Inactivation of Monazomycin-Induced Voltage-Dependent Conductance in Thin Lipid Membranes

II. *Inactivation Produced by Monazomycin Transport through the Membrane*

ERIC J. HEYER, ROBERT U. MULLER, and ALAN FINKELSTEIN

From the Departments of Physiology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461. Dr. Heyer's present address is New York Hospital, Cornell Medical Center, New York 10021. Dr. Muller's present address is the Department of Physiology, Downstate Medical Center, State University of New York, Brooklyn, New York.

ABSTRACT At sufficiently large conductances, the voltage-dependent conductance induced in thin lipid membranes by monazomycin undergoes inactivation. This is a consequence of depletion of monazomycin from the membrane:solution interface, as monazomycin crosses the membrane to the opposite (*trans*) side from which it was added. The flux of monazomycin is directly proportional to the monazomycin-induced conductance; at a given conductance it is independent of monazomycin concentration. We conclude that when monazomycin channels break up, some or all of the molecules making up a channel are deposited on the *trans* side. We present a model for the monazomycin channel: approximately five molecules, each spanning the membrane with its NH_3^+ on the *trans* side and an uncharged hydrophilic (probably sugar) group anchored to the *cis* side, form an aqueous channel lined by —OH groups. The voltage dependence arises from the flipping by the electrical field of molecules lying parallel to the *cis* surface into the "spanned state;" the subsequent aggregation of these molecules into channels is, to a first approximation, voltage independent. The channel breakup that deposits monomers on the *trans* side involves the collapsing of the channel in such a way that the uncharged hydrophilic groups remain in contact with the water in the channel as they close the channel from behind. We also discuss the possibility that inactivation of sodium channels in nerve involves the movement from one side of the membrane to the other of the molecules (or molecule) forming the channel.

INTRODUCTION

In the preceding paper (Heyer et al., 1976), using long chain quaternary ammonium ions, we demonstrated a mechanism for inactivation of monazomycin-induced conductance in which the *effective voltage* across the membrane decreases during a constant *applied voltage*. In this paper we show that inactivation also results if the monazomycin concentration at the membrane surface

decreases, again in the face of a constant voltage. This is possible because, *mirabile dictu*, the membrane's permeability to monazomycin is directly proportional to the monazomycin-induced conductance.

The magnitude of inactivation produced in this way is considerable, because the steady-state conductance, g_{ss}, depends on a large (approximately fifth) power of the monazomycin concentration ([mon]$_{int}$)[1] at the membrane solution interface:

$$g_{ss} = L[K^+][mon^+]_{int}^s\ e^{nqV/kT}, \tag{1}$$

where, s and n are empirical constants approximately equal to 5, L is a proportionality constant, and q, k, and T have their usual meaning. V is the potential difference applied across the membrane, with the *trans* side (the side *not* containing monazomycin) defined as zero potential.

MATERIALS AND METHODS

The experiments follow the same procedures as described previously (Heyer et al., 1976), except that symmetrical KCl concentrations are 1.0 M. Because inactivation occurs only at relatively high membrane conductances, the access resistance in 0.1 M KCl is a potentially complicating factor; in 1 M KCl it is lowered sufficiently (to approximately 70 Ω) to avoid this problem.

RESULTS

The Phenomenon

The bottom trace in Fig. 1(a) shows the usual response of a monazomycin-treated membrane to a positive voltage step. The approach to steady state is monotonic and S shaped. Traces *b–f* are responses to increasingly greater voltage steps. In each of these, current (conductance) goes through a maximum before settling to a constant value, i.e., the monazomycin-induced conductance inactivates. The larger the voltage, the greater is the extent of inactivation as measured by the ratio of peak to steady-state conductance.

The log g_{ss}–V curve is linear at relatively low conductances ($g_{ss} < 10^{-6}$ mho) and then bends towards the voltage axis at higher conductances (Fig. 2). Over the linear range (where the expression

$$g_{ss} \propto e^{nqV/kT} \tag{2}$$

holds), the kinetic response is always S shaped. As the curve begins to deviate from an extrapolation of the linear portion, we observe the first hints of inactivation. As this deviation increases in magnitude, so does the amount of

---

[1] [mon$^+$]$_{int}$ is the concentration of monazomycin at the *cis* "electroneutral interface" as defined in the previous paper; it is related to [mon$^+$]$_o$, the concentration in solution at the membrane surface, through the relation:

$$[mon^+]_o = [mon^+]_{int}e^{-q\psi_o/kT},$$

where ψ_o is the surface potential. We assume (for the same reasons as discussed in the preceding paper for quaternary ammonium ions) that this equilibrium relation continues to hold even during transport of monazomycin through the membrane.

inactivation seen in current versus time records. (A similar correlation between bending of the $\log g_{ss}$–V curve and inactivation occurs in quaternary ammonium-induced inactivation (Heyer et al., 1976).) In Fig. 3 we see that it takes time to recover from inactivation. The records are responses to the same positive voltage step at various times after returning V to zero. The kinetic response is maximal at times longer than 30 s.

The theory and experiments that follow attempt to explain the processes responsible for inactivation. We show that depletion of monazomycin from the *cis* membrane surface (as a consequence of its crossing the membrane) quantitatively predicts the shape of the $\log g_{ss}$–V curve. From this we then make several
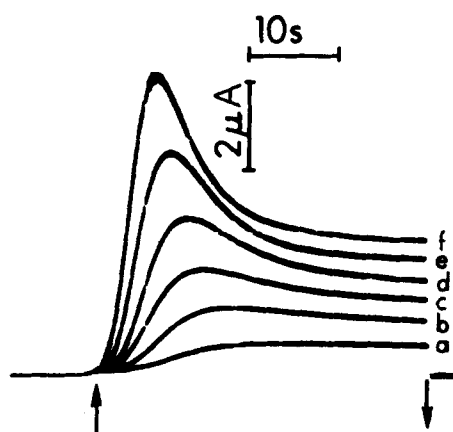

FIGURE 1. Current responses of a monazomycin-treated thin lipid membrane to increasing positive voltage steps. For each trace the voltage was applied at the upward arrow and removed at the downward arrow; at least 2 min intervened between records. The response (trace a) to a potential of $+130$ mV is monotonic. Traces b–f show progressively greater amounts of inactivation. The voltages used for b–f were, in order, 136, 140, 142, 147, and 152 mV. *Cis* monazomycin concentration $= 0.1$ μg/ml; aqueous solutions are unbuffered 1.0 M KCl; membrane area $= 1$ mm^2. The solutions were not stirred while these records were taken.

strong inferences about the nature of the ionic pathway created by monazomycin.

Theory

The steady-state conductance (g_{ss}) of a monazomycin-treated thin lipid membrane is given by Eq. 1. In the absence of a flux (Φ_{mon}^{memb}) of monazomycin through the membrane, or with perfect stirring, the interfacial concentration of monazomycin $([mon]_{int})$ would remain constant at the bulk aqueous concentration $([mon]_{bulk})$. On the other hand, given a sufficiently large flux and an aqueous diffusion barrier (the unstirred layer), $[mon]_{int}$ must decrease.

We define an ideal conductance (g_{ideal}) as the conductance that would be reached at a given voltage if $[mon]_{int}$ always equaled $[mon]_{bulk}$. Then,

$$g_{ideal} = L[K^+][mon^+]_{bulk} e^{nqV/kT}. \tag{3}$$

This is merely the equation of the linear part of Fig. 2 and its extrapolation to higher voltages. Dividing Eq. 1 by Eq. 3 at any given voltage yields:

$$\left(\frac{g_{ss}}{g_{ideal}}\right)^{1/8} = \frac{[mon]_{int}}{[mon]_{bulk}}.$$

(4)

Eq. 4 attributes the deviation of the empirical curve of Fig. 2 from a straight line to a decrease of interfacial monazomycin concentration.
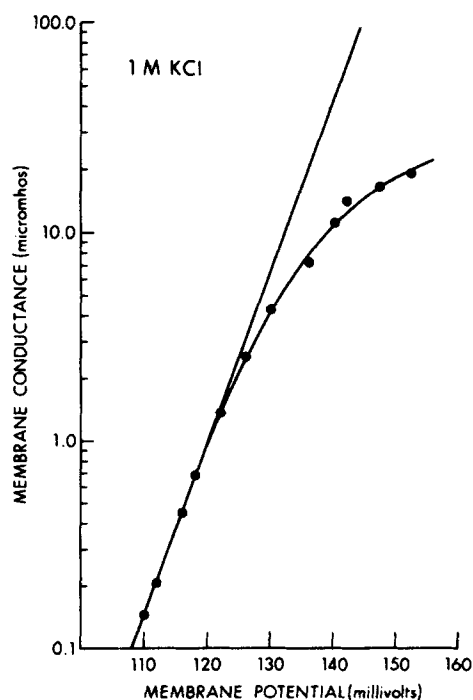

FIGURE 2. Steady-state g-V characteristic showing bending towards the voltage axis at high conductances. (The curve is drawn from Eq. 10 A with $B' = 2.3$ gl^{-1} Ω; the straight line is extended from the low conductance region.) Cis monazomycin concentration = 0.1 μg/ml; aqueous solutions are unbuffered 1.0 M KCl; membrane area = 1 mm^2.

In the steady state, the flux of monazomycin through the membrane must equal the flux through the cis aqueous unstirred layer, Φ_{mon}^{aq}, which is given by Fick's first law:

$$\Phi_{mon}^{memb} \equiv \Phi_{mon} = \Phi_{mon}^{aq} = \frac{D_{mon}A}{\Delta x}\left([mon]_{bulk} - [mon]_{int}\right),$$

(5)

where D_{mon} is the diffusion constant of monazomycin in water, A is the area of the membrane, and Δx is the thickness of the cis unstirred layer. Combining Eq. 4 and Eq. 5 yields:

$$\Phi_{mon} = \frac{D_{mon}A}{\Delta x}[mon]_{bulk}\left[1 - \left(\frac{g_{ss}}{g_{ideal}}\right)^{1/8}\right],$$

(6)

and substituting from Eq. 3 and rearranging gives:

$$g_{ss} = L[K]\left([mon]_{bulk} - \frac{\Phi_{mon}\Delta x}{D_{mon}A}\right)^s e^{nqV/kT}. \tag{7}$$

Eq. 7 reduces to Eq. 3 as the monazomycin flux approaches zero.

Upon substituting experimentally determined values of g_{ss}/g_{ideal} into Eq. 6, we find that Φ_{mon} is a linear function of g_{ss} (Fig. 4), i.e.,
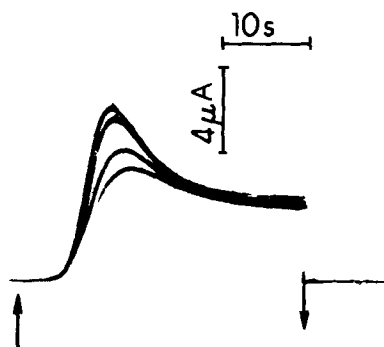
$$\Phi_{mon} = Bg_{ss}. \tag{8}$$

FIGURE 3. Recovery from inactivation. Before these records were taken, a stimulus of +142 mV (the same used for each trace in the figure) yielded a response essentially the same as the two largest. After the steady state was reached, the membrane potential was returned to 0 for 5 s and then reapplied; the bottommost trace resulted. When the conductance again reached the (same) steady state, the potential was returned to 0, this time for 15 s, and then reapplied; the second lowest trace resulted. The same procedure was followed for the two largest traces with the potential held at 0 for 30 and 60 s, respectively. (Actually, the largest response followed the 30-s hold. The difference in the two responses is not significant, and represents the normal deviation in the maximum response for a given stimulus.) (The arrows mark the points of application and removal of the stimulus.) *Cis* monazomycin concentration = 0.1 μg/ml; solutions are unbuffered 1.0 M KCl; membrane area = 1 mm².

(Note in Fig. 4 that the plot of Φ_{mon} vs. g_{ss} is the same straight line at four different values of $[mon]_{bulk}$, even though the voltage necessary to obtain a given value of g_{ss} is different for each monazomycin concentration [Fig. 5].) Substituting Eq. 8 into Eq. 7 gives:

$$g_{ss} = L[K]\left([mon]_{bulk} - \frac{Bg_{ss}\Delta x}{D_{mon}A}\right)^s e^{nqV/kT}, \tag{9}$$

as the functional relation between g_{ss} and V. Thus, a flux of monazomycin through the membrane directly proportional to steady-state conductance (Eq. 8) produces just the right amount of depletion to account for the bending of the experimental curve away from the ideal.

We can summarize the quantitative aspects of depletion inactivation in an alternative way. At a given conductance, we have upon dividing Eq. 9 by Eq. 3:

$$e^{-nq\Delta V/kT} = \left(1 - \frac{Bg_{ss}\Delta x}{D_{mon}A[\text{mon}]_{\text{bulk}}}\right)^s,$$ (10)

where ΔV is the voltage displacement of the experimental $\log g_{ss}$-V curve from the ideal. If we assume that $s = n$, this reduces to:

$$e^{-q\Delta V/kT} = 1 - \frac{Bg_{ss}\Delta x}{D_{mon}A[\text{mon}]_{\text{bulk}}} = 1 - \frac{B'g_{ss}}{[\text{mon}]_{\text{bulk}}}.$$ (10 A)

The only unknown quantity in this equation, $B' \equiv B \,\Delta x/D_{mon}A$, is determined from a single point in the region of bending of the empirical $\log g_{ss}$-V curve.
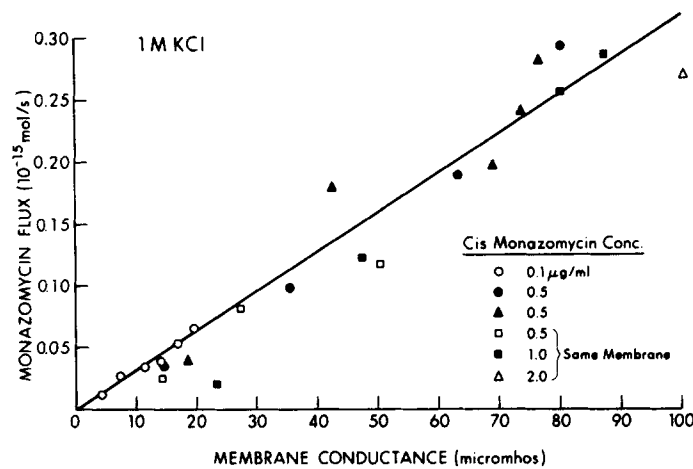

FIGURE 4. The linear relationship between monazomycin flux and steady-state conductance. Experimental values of g_{ss}/g_{ideal} were substituted into Eq. 6 and the resulting values of ϕ_{mon} are plotted against g_{ss}. ($D_{\text{mon}} = 3.5 \times 10^{-6}$ cm²/s [estimation based on the molecular weight of 1,200 for monazomycin]; $A = 10^{-2}$ cm²; $\Delta x = 2 \times 10^{-2}$ cm; $s = 5$.) Note that the data were obtained over a 20-fold concentration range of monazomycin and from several different membranes; nevertheless, the relationship is linear and extrapolates back to zero flux at zero conductance. All points were obtained from 1-mm² membranes in unbuffered 1.0 M KCl.

Thus, the deviation from linearity of the $\log g_{ss}$-V curve is predicted from Eq. 10 A. In Figs. 2, 5, 9, and 10 we see that the theoretical curves fit the data well.

Tests of the Theory

INACTIVATION DIMINISHES AS MONAZOMYCIN CONCENTRATION IS IN-CREASED   If our theory is correct, the deviation (ΔV) of $\log g_{ss}$ from $\log g_{\text{ideal}}$ at any value of g_{ss} must diminish at higher concentrations of monazomycin (see Eq. 10 A). (This is intuitively obvious. Since $[\text{mon}]_{\text{bulk}} - [\text{mon}]_{\text{int}}$ is directly proportional to flux [Eq. 5], the higher $[\text{mon}]_{\text{bulk}}$, the smaller will be the fractional decrease of $[\text{mon}]_{\text{int}}$.) This is confirmed in Figs. 5 and 10. Also, inactivation (as measured by the ratio of peak to steady-state conductance in the current vs. time records) diminishes as $[\text{mon}]_{\text{bulk}}$ is increased.

STIRRING OF THE AQUEOUS SOLUTIONS   As we have said, the very possibility of depletion depends on the existence of an unstirred layer in the *cis* compartment. Since the thickness of this unstirred layer depends on the stirring rate, decreases of stirring rate in the *cis* compartment should reduce [mon]$_{int}$ and hence decrease the steady-state conductance at constant voltage. In Fig. 6, conductance had been in a steady state for many seconds during stirring of the *cis* compartment only. When stirring was stopped, conductance declined in time
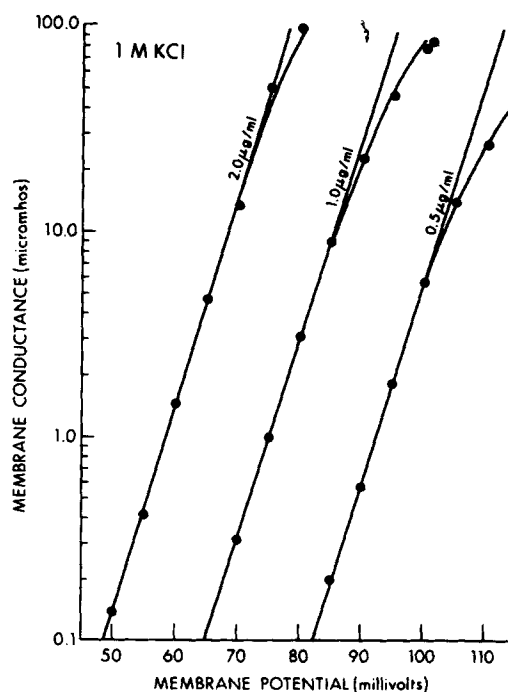

FIGURE 5.   Reduced bending of log g_{ss}-V characteristic (i.e., less inactivation) with increasing monazomycin concentration in the *cis* compartment. The three sets of data points were obtained from the same membrane with three successive additions of monazomycin. (The same value of B' [1.9 gl^{-1} Ω] was used to calculate the three curves from Eq. 10 A. The straight lines are extended from the low conductance regions.) Membrane area = 1 mm^2; solutions are unbuffered 1.0 M KCl.

to a new constant level; when stirring was resumed, it rose back to the initial value. On the other hand, as expected, stirring of the *trans* compartment had no effect on g_{ss}.

APPEARANCE OF MONAZOMYCIN IN THE TRANS COMPARTMENT   We can demonstrate that monazomycin reaches the *trans* compartment in amounts and under circumstances consistent with our theory. A membrane driven to a very high conductance level with a positive voltage shows, subsequently, an anomalous, transient conductance increase when V is switched to a high negative value (Fig. 7). (See also, Muller and Finkelstein, 1972 a.) This "reverse turn-on"

conductance is anomalous in the sense that the negative stimulus alone (i.e., not preceded by a large positive stimulus) produces no change from unmodified-membrane conductance.[2] The peak of the reverse turn-on conductance increases, as the conductance for the conditioning positive stimulus is increased
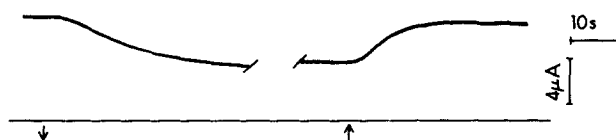
FIGURE 6.   The effect of stirring the *cis* aqueous solution on steady-state conductance. At the beginning of the record the membrane current (conductance) has been in a steady state (in response to a 110-mV applied voltage) with only the *cis* solution stirred. (The thin horizontal line marks the zero-current base line.) At the downward arrow, the stirrer was turned off; we see that the conductance declined to a smaller value. After many seconds at this level, the stirring of the *cis* solution was resumed (at the upward arrow) and the conductance then rose to its original level. (The experiment was repeated on this membrane, this time turning on and off only the *trans* stirrer. The conductance did not change during these manipulations.) *Cis* monazomycin concentration = 1.0 μg/ml; aqueous solutions were 1.0 M KCl + 0.1 mM EDTA; membrane area = 1 mm².
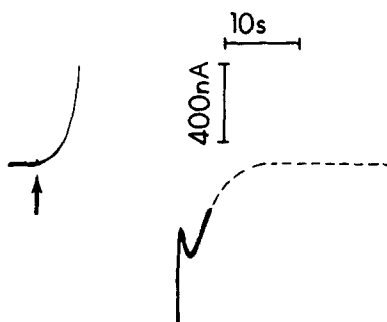
FIGURE 7.   Demonstration of reverse turn-on of conductance. At the arrow a stimulus of +60 mV was applied, resulting in a large off scale positive current. The membrane conductance attained a steady-state value of 107 μΩ⁻¹. About 15 s later (at the end of the break in the record), the membrane potential was suddenly changed to −90 mV. Initially a large negative current (off scale in the record) obtained, which decayed rapidly; before reaching zero, however, the negative current increased and then slowly decayed back toward zero. (In the record, the trace had not yet reached zero; the dashed line has been added to indicate that the current eventually decays back to zero.) The conductance at the peak of this increase in negative current is 5.5 μΩ⁻¹. *Cis* monazomycin concentration = 5 μg/ml; aqueous solutions are unbuffered 1.0 M KCl; membrane area = 1 mm².

(either by a larger stimulus or by the addition of more monazomycin). These findings strongly suggest that monazomycin crosses the membrane and attains a

---

[2] In Fig. 7, the initial very rapidly decaying transient is the fall of conductance from the value attained with the positive stimulus. (The instantaneous conductance immediately after switching voltage polarity is equal to the previous steady-state conductance [Muller and Finkelstein, 1972 a].)

finite concentration at the *trans* interface (because of trapping by the *trans* unstirred layer); once there, it can produce a conductance increase in response to a negative stimulus. This conductance increase is transient, because monazomycin diffuses from the *trans* interface into the zero-concentration bulk aqueous solution.[3]

From this interpretation of reverse turn-on conductance, we obtain a rough estimate of Φ_{mon} as follows. In the steady state, the flux of monazomycin through the *trans* unstirred layer (of thickness Δy) must equal those through the membrane and the *cis* unstirred layer (Fig. 8). Thus, the *trans* interfacial concentration, $[mon]_{trans}$, is predicted from:

$$\Phi_{mon} = \frac{D_{mon}A}{\Delta y} [mon]_{trans},\tag{11}$$

(since $[mon]_{bulk, trans} = 0$). We can now compare this to the *trans* concentration needed to achieve a steady-state conductance equal to that at the peak of the reverse turn-on.

In Fig. 7, g_{ss} at $+60$ mV is 107 $\mu\Omega^{-1}$. From Fig. 4, Φ_{mon} at this conductance is 3.4×10^{-16} mol s^{-1}. Using Eq. 11, the predicted value of $[mon]_{trans}$ is 2×10^{-7} M.[4] On the other hand, the amplitude of the peak reverse turn-on conductance at -90 mV (5.5 $\mu\Omega^{-1}$) implies[5] a value for $[mon]_{trans}$ of 7×10^{-7} M. The agreement is satisfactory, given the assumptions and the complexities of the turn-on kinetics for monazomycin.[6]

EFFECT OF CHANGING CONDUCTANCE PER CHANNEL There is a good deal of evidence that monazomycin promotes univalent cation permeability by forming channels in the membrane (Muller and Finkelstein, 1972 a).[7] The proportionality of monazomycin flux to conductance (Eq. 8), then, is actually a proportionality of flux to the number of channels. Therefore, if we were to reduce the conductance per channel and then adjust V so as to keep total membrane conductance constant, Φ_{mon} should increase. Thus, if the conductance per channel is lower, inactivation should be more pronounced at any given conductance.

A lower conductance per channel is not achieved by reducing the KCl concentration, because $[K^+]_o$ (the potassium concentration in solution at the surface) is essentially invariant to bulk KCl concentration with phosphatidylglycerol membranes (because of their large negative surface charge density [Muller and Finkelstein, 1972 b]). It *is* achieved by substituting for K^+ a less permeant cation such as tetraethylammonium (TEA$^+$). The permeability of TEA$^+$ through mon-

---

[3] That the decay of reverse turn-on conductance is due to diffusion into the *trans* compartment rather than to transport back through the membrane follows from the observations that its time-course is unchanged even if the negative stimulus is interrupted for a time, and that its rate is sensitive to stirring in the *trans* compartment.

[4] Assuming $\Delta y = 200$ μm and $D_{mon} = 3.5 \times 10^{-6}$ cm^2/s.

[5] Based on the conductance of 107 $\mu\Omega^{-1}$ at $+60$ mV with a *cis* monazomycin concentration of 4.2×10^{-6} M, and assuming that s of Eq. 1 is 5.

[6] With monazomycin present on both sides, we have found (unpublished results) that although steady-state responses for positive and negative voltages are independent, the turn-on *kinetics* are accelerated by conditioning pulses of opposite polarity.

[7] Recently, single-channel conductances have been measured (Muller and Andersen, 1975).

azomycin channels is estimated to be about 35-fold less than that of K^+, as judged by the 3.5-fold increase of g_{ss} at constant voltage when 0.01 M KCl is added to symmetrical 0.1 M TEA-Cl solutions.

In Fig. 9, we see that our prediction is qualitatively correct in that bending of the $\log g_{ss}$-V curve (and kinetic inactivation) takes place at much lower conduct-
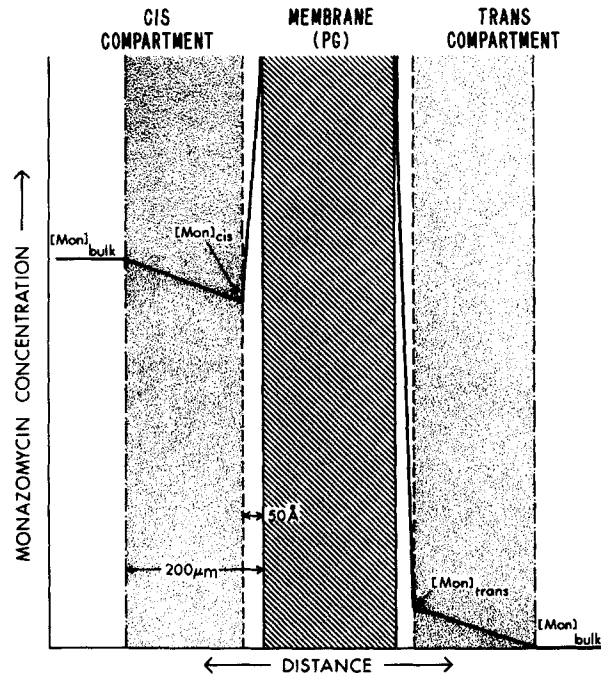

FIGURE 8. Diagram of the steady-state monazomycin concentration profiles in the *cis* and *trans* compartments at some finite monazomycin-induced conductance. The concentration profiles in the two unstirred layers (which extend from the — · lines to the membrane surfaces) are linear up to the electroneutral interfaces (indicated by the dashed lines); this is virtually the entire unstirred layer thickness. The concentrations in the space-charge regions (between the dashed lines and the membrane surfaces) rise to large (off scale) values because of the large negative surface potentials. Note that distances are not to scale; the 50-Å space-charge region is highly exaggerated with respect to the 200-μm unstirred layer thickness.

ances in 1.0 M TEA Cl than in 1.0 M KCl. In fact, good inactivation is seen in 0.1 M TEA Cl at conductances well below those where access resistance becomes a problem (Fig. 10).[8]

There is, however, a quantitative discrepancy. Although our best estimate of the decrease in conductance per channel is a factor of 35, calculated monazomycin fluxes are about 450 times greater than those found in equimolar KCl (compare Fig. 11 with Fig. 4). We cannot account for this 13-fold discrepancy at

[8] All of the KCl experiments were repeated with TEA Cl; the results were qualitatively identical.

present, but we can suggest two possibilities. First, monazomycin channels may have shorter lifetimes in the presence of TEA^+. Were this true, the number of channels breaking up per unit time at a given conductance, and hence Φ_{mon} (see Discussion), would be greater. A second possibility is that TEA^+ might bind (weakly) to the membrane and reduce the negative surface charge density; consequently, $[TEA^+]_o$ could be much lower than $[K^+]_o$ at equal bulk concentrations. Thus, the conductance per channel in 1 M TEA would be lower than that in 1 M KCl by more than our estimated factor of 35.
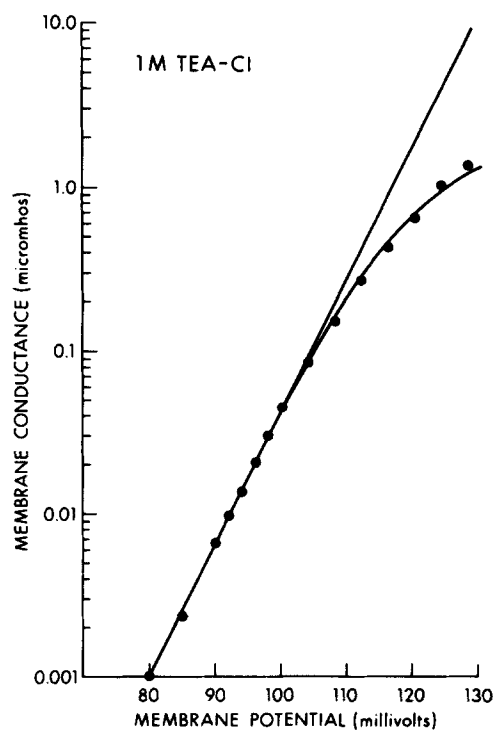

FIGURE 9. Steady-state g-V characteristic for a membrane in 1.0 M TEA-Cl. (The curve is drawn from Eq. 10 A with $B' = 1.078 \times 10^3$ gl^{-1} Ω; the straight line is extended from the low conductance region.) *Cis* monazomycin concentration = 4 $\mu g/ml$; membrane area = 1 mm^2.

DISCUSSION

The Mechanism of Pore Formation

It is very interesting that monazomycin flux varies linearly with conductance. It might be thought that this can be explained by the ability of monazomycin from solution to pass through its own channel. This is most unlikely for two reasons: first, although large lipophilic ions such as some quaternary ammonium cations easily pass through the channel (Heyer et al., 1976), relatively small, not strongly binding ions such as TEA^+ are poorly permeant. Since monazomycin is a large (mol wt $\approx$ 1,200) ion that does *not* bind significantly to the membrane, it should

be effectively excluded from the channel. Second, for a given number of channels, Φ_{mon} should increase linearly with monazomycin concentration. We find, however, that for a given number of channels (i.e., for a given conductance), Φ_{mon} is independent of [mon] (see Fig. 4). It appears, therefore, that the
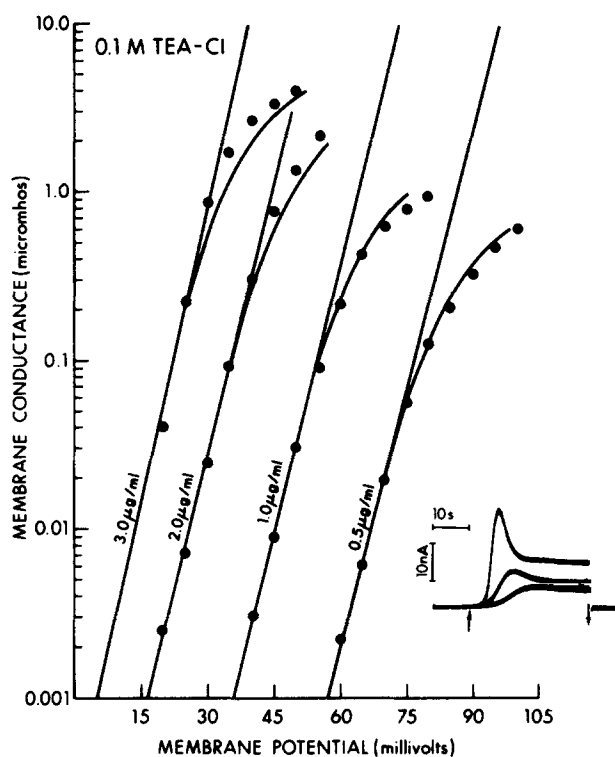

FIGURE 10. Steady-state g-V characteristics in 0.1 M TEA-Cl at several monazomycin concentrations in the *cis* compartment. The four sets of data points were obtained on the same membrane with four successive additions of monazomycin. (The same value of $B'(350 \text{ gl}^{-1} \Omega)$ was used to calculate the four curves from Eq. 10 A; the straight lines are extended from the low conductance regions. [Better fits could have been obtained by using slightly different values of B' for each curve.]) Membrane area = 1 mm². Inset: Voltage clamp responses in 0.1 M TEA-Cl with 2.5 μg/ml monazomycin in the *cis* compartment. At the first arrow successive voltage steps of 68.2, 69.2, and 71.5 mV were applied about 2 min apart. At the second arrow the voltage was returned to zero.

monazomycin crossing the membrane comes from those molecules that are part of the channel.

The following is our picture of the molecular basis for monazomycin action (Fig. 12). We postulate that the monazomycin molecule is long enough to span the bilayer, with its positively charged amino group at one end and an uncharged, but very polar region (perhaps the sugar moiety) at the other. We also imagine that one face of the long part of the molecule contains most of the

hydroxyl groups;[9] the polar faces from about five molecules form the hydrophilic lining of the pore. We think that monazomycin monomers (or small aggregates such as dimers) can achieve a metastable, high energy state (called the spanned state), with the positively charged end of the molecule at the *trans* lipid water interface and the polar end remaining at the *cis* interface.[10] Once in the spanned state, monazomycin can diffuse in the plane of the membrane and either form pores by mass action aggregation or return to its original state on the *cis* surface. (Clearly not much monomeric monazomycin can be directly involved
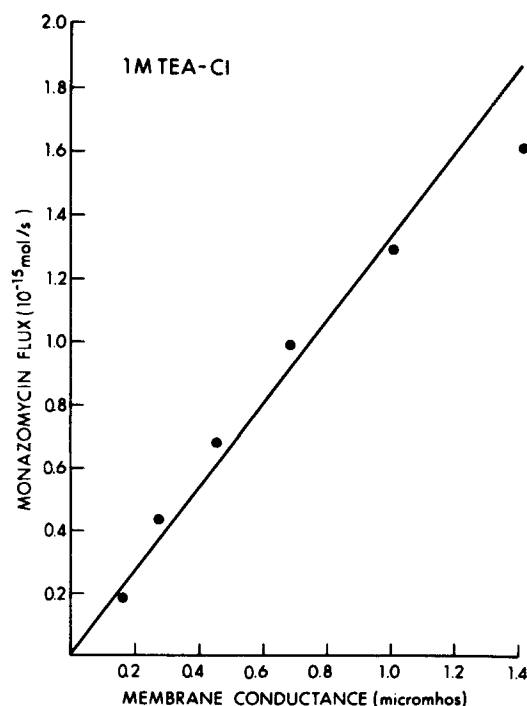

FIGURE 11. The linear relationship between monazomycin flux and steady-state conductance in 1.0 M TEA-Cl solutions. Experimental values of g_{ss}/g_{ideal} were substituted into Eq. 6 and the resulting values of ϕ_{mon} are plotted against g_{ss}. The data are from the experiment of Fig. 9.

in the *trans*-membrane flux, since this would require that flux be linear with aqueous concentration rather than with conductance.) Once formed, the chan-

---

[9] This we assume by analogy to the known structure of amphotericin B (Mechlinski et al., 1970), which has several other marked similarities to monazomycin. They both contain numerous hydroxyl groups, a positively charged amino group (amphotericin B also has a negatively charged carboxyl group which makes it a zwitterion), and a single sugar residue (although in amphotericin B the amino group is attached to the sugar). In addition both are derived from streptomyces cultures.

[10] The opposite orientation appears to be of much higher energy, for if sufficient monazomycin is added to achieve a significant conductance at $V = 0$ and then the same amount is added to the *trans* side, the total conductance only doubles. Thus it appears that pore formation requires all of the monomers to be oriented in the same way, and that primarily only one orientation occurs via mass action. Otherwise, we should observe cooperativity between monazomycin molecules from opposite sides of the membrane.

nel breaks up in such a way that some or all of its components wind up in the *trans* solution. One may suppose that the ability of the uncharged, polar end to get through the membrane once a channel has formed is a result of this part of the molecule traversing the polar interior of the pore as the pore collapses.[11]

In this scheme, voltage-dependent conductance arises because the membrane potential lowers the free energy difference between the aqueous and spanned states. The sign of the potential which increases conductance is indeed the one required to promote movement of the positively charged end of monazomycin
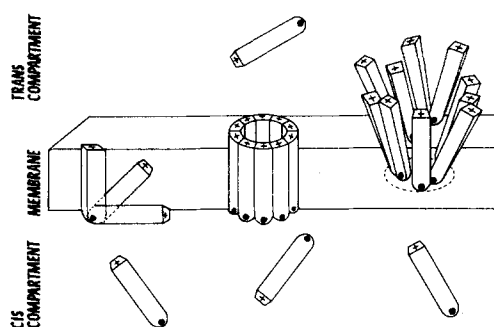

FIGURE 12. A model for the formation and breakup of monazomycin channels. The panel on far left depicts the orientation of a monazomycin molecule into the spanned state by the electric field. In this state the positively charged end sits at the *trans* membrane:water interface, and the uncharged polar end (designated by ●) remains at the *cis* membrane:water interface. In the middle panel several (probably five, but depicted in the figure as 10 for artistic purposes) spanned molecules have aggregated to form an aqueous pore. This pore could break up by disaggregation of the channel back into individual spanned molecules and a return of these molecules to the *cis* solution by reversal of the process shown in the left panel. Alternatively, the pore can break up by the process depicted in the panel on the far right, resulting in the transfer of the monazomycin molecules to the *trans* solution. Note that as the pore collapses, the uncharged polar end remains in contact with the water of the pore. Thus, this end always finds itself in a favorable polar environment; i.e., the collapsing state is energetically feasible, since the polar end does not have to move through a hydrocarbon medium. Note, also, that the opposite process is unlikely, because the positively charged ends would have to be brought into close contact, which is electrostatically unfavorable.

to the *trans* interface. What the potential is thought to do is to increase the concentration of monazomycin in the spanned state; the formation of pores by aggregation of these spanned monomers or dimers is taken, as a first approximation, to be voltage independent. (This mechanism for channel formation is essentially the same as that postulated by Boheim [1974] for alamethicin and by Bauman and Mueller [1974] for alamethicin and monazomycin. Both alamethicin and monazomycin channels are structurally analogous to the nonvoltage-dependent amphotericin B channel [Finkelstein and Holz, 1973]; being a shorter

[11] This description of monazomycin flux across the membrane is incomplete (see Appendix).

molecule, however, amphotericin B spans the membrane more readily as a head-to-head dimer than as a monomer [Marty and Finkelstein, 1975].)

Monazomycin as a Permeant Species

The ability of monazomycin to promote its own translocation raises an interesting permeability mechanism for consideration. Since the number of channels depends on the fifth power of concentration and since flux in turn depends on the number of channels, the permeability coefficient (P_d) of monazomycin is, at fixed voltage, a fifth-power function of its aqueous concentration. The function is so steep that it appears to have a threshold, when plotted on linear coordinates. This constitutes a new permeability process in which individual molecules are impermeant but capable of forming permeant aggregates.

Can "Depletion" be the Mechanism for Inactivation of Na^+ Conductance in Nerve?

It is almost certain that the molecules responsible for voltage-dependent Na^+ conductance in nerve are permanently associated with the plasma membrane (at least on the time scale of action potentials) and hence do not pass from axoplasm to external solution through unstirred layers, etc. In this sense they are obviously different from monazomycin. But the depletion mechanism presented for monazomycin is, in principle, not dependent on movement of monazomycin between aqueous solutions and membrane surfaces. In fact, inactivation would be much more pronounced if no such movement occurred. Thus, if monazomycin were permanently confined to the membrane phase, inactivation would occur as monazomycin was transferred from the *cis* to the *trans* surface, unattenuated by replenishment of the *cis* surface by molecules from solution.[12] Could such a transmembrane transfer by the molecule, or molecules, comprising a sodium channel be the mechanism for its inactivation? We see no evidence, at present, against this.

It might be objected that the absence of reverse turn-on of the sodium conductance upon repolarization from a depolarized, inactivated state argues against this mechanism, but this is not so. In the first place, if the kinetics of channel formation and breakup starting from the *trans* side (rather than the *cis* side) were such that the latter was much faster than the former, no significant conductance would be seen on repolarization. (This difference in the kinetics of the forward formation and reverse formation of channels might arise from asymmetries in membrane composition and structure.) In the second place, if, when the channel breaks up, not all of the subunits end up on the *trans* side, no reverse turn-on would be expected. Regardless of the details, the point is that there are no inconsistencies between the existing data and a mechanism of inactivation that involves the transfer of a subunit, or the entire channel, across the membrane.

It should be noted that this proposed mechanism ties the inactivation process more or less directly to the activation phenomenon, although the formation of a

---

[12] Such a situation may occur with excitability-inducing material (EIM) (Ehrenstein and Lecar, 1975; Lecar et al., 1975). Inactivation in that system is probably closely related to that seen with monazomycin. The major difference is that the subunits of EIM do not break up and pass into bulk solution.

conducting channel need not be a prelude to inactivation in the squid axon. (Thus, if the subunits could cross the membrane as monomers or dimers without first becoming part of a complete channel, inactivation could occur in the absence of a conductance turn-on. In the monazomycin case, however, it appears that the molecules cross the membrane primarily from a completed channel state.) In the Hodgkin and Huxley equations (1952), the inactivation process (h) is independent from the activation process (m). We do not feel, however, that this formalism can be translated literally into a physical model. Alternative mathematical formalisms, and hence different physical models, can equally well fit the empirical data (e.g. see Hoyt, 1963); in fact there is now good evidence that the inactivation process *is* coupled to the activation process (Goldman, 1975). Data on the effect of inactivation on sodium "gating current," which are beginning to appear (Armstrong and Bezanilla, 1974; Keynes and Rojas, 1974), should help clarify whether the mechanism we are suggesting is reasonable.

APPENDIX

The description of monazomycin flux across the membrane, as presented in the text and in Fig. 12, is incomplete. Imagine a membrane separating asymmetrical KCl solutions, $[K^+]_{trans} > [K^+]_{cis}$, with monazomycin initially on the *cis* side. Assume, also, that the monazomycin-induced permeability is ideally cation selective. Monazomycin channels will form in the face of the resulting diffusion potential and break up, depositing monazomycin on the *trans* side. (For every mon^+ that moves from the *cis* to the *trans* side, a compensating K^+ moves in the opposite direction.) Monazomycin will continue to move from the *cis* to the *trans* side until its concentration satisfies the Boltzmann distribution:

$$[mon^+]_{trans} = [mon^+]_{cis}e^{qV/kT}. \tag{1 a}$$

At this point thermodynamic equilibrium is reached and there is no further net flux of mon^+ (or K^+).

Let us now see how our model of monazomycin transport describes the equilibrium state. At equilibrium, the flux of monazomycin from the *cis* to the *trans* side:

$$C[mon^+]_{cis}^s e^{nqV/kT}, \tag{2 a}$$

must equal the flux from the *trans* to the *cis* side:

$$C[mon^+]_{trans}^s e^{-nqV/kT}, \tag{3 a}$$

where C is a proportionality constant; that is,

$$[mon^+]_{cis}^s e^{nqV/kT} = [mon^+]_{trans}^s e^{-nqV/kT}, \tag{4 a}$$

or,

$$[mon^+]_{trans} = [mon^+]_{cis}e^{2nqV/skT}. \tag{5 a}$$

Eq. 5 a is the equilibrium condition derived from our kinetic model. Since Eq. 1 a is demanded by thermodynamics, we are forced to conclude that:

$$s = 2n. \tag{6 a}$$

Eq. 6 a is a disaster on three counts. In the first place, it contradicts our experimental finding that $s = n$. In the second place, our model, in which the spanned state is achieved

by moving the monazomycin charge all the way across the membrane (that is, through the entire potential drop, V), demands that $s = n$. (If our model required the monazomycin charge to move only halfway through the membrane [that is, through a potential drop of $V/2$], then we would expect that $s = 2n$.) In the third place, merely the knowledge that monazomycin crosses the membrane cannot require a definite relation between s and n. As we just observed, the relation between s and n will be determined by the fraction of the total transmembrane potential difference operating on the monazomycin charge. (The experimental result that $s = n$, therefore, supports a model in which the monazomycin charge moves completely across the membrane.)

Our model of monazomycin transport is incomplete, because the principle of microscopic reversibility demands that the reverse of the break-up reaction that deposits monazomycin on the *trans* side can also occur. This means that in addition to the mechanism we have described for channel formation, there must also exist a channel-forming mechanism that involves bringing the uncharged polar end of the molecules through the membrane from the *cis* to the *trans* side. Thus, some channels will be formed with an orientation opposite to that shown in Fig. 12. (Since the mechanism for forming these channels is voltage *independent*, it will not contribute to the voltage-*dependent* inactivation described in this paper.) When this channel-forming mechanism is taken into consideration, the analysis of the equilibrium state leads to $s = n$ for our model and always to the Boltzmann distribution regardless of the model.

REFERENCES

ARMSTRONG, C. M., and F. BEZANILLA. 1974. Charge movement associated with the opening and closing of the activation gates of the Na channels. *J. Gen. Physiol.* **63**:533.

BAUMANN, G., and P. MUELLER. 1974. A molecular model of membrane excitability. *J. Supramol. Struct.* **2**:538.

BOHEIM, G. 1974. Statistical analysis of alamethicin channels in black lipid membranes. *J. Membr. Biol.* **19**:277.

EHRENSTEIN, G., and H. LECAR. 1975. The double negative resistance of EIM and its relation to inactivation in excitable membranes. *Biophys. J.* **15**:167a.

FINKELSTEIN, A., and R. HOLZ. 1973. Aqueous pores created in thin lipid membranes by the polyene antibiotics nystatin and amphotericin B. *In* Membranes vol. 2. Lipid Bilayers and Antibiotics. G. Eisenman, editor. Marcel Dekker Inc. New York. 377.

GOLDMAN, L. 1975. Quantitative description of the sodium conductance of the giant axon of *Myxicola* in terms of generalized second-order variable. *Biophys. J.* **15**:119.

HEYER, E. J., R. U. MULLER, and A. FINKELSTEIN. 1976. Inactivation of monazomycin-induced voltage-dependent conductance in thin lipid membranes. I. Inactivation produced by long-chain quaternary ammonium ions. *J. Gen. Physiol.* **67**:703-729.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* **117**:500.

HOYT, R. C. 1963. The squid giant axon. Mathematical models. *Biophys. J.* **3**:399.

KEYNES, R. D., and E. ROJAS. 1974. Kinetics and steady-state properties of the charged

system controlling sodium conductance in the squid giant axon. *J. Physiol. (Lond.).* **239**:393.

LECAR, H., G. EHRENSTEIN, and R. LATORRE. 1975. Mechanism for channel gating in excitable bilayers. *Ann. N.Y. Acad. Sci.* **264**:304.

MARTY, A., and A. FINKELSTEIN. 1975. Pores formed in lipid bilayer membranes by nystatin. Differences in its one-sided and two-sided action. *J. Gen. Physiol.* **65**:515.

MECHLINSKI, W., C. P. SCHAFFNER, P. GANIS, and G. AVITABILE. 1970. Structure and absolute configuration of the polyene macrolide antibiotic amphotericin B. *Tetrahedron Lett.* **44**:3873.

MULLER, R., and O. S. ANDERSEN. 1975. Single monazomycin channels. Fifth International Biophysics Congress. Copenhagen. 111. (Abstr.).

MULLER, R. U., and A. FINKELSTEIN. 1972 a. Voltage-dependent conductance induced in thin lipid membranes by monazomycin. *J. Gen. Physiol.* **60**:263.

MULLER, R. U., and A. FINKELSTEIN. 1972 b. The effect of surface charge on the voltage-dependent conductance induced in thin lipid membranes by monazomycin. *J. Gen. Physiol.* **60**:285.