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Rapid Report

The effect of amphotericin B on the K-channel activity of MDCK cells

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By using the whole-cell patch technique, it is shown that the total outward current is increased, as a function of time, after the addition of amphotericin B to the bathing solution. The whole-cell current is shown to be primarily a K-channel current by the blockage of this current upon application of TEA to the bathing solution. Single K-channel studies, using the outside-out patch-clamp technique, reveal that the single K-channel opening probability increases by a factor of six after the addition of amphotericin B. In addition, single K-channel voltage dependent studies, using the inside-out patch-clamp technique, demonstrate that this increase in opening probability is due to an increase in the amplitude of $P_o(v)$. In contrast to the present belief that amphotericin B simply creates pores in a cell's membrane, these results suggest that amphotericin B can also influence the function of the cell's K-channel proteins.

The direct actions of amphotericin B are believed to occur in the lipid membranes [1] and are implicated in producing alterations in cellular ion permeability. The direct membrane action of amphotericin B has been explained by the sterol hypothesis [1]. According to this hypothesis, amphotericin B binds to sterol molecules such as cholesterol in the membrane of the cell. The resulting complexes form aqueous pores having a diameter of 40–105 nm, which results in an increase in ion permeability [2–4].

Evidence will now be presented which suggests that potassium ion permeability can also be altered by the influence of amphotericin B on a cell's potassium channels (K-channels), rather than just by the formation of pores.

Fig. 1 shows the effect of amphotericin B on isolated K-channels. In all cases, the channel opening probability increased after the addition of amphotericin B. The mean opening probability increases from 11.0 ± 19.1% (mean ± S.D.) before the addition of amphotericin B to 67.1 ± 27.6% ($P < 0.05$) 45 min after the addition of amphotericin B. Fig. 1 demonstrates that little or no channel activity can be present prior to application of amphotericin B. After the application of amphotericin B, channel activity increases. These channels can be blocked with 10 mM TEA (tetraethylammonium chloride), which suggests that they are K-channels [5]. It was also observed, that for some outside-out patches, which exhibited no channel activity before application of amphotericin B, subsequent addition of amphotericin B did not cause any increase in channel activity. This result suggests that when no K-channels are present the current increase (due to pore formation induced by amphotericin B) is too small to be detected. In addition, it is observed that if TEA is added before amphotericin B, the K-channel activity is never expressed. This indicates that amphotericin B is only producing an effect on unblocked K-channels.

Fig. 2 shows the mean opening probability of K-channels, as a function of voltage, obtained from inside-out patches after the addition of amphotericin B. If no amphotericin B was added, the open probability remained zero because the channels were allowed to run down and the free Ca^{2+} concentration was maintained at 130 nM. The channels were shown to be K-channels by their reversible blockage with TEA. As can be seen, the opening probability of the K-channels, as a function of voltage, is greater after the addition of amphotericin B than before. This suggests that it is a change in shape and amplitude that is produced by amphotericin B, rather than a shift in the $P_o(v)$ curve along the voltage axis.

Fig. 3 shows an example of the time-course of the effect of amphotericin B on whole-cell current as a function of membrane potential. As can be seen from Fig. 3, after amphotericin B is added, the whole-cell

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current increases as a function of time. Fig. 4 shows the mean whole-cell current before amphotericin B is added and 30 min after the addition of amphotericin B. This whole-cell current can be blocked by the addition of 10 mM TEA. This implies that the whole cell current is primarily due to K-channels. In addition, whole-cell patches are sometimes obtained in which only a small baseline leakage current is observed before and after the addition of amphotericin B. This result implies that the presence of amphotericin B, when no K-channels are open, is insufficient to produce a detectable current.

The single-channel data suggest that the reason for the increase in whole-cell current, after the addition of amphotericin B, is primarily due to an increase in the open probability of the individual K-channels. Thus, the ensemble effect of the increase in open probability of the individual single K-channels results in the observed whole-cell current increase.

Several investigators have reported that nystatin, which is structurally similar to amphotericin B [6,7], and amphotericin B [8] can permealize cells to ions after their addition to the exterior of a cell's surface

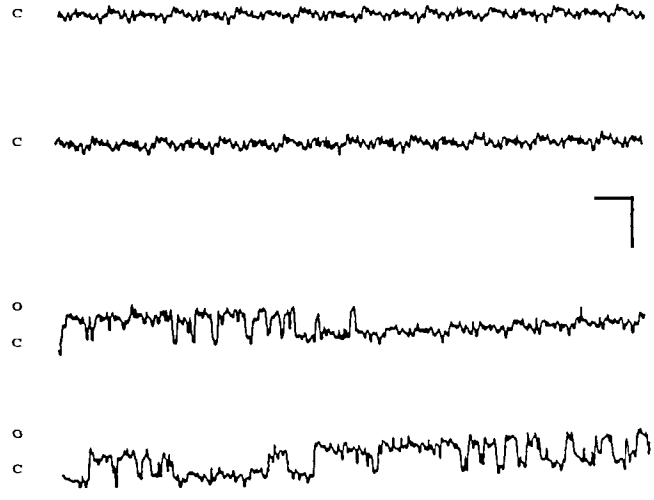

Fig. 1. Effect of amphotericin B on an outside-out patch that originally showed low channel activity at a holding potential of 80 mV. Plot a is before the addition of a saturated solution of amphotericin B. Plot b is approx. 45 min after the addition of amphotericin B. Small 'o' refers to the channel being open and small 'c' refers to channel being closed. The calibration bars are 5 pA and 10 ms. The bathing solution used in the single-channel patch-clamp experiments contained (mM): NaCl, 145; KCl, 5; $CaCl_2$, 2; $MgCl_2$, 1; glucose, 10 and Hepes (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 10. The pipette solution contained (mM): NaCl, 10; KCl, 145; $CaCl_2$, 1.54; $MgCl_2$, 1; EGTA (ethylene glycol bis(β-aminoethylether)-*N,N,N',N'*-2-tetraacetic acid), 2.3; glucose, 10 and Hepes, 10. Solution pH was adjusted to 7.4 ± 0.1 by the addition of NaOH (bathing solution) or KOH (pipette solution). A saturated solution of amphotericin B ($6.8 \cdot 10^{-8}$ M) was used. MDCK cell clone C-7 was used and obtained from Dr. Marelino Cereijido, as a kind gift, at the Department of Physiology, Biophysics and Neuroscience at the Center of Investigation in Mexico. Patch-clamp was formed as described in Ref. 12.

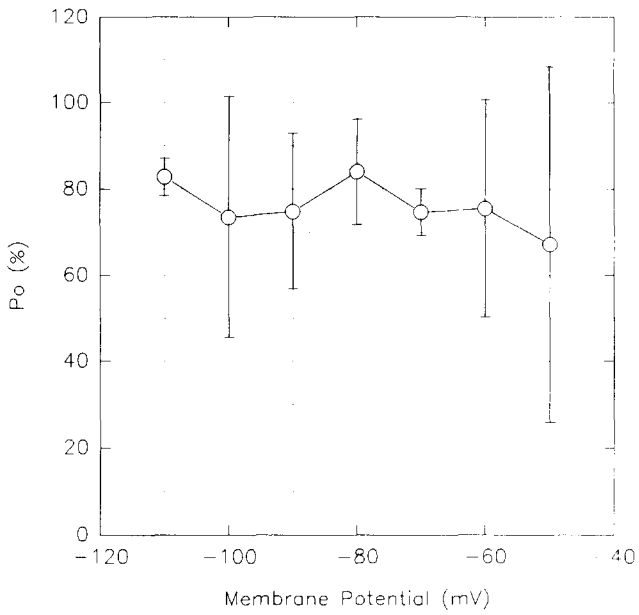

Fig. 2. The mean opening probabilities along with associated standard deviations are given for inside-out patches as a function of membrane potential. The data shown was obtained after the addition of amphotericin B. Since the channel was allowed to run down and Ca^{2+} was maintained at 130 nM, the opening probability without the presence of amphotericin B was zero. The solution used in the inside-out patch-clamp experiments was a symmetrical K^+ solution which contained (mM): NaOH, 10; KOH, 141; methanesulfonic acid, 154; $Ca(OH)_2$, 1.54; $Mg(OH)_2$, 1; EGTA, 2.3; glucose, 10 and Hepes, 10. Same method of pH adjustment, cell type, amphotericin B concentration and techniques reference as described in Fig. 1.

without apparently altering ion-channel activity. In those experiments, amphotericin B or nystatin was used to permealize the cell membrane directly under the patch pipette. This enables whole-cell recordings to be conducted over a longer period of time before wash-out of the cell's contents occurs [6]. However, our whole-cell experiments demonstrated that when channel activity is not expressed, before or after the addition of amphotericin B, only a small baseline leakage current is observed. Therefore, our results suggest that if amphotericin B is increasing the cell's permeability to ions, thereby producing an increase in whole-cell current, this phenomenon represents only a minor effect relative to the effect of amphotericin B on the K-channel activity. The apparent discrepancy between our results and those given in Refs. 6–8, can be explained in terms of two observations. First, the molar concentration of amphotericin B used by Rae et al. [8] was 3800-times that used in our experiments and the nystatin molar concentration used by Korn et al. [7] was 2600-times the molar concentration of amphotericin B we used. These higher concentrations were obtained by dissolving nystatin and amphotericin B in dimethylsulfoxide. Therefore, these investigators observed a measurable increase in ion permeability, due to pore formation, presumably because their ampho-

tericin B or nystatin concentrations were three orders of magnitude greater than ours. Second, in the work reported on both nystatin [6,7] and amphotericin B [8] the nystatin or amphotericin B was only present in the pipette solution and the experiments were done in the cell-attached mode (the pipette formed a seal with the cell membrane without subsequent membrane rupture). Horn [9] has shown that amphotericin B does not diffuse outside the cell-attached patch. Since this constitutes only a small fraction of the cell membrane surface area (for a 20-μm cell and a 1-μm patch pipette the fraction equals 0.0025), any effect on the channels present in the patch would be of little consequence in their whole-cell recordings.

One can speculate that the resulting increase in opening probability of the K-channels is the result of a direct interaction between the amphotericin B molecule(s) and the K-channel membrane spanning protein, producing a conformational change in the channel protein which could increase the opening probability. Alternatively, it is known that amphotericin B is incorporated into the cell's lipid bilayer and

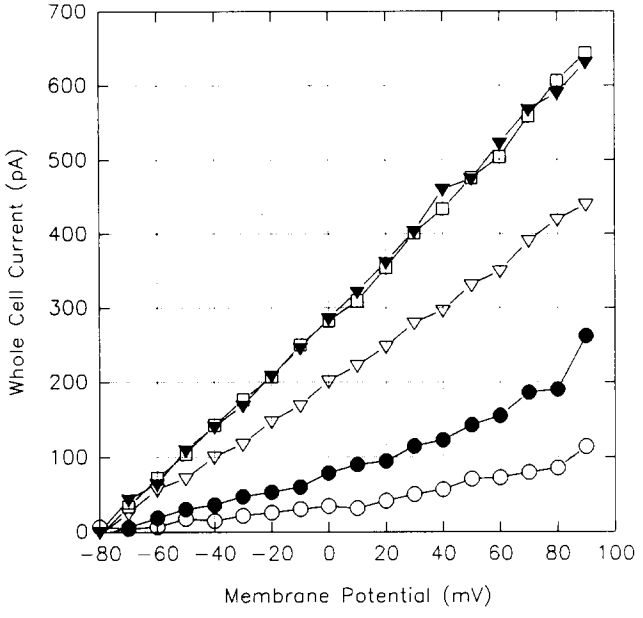

Fig. 3. Whole-cell current before and after amphotericin B treatment, control at $t = 0$ min (○), amphotericin B added at $t = 1$ min, $t = 2.5$ min (●), $t = 12$ min (▽), $t = 21$ min (▼) and $t = 30$ min (□). If no amphotericin B was added, the whole-cell current, as a function of membrane potential, remained like the control data obtained at $t = 0$ min. The bathing solution used in the whole-cell patch-clamp experiments contained (mM): NaOH, 146; KOH, 5; methanesulfonic acid, 154; $Ca(OH)_2$, 2; $Mg(OH)_2$, 1; glucose, 10 and Hepes, 10. The pipette solution contained (mM): NaOH, 10; KOH, 141; $Ca(OH)_2$, 1.54; $Mg(OH)_2$, 1; EGTA, 2.3; glucose, 10 and Hepes, 10. Same method of pH adjustment, cell type, amphotericin B concentration and techniques reference as described in Fig. 1.

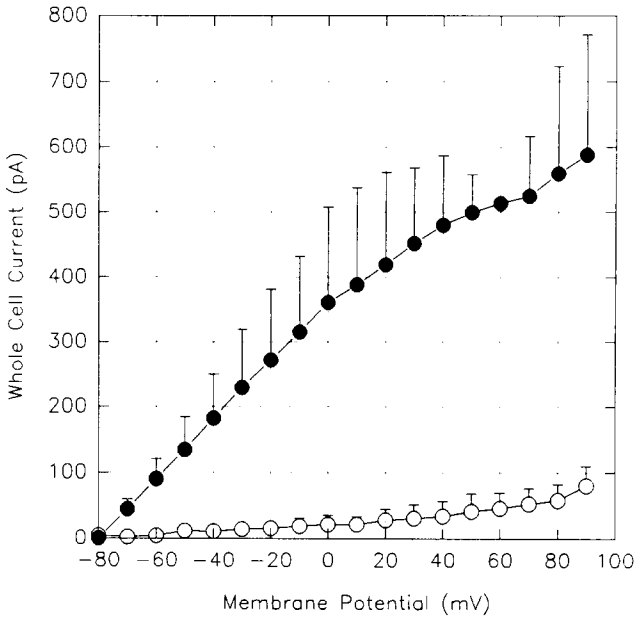

Fig. 4. Whole-cell current before and after amphotericin B treatment, control at $t = 0$ min (○) and $t = 30$ min (●) after the addition of amphotericin B. By using Student's t-test, all control vs. amphotericin B data points, except at -80 mV, were shown to be statistically different ($P < 0.02$). The mean whole-cell current is given along with the associated standard deviations. If no amphotericin B was added, the whole-cell current, as a function of membrane potential, remained like the control data obtained at $t = 0$ min. The bathing solution, pipette solutions and amphotericin B concentrations are the same as those given in Fig. 3.

preferentially associates with sterols like cholesterol [1]. This could modify the lipid environment around the K-channel proteins, resulting in an alteration in the channel protein's conformation, which could produce an increase in opening probability.

These results may have implications in the explanation of amphotericin B's nephrotoxicity [10,11].

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