. 6 was used.

3.3. Effects of amphotericin B on whole-cell currents

We first characterized the K^+ current profile in the cloned MDCK cells using the whole-cell voltage clamp technique. The cells were held at −80 mV, and a series of 1-s depolarization pulses from −80 to +90 mV at 10-mV intervals were then applied. Two types of current responses were observed in MDCK cells (Fig. 6A), and hence these cells were classified into two groups, Types I and II. In 30% of the cells, depolarization pulses induced no detectable currents; these cells were referred to as the Type I cells. The remaining 70% of the cells, referred to as the Type II cells, exhibited significant whole-cell conductance when depolarization pulses were applied. We noticed that in many, but not all, Type II cells, whole-cell currents run down over time (Fig. 6B), indicating possible loss of cytoplasmic factors required for channel regulation. Also, the depolarization pulses did not always evoke similar patterns of whole-cell currents in all Type II cells. Some cells responded to the applied voltage pulses with non-inactivating K^+ currents, some cells with delayed-activating K^+ currents and some with inactivating K^+ currents. Since these cells came from a cloned cell line, such variations may be due to changes in channel expression regulated by factors such as the cell cycle and the level of maturity of the monolayer reached during culturing[23].

We investigated the effects of amphotericin B on whole-cell currents of both types of cells. In Type I cells, amphotericin B increased the whole-cell currents at all voltages tested. One such example is illustrated in Fig. 7. As shown in this figure, the same series of voltages were applied to the cell over time both before and during incubation with amphotericin B. The mean responses from three cells are shown in Fig. 8. It is evident that in Type I cells amphotericin B caused a dramatic increase in whole-cell conductance.

Detailed examination of the increases in whole-cell conductance in various Type I cells reveals that amphotericin B brought about different patterns of current increases in these cells (not shown). In that illustrated in Fig. 7A, amphotericin B induced what may be regarded as outwardly-rectified K^+ currents. Other types of currents observed in Type I cells after incubation with amphotericin B included inwardly-rectified K^+ currents, and A-like K^+ currents. We further noted that the characteristics of these amphotericin B-induced currents were very similar to some whole-cell currents seen in Type II cells prior to their incubation with amphotericin B. The similarity in these whole-cell currents suggests that at low concentrations, amphotericin B may only be able to modify the activity of ion channels already present in the membrane, instead of creating new conduction pathways. Observed amphotericin B-induced conductance changes in Type II cells (see below) provide support for this idea because increased whole-cell conductance profiles retained their characteristic shapes (inactivation, inward or outward rectification) after amphotericin B treatment. Amphotericin B increased Type II whole-cell currents in six out of eight cells tested. One example of such whole-cell responses is illustrated in Fig. 9. The mean conductance for the six responsive cells increased from 2.10 ± 1.53 (mean ± S.D.) to 7.14 ± 4.61 nS ($P < 0.05$). This represents an increase of 340%.

4. Conclusions

It is well known that amphotericin B increases the permeability to ions of fungal cell membranes. Several lines of evidence suggest that this is due to the formation of aqueous pores created by the interaction of amphotericin B with the fungal sterol molecule, ergosterol [5]. Because amphotericin B also interacts with cholesterol, this interaction has long been cited to account for the toxicity of amphotericin B in mammalian cells [24].

However, a detailed examination of the properties of these amphotericin B pores reveals evidence against such a mechanism. For example, the selectivity of amphotericin B pores does not help to explain the amphotericin B toxicity seen in isolated cells. These pores are found to be anion-selective. If these pores do form in cell membranes as a result of amphotericin B treatment, with extracellular fluid containing high levels of Na^+ and Cl^-, the concentration gradient of chloride ions should drive the extracellular chloride into the cells through these pores. This would hyperpolarize the cells, and would lead to an increase in Na^+ influx as well as a decrease in K^+

efflux. However, this prediction does not agree with the experimental observation of K^+ leakage.

One could also consider a K^+ leakage brought about by Cl^- accumulation. When intracellular Cl^- accumulates above its electrochemical equilibrium due to the presence of a Na–K–Cl pump, opening of anion-selective channels could lead to efflux of Cl^-, causing membrane depolarization and thus activating voltage-gated K^+ channels. This could account for the K^+ loss reported in the literature. However, we examined the membrane potential before and after incubation with amphotericin B, and found that the resting membrane potential was altered from -38.4 ± 16.4 to -53.7 ± 26.6 mV ($n = 7$), indicating that activation of K^+ channels is unlikely, so that this mechanism cannot be used to explain the K^+ leakage. We thus concluded that anion pores in the cell membrane play no part in the cellular K^+ leakage caused by amphotericin B.

Two alternative hypotheses may explain the cation leakage. The first hypothesis is that the sterol molecules resident in cell membranes may participate in creating proper environments for normal membrane protein function. If amphotericin B, through its interaction with membrane sterol, alters the interaction of the sterol molecules with certain membrane proteins, then protein functioning in the cell membrane would be expected to change. The other hypothesis involves direct interaction between amphotericin B and membrane proteins, again leading to changes in the function of proteins. The cation leakage reported in the literature may be understandable by reference to these two hypotheses. Although our data support the two alternative possibilities, the design of our study precluded the identification of any evidence supporting one of these hypotheses over the other.

We voltage clamped MDCK cells to study how amphotericin B altered the membrane conductance of these cells. The results of our studies support the idea that it is the endogenous ion channels that are affected by amphotericin B. At the whole-cell level, large increases in membrane conductance were demonstrated when MDCK cells were incubated with amphotericin B. Since the characteristics of the increased membrane conductance resembled those seen in some of these cells under controlled conditions, it was thought that these increases might be a result of higher activity of the endogenous channels. By replacing Cl^- ions with gluconate ions, and using a specific K^+ channel blocker, it was confirmed that the activity of K^+ permeable channels was due to amphotericin B. However, it was unclear whether these channels were amphotericin B pores or endogenous K^+ channels.

One hint from an experiment using LLP-CK_1 cells (a cell line generally used as a model for the kidney proximal tubular cells) suggested that the endogenous proteins were the more likely candidate [25]: if whole-cell currents were abolished by the channel blocker TEA before adding amphotericin B, then the amphotericin B did not affect the whole-cell currents. This indicated that after eliminating the ionic conductance mediated through channel proteins, amphotericin B had no effect on the ion permeability of the lipid membrane. Blocker studies in MDCK cells have also suggested this possibility. In MDCK cells, TEA was demonstrated to block the amphotericin B-induced whole-cell currents (data not shown). Results from single-channel studies confirmed that amphotericin B does indeed modify the activity of these endogenous channels. For example, amphotericin B had minimal effects on outside-out patches lacking endogenous channels, but led to greater single-channel activity in outside-out patches with endogenous channels (Fig. 1; Table 1). The evidence for the minimal effects of amphotericin B in lipid membranes in the first case is the contrast between the absence, after treatment, of detectable changes in either the DC levels of the currents or the noise levels associated with these currents in patches devoid of single channels, even after very long incubation times (> 2 h), compared with the post-treatment increases in single-channel activity in inside-out patches, where channel activity was observed (Figs. 2 and 3C). These data lead us to believe that the effects of amphotericin B on the ionic currents of MDCK cells involve interactions between amphotericin B and various types of potassium channels in MDCK cells.

In summary, we have shown that amphotericin B, at the concentrations studied, has a minimal effect on ion transport in lipid membranes devoid of ion channels. A very low concentration of amphotericin B was used in our study because it is the concentration likely to be present in the kidney tubules of patients receiving amphotericin B treatment. We found that at

this concentration, amphotericin B acts mainly on the ion channels present in cell membranes. The effects of amphotericin B on whole-cell currents depend on the magnitude of depolarization and vary among different cells. This may be due to the fact that the various cells we clamped expressed different channels in their membranes. Because the characteristic shapes of the whole-cell current profiles after amphotericin B treatment were similar to the shapes before the treatment, it is likely that, at least within the experimental time frame, amphotericin B affected only channel activity and not channel expression. Finally, we suggest that the potassium wasting seen in amphotericin B-treated patients may be due to an increase in renal potassium secretion caused by the increase in potassium channel activity described here.

Acknowledgements

The authors wish to thank Dr. Meyer Jackson of the Department of Physiology, Dr. Shing-Yan Chiu of the Department of Neurophysiology, and Drs. Richard Peterson and Kenneth A. Connors of the Department of Pharmacy at the University of Wisconsin for their helpful comments in regard to this work. The authors would also like to thank Sansana Boontaveekul for helpful discussions. Finally, the authors wish to thank Dr. Marlino Cereijido of the Department of Physiology, Biophysics and Neuroscience at the Center of Investigation in Mexico for the MDCK cell clone C-7 and for helpful suggestions.

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