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

I. Inactivation Produced by Long Chain Quaternary Ammonium Ions

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ABSTRACT The voltage-dependent conductance induced in thin lipid membranes by monazomycin undergoes inactivation upon the introduction of quaternary ammonium ions (QA) having a long alkyl chain (e.g. dodecyltrimethylammonium [C12]) to the side containing monazomycin. That is, in response to a step of voltage the conductance rises to a peak and then falls to a much lower steady-state value. We demonstrate that the basis of this phenomenon is the ability of QA to pass through the stimulated membrane and bind to the opposite surface. As a consequence, the surface potential on that side becomes more positive, thus reducing the voltage across the membrane proper and turning off the monazomycin-induced conductance. Because the flux of QA through the membrane increases linearly with conductance, we believe that these ions pass through the monazomycin channels. QA permeability increases with alkyl chain length; remarkably, in spite of its much larger size, C12 is about 150 times more permeant than K+. It appears, therefore, that there is a hydrophobic region of the channel that favors the alkyl chain; we propose that this region is formed by the hydrophobic faces of the monazomycin molecules and the phospholipid tails. We compare QA inactivation of monazomycin channels in lipid bilayers to QA inactivation of potassium channels in the squid giant axon, and suggest that there may be a common structural feature for the two channels. It is possible that some of the inactivation phenomena in excitable cells may arise from local field changes not measurable by the recording electrodes.

INTRODUCTION

When present in micromolar amounts on one side of a lipid bilayer membrane, monazomycin (a positively charged, polyene-like antibiotic of molecular weight ~ 1,200) induces voltage-dependent conductance phenomena similar to those seen in excitable cells (Muller and Finkelstein, 1972 a). In particular, the S-
shaped rise of conductance (current) to a steady-state value in response to a positive voltage step is reminiscent of the behavior of the potassium conductance elements in the squid giant axon and the frog node of Ranvier (Hodgkin and Huxley, 1952a; Frankenhaeuser, 1962). Here and in the following paper we describe two ways of converting this monotonic response into one that resembles the sodium conductance element in nerve in that the conductance rises to a peak and then falls to a lower steady-state value; that is, there is “inactivation” of the monazomycin-induced conductance. These mechanisms of achieving inactivation are interesting because of the information they give about the structure of monazomycin channels, and because of their possible relevance to inactivation in excitable cells.

The steady-state conductance \( g_{ss} \) of a monazomycin-treated thin lipid membrane is determined by three variables: the concentration of permeant ion (e.g., \( K^+ \)) and of monazomycin (\( \text{mon}^+ \)) at the membrane surfaces (subscripted as “o”), and the potential difference across the membrane proper \( (V_m) \).

\[
g_{ss} = L [K^+]_o[\text{mon}^+]_o e^{nqV_m/kT}, \tag{1}
\]

where, \( L \) is a constant of proportionality, \( s \) and \( n \) are empirically determined constants \( (s = n = 5) \), \( k \) is Boltzmann’s constant, \( T \) is the Kelvin temperature, and \( q \) is the electronic charge \( (Muller \text{ and Finkelstein, 1972}) \). \( (kT/q = 25.6 \text{ mV at } 300^\circ \text{ K}) \). \( V_m \) is the sum of the potential difference \( (V) \) across the membrane as ordinarily measured with electrodes placed in the bathing solutions, and of the difference in the two surface potentials \( (\psi_{oc} \text{ and } \psi_{ot}) \) associated with any fixed surface charge that the membrane might have (Fig. 1).

\[
V_m = (V_c - V_t) + (\psi_{oc} - \psi_{ot}) = V + (\psi_{oc} - \psi_{ot}). \tag{2}
\]

The subscripts \( c \) and \( t \) refer to the two sides of the membrane (cis and trans), side \( c \) being the solution into which monazomycin is introduced. Note that the conductance increases when side \( c \) is made more positive; such a change in \( V_m \) is in the direction to “drive” more of the positively charged monazomycin into the membrane, with the consequence that more ion-conducting channels form. \( V \), the potential difference between sides \( c \) and \( t \) at distances greater than a few tens of angstroms from either side of the membrane, is the quantity actually controlled by “voltage clamping.” In this paper we show that inactivation of monazomycin-induced conductance occurs if \( \psi_{ot} \) becomes more positive while \( V \) is maintained constant. In this way \( V_m \) is reduced, with the expected effect on \( g_{ss} \).

MATERIALS AND METHODS

Membranes were formed at room temperature by the brush technique of Mueller et al. (1963) across a 1-mm² hole in a Teflon partition separating two Lucite compartments containing symmetrical (usually 0.1 M) unbuffered KCl solutions (pH 5.0-6.8); often 0.1 mM EDTA was present in both solutions. All membranes were formed from an n-decane solution containing 0.5% bacterial phosphatidylglycerol (PG) plus 0.5% cholesterol. After the membranes were completely black, monazomycin (from a stock aqueous solution of 30-2,000 \( \mu g/ml \)) was added to the cis compartment to a concentration of 0.1-10 \( \mu g/ml \), and records were first taken about 15 min later. Additional components (such as various quaternary ammonium compounds) were added to either or both compartments during
the course of the experiments from small volumes of concentrated aqueous solutions. If the membrane broke, we often formed another in the presence of the components already added to the solutions. Generally, both compartments were continuously stirred with magnetic fleas.

Monazomycin was a generous gift from Dr. H. Yonehara. PG was purchased from Supelco, Inc. (Bellefonte, Pa.) and was reported to be 98% pure; it was washed with 0.01 M H$_2$SO$_4$ to remove any multivalent cations, and then extracted into ether. Cholesterol, purchased from Eastman Kodak (Rochester, N. Y.) was recrystallized twice from ethanol; n-decane (99.9%) was from Chemical Samples Co. (Columbus, Ohio).

The long chain aliphatic quaternary ammonium ions (QA) used in our experiments can be represented by the formula:

$$R\overset{\text{CH}_3}{\underset{\text{CH}_2}{-(\text{CH}_2)\text{n}-\text{N}^+-R}}$$

where in a given molecule R is either CH$_3$ or C$_2$H$_5$, and n is either 8, 9, or 11. Following Armstrong's (1971) notation, we shall call the nonyl, decyl, and dodecyl ions C$_9$, C$_{10}$, and C$_{12}$, respectively, followed by either methyl or ethyl in parenthesis. Thus, dodecyltriethylammonium [CH$_3$(CH$_2$)$_{11}$N(C$_2$H$_5$)$_3$]$^+$ is designated C$_{12}$(ethyl). With the exception of C$_{12}$(methyl), which came as the chloride salt, all of the others were the bromide salt. The trimethyl compounds were purchased from Eastman Kodak, and the triethyl compounds came from Eastman Kodak via the generosity of Dr. Clay Armstrong. C$_{12}$(methyl) with one of the methyl groups tritium labeled was synthesized for us by New England Nuclear (Boston, Mass.).

All experiments were done under voltage-clamp conditions with two pairs of electrodes. One pair measured the potential difference, $V$, across the membrane and the other pair passed whatever current, $I$, was necessary to keep $V$ at the command value. The current response was displayed on an oscilloscope face. Ag/AgCl electrodes were used as the current passing electrodes, and either Ag/AgCl or calomel electrodes coupled to the solutions through saturated KCl junctions were the voltage-sensing electrodes. ($V$ is the potential of the cis compartment [which contains monazomycin] with respect to the trans compartment, whose potential is defined as zero; positive current therefore flows from cis to trans.)
The basic quantity of interest is the membrane chord conductance, \( g \). Since there is no diffusion EMF across the membrane (because the concentrations of permeant ions in the two compartments are nearly identical in all experiments), \( g \) is defined by

\[
g = \frac{I}{V}. \tag{3}^3\]

Thus following a step to constant voltage, current is directly proportional to conductance.

**RESULTS (GENERAL DESCRIPTION)**

With only monazomycin added to the cis compartment, conductance (current) rises in an S-shaped manner to a steady-state value in response to a step change of potential from zero to some positive value (Fig. 2 a inset). The steady-state conductance, \( g_{ss} \), is a steep exponential function of the voltage of the form (Muller and Finkelstein, 1972 a):

\[
g_{ss} \propto e^{nqV/kT}, \tag{1 A}\]

where \( n \) is approximately equal to 5. Thus, \( g_{ss} \) increases \( e \)-fold for a 4-6-mV increase in potential. A semilogarithmic plot of \( g_{ss} \) versus \( V \) yields a straight line (Fig. 2 a).

When micromolar amounts of either \( C_{12} \) (methyl) or \( C_{12} \) (ethyl) are also added to the cis compartment, a step change of potential from zero to some positive value causes the conductance to increase to a peak and then to decline to a much lower steady-state value (Fig. 2 b inset); in other words, the monazomycin-induced conductance inactivates. A semilogarithmic plot of \( g_{ss} \) versus \( V \) now yields a curve that is concave downward, although at small values of \( g_{ss} \), it approaches a straight line with the same slope as that obtained with monazomycin alone (Fig. 2 b). The magnitude of inactivation, as measured by the ratio of the peak conductance to the steady-state conductance, is greater where the slope of the log \( g_{ss} - V \) curve is smaller; where the log \( g_{ss} - V \) curve is linear, the kinetic response is indistinguishable from that obtained with monazomycin alone. (Inactivation is also seen in the presence of a positive EMF, even for \( V < EMF \). In this case the direction of current flow is from trans to cis [Fig. 2 c and d].)

Increasing amounts of QA result in greater bending (at a given conductance) in the log \( g_{ss} - V \) curve from the limiting straight line (Fig. 2 b). All of the above phenomena seen with \( C_{12} \) are also seen with \( C_{10} \) and \( C_9 \). The concentrations required to produce a certain amount of inactivation at a given \( g_{ss} \) are in the order \( C_{12} < C_{10} < C_9 \) (Fig. 3 a and b). There is no significant difference in effectiveness between the trimethyl and triethyl forms of these compounds.

\(^1\) An exception are the experiments shown in Fig. 2 c and d in which there is a difference in KCl concentration in the two compartments. In those experiments there is a diffusion EMF, and we have instead of Eq. 3:

\[
g = \frac{I}{V - EMF} \tag{3 A}\]

\(^2\) \( I \) is the noncapacitance current flowing through the membrane. The surge of capacitance current that flows when the membrane potential is suddenly changed occurs too rapidly to be seen in any of the figures; all discussions of voltage clamp records in this paper refer to events after the capacitance surge.
THEORY

Before proceeding further, we shall present our theory for the mechanism of QA-induced inactivation.

Effect of QA in the trans Compartment

To understand the action of these quaternary ammonium ions when added to the cis compartment, it is first necessary to understand their effect when added to the trans compartment. Addition of micromolar amounts of QA to the trans compartment produces a parallel shift of the log $g_{ss} - V$ curve to the right along the voltage axis (Fig. 4 a); no bending of the log $g_{ss} - V$ curve occurs nor is any inactivation seen in the kinetic response. We interpret this behavior as follows: because of the negative surface charge density, $\sigma$, on each face of the membrane (due to the negative charge of PG), there exists a negative surface potential, $\psi_s$, at each interface (Fig. 5 a). QA binds reversibly to the trans surface. Because QA is positive, the trans surface charge density, $\sigma_t$, is reduced (i.e., $\sigma_t$ becomes less negative). Hence, $\psi_{st}$ is also reduced. Consequently, a negative potential difference (not measurable by the recording electrodes) exists across the membrane proper and adds algebraically to any macroscopically applied positive potential (Fig. 5 b). Since monazomycin “sees” the potential difference across the membrane proper ($V_m$), the log $g_{ss} - V$ curve is shifted to the right along the voltage axis by an amount equal to the decrease of the trans surface potential. Fig. 4 b plots the shift in surface potential as a function of C12 concentration. Similar results, again requiring higher concentrations, are obtained for C10 and C8 (see Table I, column 2).

The shift in surface potential ($\Delta \psi_{st}$) as a function of quaternary ammonium concentration in the trans compartment ([QA]t) is given by:

$$e^{\Delta \psi_{st}/RT} = \frac{2\beta \sigma_t [QA]_t + [K^+] - (4\beta \sigma_t [QA]_t [K^+] + [K^+])^{1/2}}{2(\beta \sigma_t [QA]_t [K^+]^{1/2})}$$

where $\beta$ is the binding constant of QA to the membrane, $\sigma_t$ is the surface charge density in the absence of QA, $[K^+]$ is the potassium concentration, and $S$ is $2\pi/eRT$. (See Appendix I for derivation).

Effect of QA in the cis Compartment

QA, when added to the cis compartment, binds to the cis surface. The subsequent change in $\psi_{oc}$ has little effect on the log $g_{ss} - V$ characteristic for the same reasons that Mg$^{++}$ or Ca$^{++}$ added to the cis compartment have little effect. Namely, the effects of reducing $[\text{mon}^+]_o$ and increasing $V_m$, the two consequences of the change in $\psi_{oc}$, cancel each other. (See Muller and Finkelstein, 1972 b, pp. 296-298.) As we shall show, however, QA has the additional property of being able to cross the membrane to the trans compartment while the monazo-

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3 Mg$^{++}$ or Ca$^{++}$ added to the trans compartment also shift the log $g_{ss} - V$ curve to the right. However, they reduce $\psi_{st}$ not (primarily) by binding to the membrane, but rather by their screening action in the diffuse double layer (Muller and Finkelstein, 1972 b). The effective concentrations of these univalent quaternary ammonium compounds are so low as to preclude screening as the way they reduce $\psi_{st}$.
FIGURE 2. (a) Steady-state g-V characteristic with monazomycin (0.15 μg/ml) in the cis compartment; membrane area = 1 mm². Inset: Voltage clamp responses with monazomycin (2.5 μg/ml) in the cis compartment. At the vertical blip successive voltage steps of 55, 57.2, and 59.4 mV were applied about 1 min apart. Aqueous solutions are unbuffered 0.1 M KCl. (The trivial decrease in current seen in this figure for the 59.4-mV stimulus and seen in Fig. 2 b [inset] for the response in the absence of C₂ may be hints of the kind of inactivation discussed in the following paper [Heyer et al. 1976].) (b) Steady-state g-V characteristics on a single membrane with both monazomycin (0.5 μg/ml) and different amounts of C₁₂(methyl) in the cis compartment. O, [C₁₂] = 1.67 x 10⁻⁵ M; O, [C₁₂] = 2.5 x 10⁻⁵ M. (The curve fitting the solid dots is drawn from Eq. 8 with b = 5.2 μM/μΩ⁻¹; the curve fitting the open circles is drawn from Eq. 8 with b = 11.4 μM/μΩ⁻¹; the straight line is extended from the low conductance region.) Aqueous solutions are 0.1 M KCl + 0.1 mM EDTA (pH 5.5); membrane area = 1 mm². Inset: Voltage clamp records from the same membrane as in the inset of Fig. 2 a. The monotonic record was obtained about 3 min after the records in Fig. 2 a. C₁₂(ethyl) was then added to the cis compartment to a concentration of 1.67 x 10⁻⁵ M, and the biphasic record was obtained about 5 min later. The vertical blip marks the onset of the voltage
stimulus, which for both traces was 59.4 mV. (c) Voltage clamp responses with monazomycin (8.5 μg/ml) in the cis compartment and a positive diffusion EMF present (created by a KCl gradient). The cis compartment contained 0.03 M KCl and the trans compartment contained 0.1 M KCl. (Both compartments also contained 1 mM CaCl₂.) This resulted in a diffusion EMF of +21 mV. Consequently, there is a negative current at V = 0 (the trace at V = 0 is below the thin line that designates I = 0). Successive traces from bottom to top are responses to voltage steps from V = 0 to V = +16, +20, +21, and +22 mV, respectively. (The “tails” of current occur when the voltage is returned to 0.) Note that the current response is negative for V < EMF, 0 for V = EMF, and positive for V > EMF. Membrane area = 1 mm².

(d) Voltage clamp response with both monazomycin (9.5 μg/ml) and C₁₀₉(methyl) (2 × 10⁻⁸ M) in the cis compartment and a positive diffusion EMF present (created by a KCl gradient). The cis compartment contained 0.02 M KCl and the trans compartment contained 0.14 M KCl. (Both compartments also contained 0.1 mM EDTA.) This resulted in a diffusion EMF of +38.3 mV. Consequently there is a negative current at V = 0. (The trace at V = 0 is below the thin line that designates I = 0.) The trace is the response to a voltage step from V = 0 to V = +36 mV. (The tail of current occurs when the voltage is returned to 0.) Note that the current response is negative (because V < EMF) and displays inactivation. (Contrast this to the records in Fig. 2c where no C₁₀ is present.) Membrane area = 1 mm².

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**Figure 3.**

- (a) Steady-state g-V characteristic with both monazomycin (0.5 μg/ml) and C₁₀(methyl) (1.67 × 10⁻⁴ M) in the cis compartment. (The curve is drawn from Eq. 8 with b = 2.8 μM/μΩ⁻¹; the straight line is extended from the low conductance region.) Aqueous solutions are 0.1 M KCl + 0.1 mM EDTA (pH 5); membrane area = 1 mm².
- (b) Steady-state g-V characteristic with both monazomycin (0.5 μg/ml) and C₉(methyl) (5 × 10⁻⁴ M) in the cis compartment. (The curve is drawn from Eq. 8 with b = 9.2 μM/μΩ⁻¹; the straight line is extended from the low conductance region.) Aqueous solutions are 0.1 M KCl + 0.1 mM EDTA (pH 5); membrane area = 1 mm².

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Figure 4. (a) Steady-state g-V characteristics on a single membrane with monazomycin (1 μg/ml) in the cis compartment and various amounts of C₁₂(methyl) (indicated in the figure) added to the trans compartment. Aqueous solutions are 0.1 M KCl + 0.1 mM EDTA (pH 5); membrane area = 1 mm². (b) Change in surface potential of a PG membrane as a function of the C₁₂(methyl) concentration in bulk solution. The data points are from Fig. 4a; the curve is drawn from Eq. 4 with $\sigma_i = 2 \times 10^{14}$ charges cm⁻² and $\beta = 2.4 \times 10^{17}$ charges cm⁻² M⁻¹. (Actually, there is only one constant $[\beta \sigma_i]$ undetermined in Eq. 4; $\sigma_i$, however, is determined independently from surface potential changes produced by Ca⁺⁺ [Muller and Finkelstein, 1972b].)
mycin-induced conductance is turned on. Once across the membrane it binds to the trans surface, making \( \psi_{st} \) more positive. Consequently, \( V_m \) is reduced, and the monazomycin-induced conductance decreases. Thus, inactivation occurs because the reduction of the potential difference across the membrane proper (which occurs with time) lowers the membrane conductance from the value it would achieve in the absence of QA.  

**Unstirred Layers**

Since these quaternary ammonium ions bind reversibly to the membrane, there must exist a diffusion barrier near the membrane in order for inactivation to occur. Without such a barrier, the C12 which crosses the membrane from the cis compartment would be whisked away from the trans surface of the membrane and therefore could never achieve a high enough concentration to change \( \psi_{st} \).

The existence of the unstirred layer not only allows for inactivation, but also enables us to treat the steady-state aspects quantitatively. QA that crosses the membrane diffuses away from the trans surface through the aqueous unstirred layer into the bulk solution. In the steady state, the flux of QA through the unstirred layer, \( \Phi_{QA}^u \), must equal the flux through the membrane, \( \Phi_{QA}^m \). Thus,

\[
\Phi_{QA}^m = \Phi_{QA}^u = \Phi_{QA}^m = -\frac{D_{QA}A}{\Delta x} (\text{[QA]}_{\text{trans bulk}} - \text{[QA]}_{\text{trans surface}}),
\]

where, \( D_{QA} \) = diffusion constant of QA in aqueous solution; \( A \) = membrane area; \( \Delta x \) = thickness of unstirred layer; \( \text{[QA]}_{\text{trans bulk}} \) = concentration of QA in the trans compartment (far from the membrane); \( \text{[QA]}_{\text{trans surface}} \) = concentration of QA in solution at the trans surface. (Note that this is the concentration at the surface in the aqueous solution and has the dimensions of charge per unit volume. It is not to be confused with the surface concentration, which is the concentration on the membrane and has the dimensions of charge per unit area.) Since

4 When QA is added only to the trans compartment, it will of course also cross the membrane (against the electric field) and bind to the cis surface. The subsequent change in \( \psi_{st} \) has little effect on the response, for the reasons alluded to above. The determination of the equilibrium binding isotherms for QA are not affected by the flux of QA from the trans compartment; i.e., the QA concentration at the trans interface is not significantly reduced. This is evident in Fig. 4 a from the fact that all curves are straight lines.

5 This is a bona fide effect and not the result of a technical failure in voltage clamping, although, in fact, it occurs because the potential difference between the two surfaces is not constant as QA crosses the membrane and binds to the trans surface. It is perhaps possible to artifactually obtain inactivation by driving the membrane to such low resistances that the resistance in series with the membrane (the so-called access resistance of the salt solutions bathing the membrane) becomes a few percent of the membrane resistance. In that case, a fraction of the applied voltage is dropped across this resistance and does not appear across the membrane. In our experiments the access resistance in 0.1 M KCl was about 700 \( \Omega \), whereas inactivation was always achieved at resistances greater than 10^5 \( \Omega \) (with \( C_{st} \) at resistances of 10^6 \( \Omega \) [see Fig. 2 b]). Thus, at most, only a fraction of a millivolt was ever dropped across the access resistance.

6 A diffusion barrier would not have to be postulated were we dealing with compounds that desorb much more slowly from the membrane. However, since recovery from inactivation depends on the diffusion barrier, as will be shown later, the dissociation from the membrane surface is not rate limiting.
[QA]_{trans bulk} \text{ remains essentially zero throughout the experiment, Eq. 5 simplifies to:}

\[ \Phi_{QA} = \frac{D_{QA}A}{\Delta x} [QA]_{trans surface} \]  

(5 A)

For a membrane formed from a lipid having no net charge (e.g. lecithin), [QA]_{trans surface} in Eq. 5 A is simply the concentration of QA in solution at the trans membrane-solution interface.\(^7\) If the lipid is negatively charged (e.g. PG), the resulting negative surface potential introduces a complication. Eq. 5 is valid if QA moves by simple diffusion, in which case the concentration profile of QA in the uninstirred layer is linear. In the region near the membrane (i.e., in the diffuse double layer), however, the gradient of the electrical potential is also a driving force on QA, and consequently the concentration profile of QA there is highly nonlinear. Fortunately, there is a simple way of handling this problem.

\(^7\) Actually, this is true only in the limit of very small concentrations of [QA]_{trans surface}. Adsorption of QA imparts a positive surface charge to the membrane, and therefore the double-layer considerations discussed in the text for PG membranes will apply even to membranes that are uncharged in the absence of QA.
TABLE I

BINDING CONSTANTS AND PERMEABILITY COEFFICIENTS
OF LONG CHAIN QUATERNARY AMMONIUM IONS

<table>
<thead>
<tr>
<th>QA (methyl)</th>
<th>$\beta^a$</th>
<th>$P_{aq}/P_{at}$</th>
<th>$P_{aq}/P_{c}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_6</td>
<td>0.42 x 10^10</td>
<td>9.8</td>
<td>1</td>
</tr>
<tr>
<td>C_10</td>
<td>2.15 x 10^10</td>
<td>24</td>
<td>5.1</td>
</tr>
<tr>
<td>C_12</td>
<td>23.8 x 10^10</td>
<td>146</td>
<td>57</td>
</tr>
</tbody>
</table>

* $\beta$ for C_12 was calculated from the slope of the line in Fig. 14 b; similar plots are obtained for C_6 and C_10.

† Calculated from Eq. 10 with $D = 6 \times 10^{-10}$ cm$^2$ s$^{-1}$ (Blair and Kraus, 1951), $A = 10^{-1}$ cm$^2$, $\Delta x = 2 \times 10^{-2}$ cm, $[K]_{aq} = 10^{-4}$ mol cm$^{-3}$ and $1 - e^{-Vx/\theta_R}$ $\approx$ 19.5 V$^{-1}$.

(We choose this approximation for $(1 - e^{-Vx/\theta_R})/V$, since $V \approx$ 40 mV falls about in the middle of the voltage range over which most of the data were obtained. As noted in footnote 11 Eq. 9, the value of $P_{aq}/P_{at}$ is not very sensitive to voltage in the range that we operated.) $b_{aq}/C_6 = 1.72 \times 10^5$ $\Omega$; $b_{aq}/C_{10} = 4.5 \times 10^4$ $\Omega$; $b_{aq}/C_{12} = 2.56 \times 10^3$ $\Omega$. (Taken from the slopes in Fig. 12.)

Since the electrostatic potential a few Debye lengths from the surface is essentially zero, the QA concentration profile is linear from there throughout the rest of the unstirred layer. We can therefore divide the unstirred layer into two parts: the region within a few Debye lengths of the surface, where the diffuse double-layer potential is significant, and the remaining thickness of the unstirred layer, where the concentration profile of QA is linear (Fig. 6). Since the Debye length in 0.1 M KCl is approximately 10 Å and the unstirred layer thickness is approximately 200 μm, the linear concentration profile comprises almost the entire 200 μm. We define the "electroneutral interface" as the plane parallel to the membrane surface beyond which the diffuse double-layer potential can be ignored and electroneutrality can be assumed (Fig. 6). Thus $[QA]_{trans surface}$ in Eq. 5 is the concentration of QA at the trans electroneutral interface, and Eq. 5 A should be written:

$$\Phi_{QA} = \frac{D_{QA} A}{\Delta x} [QA]_{trans int}$$  (5 B)

**Calculation of $[QA]_{trans int}$ and hence $\Phi_{QA}$**

Consider a point such as A ($g_{st} = 0.1$ μmho) in Fig. 7 on the log $g_{st}$ - V curve. According to our model, the voltage displacement (13.5 mV) of this point from the straight line is the change in $\phi_{st}$ due to binding of C_12 to the trans membrane surface. If we make the assumption that C_12 at the electroneutral interface is in equilibrium with C_12 at the membrane surface, then $[C_{12}]_{trans int}$ for point A is equal to the concentration of C_12 that would have had to be added directly to the trans compartment to produce the same voltage shift of the log $g_{st}$ - V characteristic. This concentration (1.6 μM) is obtained directly from the empirical binding curve in Fig. 4 b (or from Eq. 4). (The assumption of equilibrium between the electroneutral interface and the membrane surface simply means that the kinetics of transport through the narrow space charge region [thickness <50 Å] is very rapid compared to the rate of diffusion through the rest of the unstirred layer [thickness ~200 μm].) From Eq. 5 B (with $D_{C_{12}} = 6 \times 10^{-10}$ cm$^2$/s) we then...
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FIGURE 6. Diagram of the steady-state QA concentration profiles at some finite monazomycin-induced conductance. The concentration profiles in the two unstirred layers (which extend from the \( - \cdot \) 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.

know \( \Phi_{\text{cis}} \) (in this case \( 4.8 \times 10^{-15} \) mol/s). Thus, from the displacement of \( \log g_{ss} \) from the straight line and from the binding data obtained by adding \( C_{12} \) directly to the trans compartment (Fig. 4 b), \( [C_{12}]_{\text{trans int}} \) can be calculated for any point on the \( \log g_{ss} - V \) curve. Hence the flux of \( C_{12} \) through the membrane, \( \Phi_{\text{cis}} \), can be found using Eq. 5 B for any point on the \( \log g_{ss} - V \) curve. Identical calculations are performed for \( C_{10} \) and \( C_9 \).

RESULTS (DETAILS)

Demonstration that Inactivation Depends on QA Crossing the Membrane

EFFECT ON INACTIVATION OF ADDING \( C_{12} \) TO TRANS SIDE If the mechanism of inactivation depends on \( C_{12} \) binding to the trans surface after crossing the membrane, addition of \( C_{12} \) to the trans compartment should suppress inactivation for at least two reasons. First, with \( C_{12} \) already present on the trans side, the flux of \( C_{12} \) from cis to trans will be reduced, because the concentration gradient
Figure 7. Steady-state $g-V$ characteristic with both monazomycin (3.5 \( \mu \text{g/ml} \)) and $C_{12}$ (methyl) \( (3.33 \times 10^{-5} \text{ M}) \) in the \( \text{cis} \) compartment. (The curve is drawn from Eq. 8 with $b = 12.4 \ \mu \text{M/\muΩ}^{-1}$; the straight line is extended from the low conductance region.) Aqueous solutions are unbuffered 0.1 M KCl; membrane area = 1 mm$^2$.

has been decreased. Second, even if the flux of $C_{12}$ remained the same, we see from the binding curve of Fig. 4 $b$ that equal increments of $C_{12}$ concentration produce smaller effects on $\psi_{in}$ as the concentration of $C_{12}$ increases. Experimentally we find that inactivation (as measured by the ratio of peak to steady-state conductance) is decreased or eliminated as $C_{12}$ is added to the \( \text{trans} \) side. Concomitant with this is a decrease or disappearance of bending in the $\log g_{in}-V$ plot (Fig. 8).

Recovery from inactivation and the effect of stirring. According to our model, recovery from inactivation occurs as QA diffuses away from the \( \text{trans} \) interface, through the unstirred layer, and into the bulk solution. Thus, the time required for recovery from inactivation should be a function of the thickness of the \( \text{trans} \) unstirred layer and therefore of the rate at which the \( \text{trans} \) solution is stirred. In Fig. 9, the initial stimulus produced a large inactivation response. When the stimulus was removed for 1 s and then applied again, the current approached the same steady-state value monotonically; i.e., without showing any inactivation. When the stimulus was interrupted for about 5 s, the ensuing response showed a small amount of inactivation, but the peak current was much smaller than when the stimulus was first applied. In fact, if the solutions are stirred, it is necessary to wait approximately 2 min after a previous steady state before the peak current reaches the same height as for the first application of the
stimulus. This is consistent with an unstirred layer thickness of about 200 μm. If the solutions are not stirred, recovery time increases to about 5 min, indicating, as expected, an increase in unstirred layer thickness.

A dramatic demonstration of the importance of the trans unstirred layer is the change in recovery time that occurs when the solution in only the trans compartment is stirred. The experiment is as follows (Fig. 10). For a large positive potential step the conductance increases rapidly to a peak and then falls to a steady-state level. If at this time the potential is suddenly decreased to a smaller value, the conductance decreases to a minimum and slowly increases to the new steady-state value. (In the absence of C₁₂, the conductance simply decreases monotonically; this reflects the relaxation of the monazomycin-induced con-

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*This value is about twice that found by Holz and Finkelstein (1970) (assuming equal thicknesses for the cis and trans layers). Because of membrane fragility in the presence of C₁₂, however, stirring rates in our experiments were considerably slower than those employed in their studies.*
Recovery from inactivation takes time. These current vs. time records (lower traces) were obtained on a membrane in unbuffered 0.01 M KCl with 0.5 μg/ml monazomycin and 3.5 × 10⁻⁵ M Cn(ethyl) in the cis compartment. (Upper traces are the applied voltage vs. time.) Upon application of an 80-mV positive voltage step (positive steps are, unfortunately, shown by a downward deflection), a record showing significant inactivation is obtained (trace on far left). If after a steady state is reached the voltage is dropped to 0 and “immediately” reapplied, no inactivation is seen (middle trace). On the other hand, if a 5-s interval is allowed before reapplying the pulse, signs of inactivation are seen (trace on far right). (It requires about 2 min at zero voltage before the original record [the trace on far left] is reproduced.)

Effect of stirring on the time for recovery from inactivation. (a) The conductance has reached a steady-state value in response to a 90-mV stimulus. At this point (the beginning of the record) the potential is suddenly reduced to 50 mV. The current rapidly declines from a high (off-scale) value, goes through a minimum, and then slowly increases to a new steady-state value. As explained in the text, this slow increase is a reflection of recovery from inactivation. The “noisy” record and the smooth record were taken with and without stirring in the cis compartment, respectively. We see that they are virtually superimposable; i.e., recovery from inactivation is not affected by stirring in the cis compartment. Monazomycin concentration in cis compartment = 1.5 μg/ml; Cn(ethyl) concentration in cis compartment = 1.67 × 10⁻⁴ M; aqueous solutions are unbuffered 0.1 M KCl. (b and c) The same conditions as in a except that the Cn(ethyl) concentration is 8.3 × 10⁻⁵ M and the voltage is stepped down from 104 to 66 mV. In record b there is no stirring and in record c there is stirring in the trans compartment. We see that the recovery rate is markedly increased by stirring in the trans compartment.

The conductance from one steady state to another, and the rate of this process is unaffected by stirring.) The time-course of the conductance increase from the minimum reflects the diffusion of Cn away from the trans interface (as the trans concentration of Cn declines to the value appropriate for the lower steady-state conductance associated with the smaller applied potential). We see in Fig. 10 that...
the time-course of the conductance increase is unaffected by stirring in the cis compartment but is markedly decreased with stirring in the trans compartment.

**Reversibility of QA Binding** Implicit in our theory is that QA binds reversibly to the membrane surface. This is confirmed by the observation that when a solution in the trans compartment containing C_{16} (which binds much more strongly than even C_{12}) is exchanged for one containing no C_{16}, membrane conductance (at a given voltage) increases within a minute (the time for the exchange) to the steady-state value appropriate for \([C_{16}]_{\text{trans}} = 0\).

**Quantitative Aspects of Inactivation**

**Flux of QA** In Fig. 11 we have the interesting result that the steady-state flux of QA through the membrane varies linearly with the steady-state conductance:

\[ [QA]_{\text{trans int}} = \frac{\Delta x}{R_{QA}} \phi_{QA} = b_{QA} g_{ss}. \]  

(At very high values of \(g_{ss}\), \(\phi_{C_{16}}\) becomes less than linear. This results from the decrease in the concentration difference of C_{12} across the membrane, because of C_{12} depletion and buildup at the cis and trans electroneutral interfaces, respectively. We do not see this phenomenon with C_{9} and C_{10} at comparable values of \(g_{ss}\), because these compounds are much less permeant and hence their concentration differences across the membrane are not lowered appreciably by their...
flux through the membrane.) In addition, the proportionality constants, \( b \), vary linearly with the QA concentrations in the cis compartment:

\[
b_{QA} \propto [QA]_{cis}.
\]  

(Fig. 12). Thus, QA flux through the membrane depends linearly on both the number of monazomycin channels in the membrane and the amount of QA in the cis compartment.\(^9\)

We can summarize the quantitative aspects of QA-induced inactivation in a different way. Combining Eqs. 4 and 6 we have:

\[
e^\psi_{adot} = \frac{2S\beta\sigmabg_{st} + [K^+] - (4S\beta\sigma bg_{st}[K^+] + [K^+]^{1/2})}{2(S\beta\sigma bg_{st})^2[K^+]^{-1}}
\]  

(8)

The only undetermined constant in this equation is \( b \).\(^{10\text{a}} \) This is found from a single experimental point in the nonlinear region of the log \( g_{st}-V \) curve. (The voltage displacement of any point from the extrapolated linear curve directly measures \( \Delta\psi_{adot} \), and hence \( b \) is calculated from Eq. 8.) Thus the dependence of \( \Delta\psi_{adot} \) on \( g_{st} \) (and hence the deviation from linearity in the log \( g_{st}-V \) curve) is predicted by Eq. 8. In figures 2b, 3, 7, and 8 we see that the theoretical curves fit the data quite well.

\(^9\) Since the concentration of QA at the membrane surface increases less than linearly with the amount in solution (because of the reduction in surface potential as binding occurs), one might expect the flux also to increase less than linearly with the amount in solution. However, because of the reduction in surface potential, the surface concentration of permeant ion (K\(^+\)) and hence the conductance per channel must be reduced; therefore to achieve a given conductance at higher QA concentrations, more channels must exist. This effect alone would cause flux to increase more than linearly with QA concentration. The two effects theoretically will cancel each other, and thus lead to a linear relationship between QA concentration and QA flux.

\(^{10\text{a}}\) \( \beta \) and \( \sigma_1 \) are determined from independent experiments, the former from the voltage shifts of the log \( g_{st}-V \) characteristic produced by additions of QA to the trans compartment, and the latter from the voltage shifts produced by the additions of Mg\(^++\) or Ca\(^++\) to the trans compartment (Muller and Finkelstein, 1972 b). It is not necessary, however, to know their values in order to use Eq. 8, since \( \beta\sigma_1 b \) can be treated as a single, undetermined constant.
PERMEABILITY OF QA IONS THROUGH MONAZOMYCIN-TREATED MEMBRANES

The ratio of the permeability coefficient of QA, \( P_{QA} \), to that of K\(^+\), \( P_K \), is given by (see Appendix II):

\[
P_{QA} = \frac{[K]_{cis} \frac{F \Phi_{QA} (1 - e^{-FV/RT})}{[QA]_{cis} \delta_{ss}}} {V}.
\]

Combining this with Eq. 6 we have:

\[
\frac{P_{QA}}{P_K} = \frac{b_{QA} [K]_{cis} (D_{QA} \Phi_A) (1 - e^{-FV/RT})}{[QA]_{cis} \Delta \sigma} V.
\]

Since \( b_{QA}/[QA]_{cis} \) is a constant (Eq. 7),\(^{13} \) we can immediately calculate \( P_{QA}/P_K \) (Table I, column 3).

The results in Table I, column 3 are striking, for they show that these large quarternary ammonium ions are much more permeant than the potassium ion. In fact, \( P_{QA} \) increases as molecular size increases from C\(_9\) to C\(_{12}\), with C\(_{12}\) about 150 times more permeant than K\(^+\) (C\(_2\), i.e. TEA\(^+\), is only about 1/35th as permeant as K\(^+\) [Heyer et al., 1976]; thus C\(_{12}\) is some 5,000 times more permeant than TEA\(^+\).) This result was so surprising that we felt compelled to verify it directly with a tracer flux experiment. The experiment in Fig. 13 indeed confirms that \( P_{C_{12}} \) is about 150 times larger than \( P_K \).

DISCUSSION

Binding of QA Ions to the Membrane

Before discussing QA-induced inactivation, it is of some interest to consider simply QA binding to lipid bilayers. Operationally, we measure binding by the voltage shift of the log \( g_{ss}-V \) curve upon addition of QA to the trans compartment.\(^{13} \) Assuming that this shift results from a reduction in the trans negative surface charge density, \( \sigma_t \), we can calculate \( \Delta \sigma_t \) (Eq. 5 a, Appendix I) and hence determine the number of QA ions bound per unit area of membrane as a function of bulk QA concentration (Fig. 14 a). Finally, from the Boltzmann distribution (Eq. 4 a, Appendix I) we can calculate the concentration of QA in solution at the membrane surface, [QA]\(_{tot}\), and hence plot the amount of QA bound as a function of [QA]\(_{tot}\) (Fig. 14 b). The last gives the particularly pleasing and simple result that the amount of QA bound varies linearly with the QA concentration in solution at the surface; hence a true binding constant (\( \beta \)) for QA

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\(^{11} \) Eq. 9 is relatively insensitive to V. From 0 to 100 mV, a greater voltage range than we used, \((1 - e^{-FV/RT})/V \) changes by only a factor of 4 (from \( F/RT \) to \( F/4RT \)). Most of our data were obtained with \( V \approx 40 \) mV, thus making \((1 - e^{-FV/RT})/V \approx F/2RT \).

\(^{13} \) Actually, \( b_{QA} \) (i.e., \( \Phi_{QA} \)) should have a weak direct voltage dependence of the form \( V/(1 - e^{-FV/RT}) \) as opposed to its strong indirect voltage dependence through \( g_{ss} \) (see Appendix II). Our data are not sufficiently accurate to measure this, as they are dominated by the conductance dependence of \( \Phi_{QA} \).

\(^{13} \) The analysis of the inactivation experiments does not depend on the material in this section or on any assumptions as to how QA acts from the trans side. All that is required is the empirically determined plot of \( \Delta \phi_{tot} \) versus bulk QA concentration (Fig. 4 b).
Direct measurement of Cn permeability by a tracer flux experiment. The membrane (1 mm²) was formed in 0.1 M KCl + 0.1 mM EDTA (pH 5). Monazomycin (2 μg/ml) was added to the cis compartment and a log g_m-V curve was determined to establish that the system was well behaved. 40 μCi of tritiated Cn(methyl) (specific activity = 740 mCi/mm) were added to the cis compartment. (This made the Cn concentration in the cis compartment 1.8 x 10⁻⁵ M.) Two 10-μl samples were taken from the cis compartment for counting and 50-μl samples were periodically taken from the trans compartment for counting and replaced by 50 μl of "cold" solution to prevent bulging of the membrane. Both compartments were continuously stirred throughout the experiment. During the first 21 min the membrane potential was held at 0 mV. At this potential the conductance was less than 2 x 10⁻⁹ Ω⁻¹. We see that no discernible number of counts appeared in the trans compartment during this time. The membrane potential was then clamped at between 39-40.6 mV to maintain a conductance of 10⁻⁷ Ω⁻¹. During this time the counts in the trans compartment rose linearly. Specific activity of the cis compartment = 4.43 x 10⁶ cpm/ml; volume of trans compartment = 3.0 ml. From these data and the slope of the line (0.37 cpm/min/0.05 ml) we obtain from Eq. 9 that $P_{C_{12}}/P_k = 173$.

(true in the sense that the relevant QA concentration, that at the surface, is used in the calculation) can be determined (Table I, column 2). As expected from the lipid solubility of aliphatic molecules, the binding sequence is $C_{12} > C_{10} > C_8$. (There are no significant differences between the pairs of methyl and ethyl derivatives.) From the relative values of β, we see that each methylene group lowers the binding free energy by about 800 cal, in agreement with hydrocarbon solubility and binding data at hydrocarbon:water interfaces (Tanford, 1973). The absolute values of the β's are about 1,000-fold smaller than those reported at the hydrocarbon:water interface (Haydon and Phillips, 1958). This difference probably results from cholesterol in the bilayer membrane. If cholesterol is included in a PG monolayer at an air:water inter-

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14 In these calculations we have assumed that QA ions affect surface potential only through their positive charge. However, they also introduce a dipole potential at the hydrocarbon:water interface (Davies, 1951; Haydon, 1962), which is surprising since they are nearly symmetrical and hence do not have significant dipole moments. We have neglected this component both for simplicity and because it is not clear to us that monazomycin "sees" this contribution to the surface potential.
Figure 14. (a) Change in surface charge density ($\Delta \sigma$) of a PG membrane as a function of $C_{12}$ (methyl) concentration in bulk solution. The data points are those in Fig. 4b with the ordinate changed from $\Delta \psi$ to $\Delta \sigma$ according to Eq. 5a, Appendix I. The curve is drawn from Eq. 6a, Appendix I, with $\sigma_1 = 2 \times 10^{14}$ charges cm$^{-2}$ and $\beta = 2.4 \times 10^{17}$ charges cm$^{-2}$ M$^{-1}$. (b) Change in surface charge density ($\Delta \sigma$) of a PG membrane as a function of $C_{12}$ (methyl) concentration in solution at the membrane surface ($[C_{12}]_b$). The data points are those of Fig. 14a, with the abscissa changed from $[C_{12}]_{bulk}$ to $[C_{12}]_b$ according to Eq. 4a, Appendix I. The slope of the line gives the value of the binding constant, $\beta$, to be $2.4 \times 10^{17}$ charges cm$^{-2}$ M$^{-1}$. 
face, $\beta$ for $C_{12}$ is much smaller than it is for monolayers without cholesterol. This small value of $\beta$ is consistent with that found for PG:cholesterol membranes (unpublished surface potential measurements).

**Mechanism of QA Permeation through Monazomycin-Treated Membranes**

Given the linear dependence of QA permeability ($P_{QA}$) on steady-state conductance ($g_{st}$), despite the complex dependence of $g_{st}$ on monazomycin concentration and membrane potential, it is difficult to avoid the conclusion that QA transport is intimately associated with the monazomycin channels. There are at least two other possibilities, but we think them unlikely: First, QA might move through the membrane in association with monazomycin which crosses upon breakup of channels (Heyer et al., 1976). Since, however, the $C_{12}$ flux at a given conductance (and a given $C_{12}$ concentration) can be thousands of times larger than the monazomycin flux at the same conductance (see Heyer et al., 1976), this means that thousands of $C_{12}$ ions can associate with one monazomycin ion, which is not very reasonable. Second, QA might cross through bilayer regions disrupted by either monomeric monazomycin or by various nonconducting aggregates (dimers, trimers, etc.). The linearity of $P_{QA}$ with $g_{st}$ argues against this, for it is unlikely that the number of these disrupting aggregates show the same voltage dependence as the number of conducting channels.

We therefore conclude that QA either passes through the lumen of the channel or crosses the modified region of the bilayer immediately adjacent to the channel. We think the second of these possibilities is less likely, since one might expect that any amphipathic molecule which bound to the membrane could move by this mechanism. Yet clearly this is not the case. Tetracaine binds (Muller and Finkelstein, 1972 b), but does not produce inactivation; i.e., it does not traverse the membrane in conjunction with monazomycin-induced conductance.

If QA indeed moves through the channels, interesting issues are raised. First, one might expect $P_{QA}$ to decrease with molecular size. On the contrary, as size increases (by lengthening the aliphatic chain), $P_{QA}$ also increases (Table 1, column 5). Second, although these organic cations are much larger than potassium, they are all, surprisingly, much more permeant (Table 1, column 3) (although TEA itself is about 35 times less permeant than $K^+$). Third, their relative permeabilities parallel their relative binding constants to the membrane (compare columns 4 and 5 of Table 1).

Formally, large partition coefficients for QA between channel and surrounding aqueous solution explain these observations. This must mean that there is a hydrophobic region associated with the channel to which QA ions bind. Our model of how these ions traverse the channel is shown in Fig. 15. The hydrophobic region to which the aliphatic chain of the ion binds includes the bilayer itself; the polar amino end of the ion is in the lumen of the aqueous channel. The tail of the ion lies between the monomeric subunits (i.e., the individual monazomycin molecules) that form the channel. QA ions that pass through the channel in this way may have entered either directly from solution, or by first binding to the lipid and then slipping in between the monazomycin monomers.
Figure 15. Our fantasy of how a long chain quaternary ammonium ion traverses a monazomycin channel. The channel is composed of several (probably five, but for artistic purposes indicated as six in the figure) monazomycin molecules packed together with lipids (see Heyer et al., 1976). The charged end of the QA ion passes down the lumen of the channel, whereas its hydrocarbon tail slides through the hydrophobic region formed by the nonpolar faces of the monazomycin molecules and the hydrocarbon tails of the phospholipids.

Biological Implications

QA Inactivation of Potassium Channels in Nerve. Our initial motive for these experiments was Armstrong's observations that QA ions inactivate the voltage-dependent potassium conductance of squid giant axons when injected into the axoplasm (Armstrong, 1971). He postulated that these ions "plug" the channel and thus prevent K⁺ transport. The strongest evidence supporting this interpretation is the ability of both hyperpolarization and increased external K⁺ concentration to speed recovery from inactivation, presumably by sweeping out QA from the channel (Armstrong, 1971). Our failure to observe this same phenomenon is one of many reasons for precluding QA "plugging" of monazomycin channels as the inactivation mechanism.

Despite differences in detail between QA inactivation of K⁺ channels and monazomycin channels, there are interesting similarities. QA enters both channels. It plugs the former whereas it passes through the latter. Armstrong (1971) has proposed that the K⁺ channel consists of a wide part, facing the axoplasmic side of the membrane, that can accommodate the large TEA ion (and its derivatives) and the hydrated potassium ion, and a narrow part, facing the outside, that can only accommodate a dehydrated (or partially dehydrated) potassium ion. Armstrong and Hille (1972) have further proposed that it is this wide part that is gated by voltage. If this model is correct, then monazomycin-induced channels phenomenologically correspond to the wide part of K⁺ channels.

This correspondence is even stronger. On the basis of the relative effectiveness of QA blockers, Armstrong (1969) concludes that there is a hydrophobic

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15 Only the triethyl derivatives are effective on the axon.
portion of the K⁺ channel that binds the long aliphatic chain. We have noted that
the monazomycin channel is much more permeable to long chain QA ions than
to K⁺, and we have suggested that the QA aliphatic chain extends between
monazomycin monomers into the surrounding bilayer region. Perhaps, then,
there is more than a formal correspondence between the monazomycin-induced
channels and the wide part of the K⁺ channel. The latter could also be built up of
voltage-dependent subunits, a suggestion made by Baumann and Mueller (1974)
on other grounds, and the hydrophobic region of the channel would be, as in
the monazomycin system, composed of the exterior aspect of the subunits and
the bilayer region immediately surrounding the channel.

One aspect of QA action in nerves is puzzling. Aside from their specific
interaction with the K⁺ channels, we would expect them to bind (as in our
membranes) to the bilayer of the axonal membrane. Thus, their addition to the
outside medium should shift the parameters (m, h, and n) of both the voltage-
dependent sodium and potassium conductances to the right along the voltage
axis (as does Ca⁺⁺ and Mg⁺⁺ [Frankenhaeuser and Hodgkin, 1957]), and their
injection into the axoplasm should shift these parameters to the left. Arm-
strong's data show neither of these effects. Possibly because of adsorption to
surrounding Schwann cell membrane, these ions do not achieve a sufficiently
high concentration at the outside of the axonal membrane. Also, smaller surface
potentials (particularly at the inner surface) on the axonal membrane than on
the phosphatidylglycerol bilayers would lead to smaller surface concentrations of
these ions and hence less binding. Finally, the hydrocarbon portion of the
axonal bilayer may be less favorable for adsorption of these ions (note our
remarks on the effect of cholesterol on binding). Nevertheless, at sufficient
concentrations, C₁₂, particularly, should produce the predicted shifts along the
voltage axis of m, h, and n.

RELATION TO NATURALLY OCCURRING INACTIVATION IN EXCITABLE CELLS
Inactivation in biological voltage-dependent systems is fairly common. Besides
the popular inactivation of sodium conductance in nerve (Hodgkin and Huxley,
1952 b), there is a long-term inactivation of both sodium and potassium con-
ductances upon prolonged depolarization (Bezanilla and Armstrong, 1974;
Ehrenstein and Gilbert, 1966). Other voltage-dependent conductances also dis-
play inactivation. Can the mechanism of QA inactivation of the monazomycin
system be relevant to any of these? The particular aspect of QA inactivation that
we are referring to is the creation of an internal electric field, not recorded by
external electrodes, that is “seen” by the voltage-sensitive elements. Taking the
familiar Na⁺ inactivation as an example, what is required is the movement of a
molecule through the bilayer in the vicinity of the sodium channel such that
either the charge or dipole moment of the molecule creates a hyperpolarizing
field to turn off the sodium system. Indeed, if the sodium channel is built up of
subunits, these subunits themselves, which must be charged or have a large
dipole moment, could act in this capacity and inactivate themselves. The long-
term inactivation of the sodium and potassium conductances may be due to the
movement of charged particles generally present in the bilayer and not specifi-
cally associated with these systems.
APPENDIX I

Derivation of the Effect of QA Binding on the trans Surface Potential, $\psi_{st}$, and the Surface Charge Density, $\sigma$, of a Negatively Charged Membrane

The solution of the Poisson-Boltzmann equation for large negative surface potentials in the presence of only uni-univalent salts (the case of interest for our experiments) is (see Muller and Finkelstein, 1972 b):

\[ S_0 e^\sigma = [K^+][e^{-\sigma/T}], \quad (1a) \]
\[ S_0 e^\sigma = [K^+][e^{-\sigma/T}], \quad (2a) \]

where, $S = (2\pi/\varepsilon T)$ ($\varepsilon$ is the dielectric constant of water), and we have used $[K^+]$ for the total cation concentration, since in our experiments $[K^+] \gg [QA^+]$. The subscript "i" refers to initial values before the addition of QA. $\sigma$ is related to $\sigma_i$ by:

\[ \sigma = \sigma_i - \beta[QA^+]_{st}, \quad (3a) \]

where $[QA^+]_{st}$ is the concentration of QA in solution at the membrane surface, and $\beta$ is the binding constant of QA + to the membrane. (We assume in Eq. 3 a that the total number of binding "sites" is large compared to the number of sites occupied by QA.) From the Boltzmann distribution we have:

\[ [QA^+]_{st} = [QA^+]_{t}e^{-\sigma/T}, \quad (4a) \]

where $[QA^+]_{t}$ is the bulk concentration of QA in the trans compartment. Combining Eqs. 1 a through 4 a, gives Eq. 4 of the text. We also obtain from Eqs. 1 a and 2 a the relation between the change in surface potential and the change in surface charge density:

\[ \Delta \sigma_t = \sigma_i - \frac{[K^+]}{\beta\sigma_i} \left(1 - e^{\sigma_i/T}\right), \quad (5a) \]

where,

\[ \Delta \sigma_t = (\sigma_t - \sigma_i), \]
\[ \Delta \psi_{st} = (\psi_{st} - \psi_{st}). \]

For the phosphatidylglycerol:cholesterol membranes used in our experiments,

$\sigma_i = 2 \times 10^{14}$ charges cm$^{-2}$,
\[ \psi_{st} = 144 \text{ mV}. \]

(See Muller and Finkelstein, 1972 b for the method of obtaining these values.) Substituting Eq. 4 into this we obtain the relation between $\Delta \sigma_t$ and $[QA^+]_{t}$:

\[ [QA^+]_{t} = \frac{[K^+]}{\beta\sigma_i} \left(1 - \frac{\Delta \sigma_t/\sigma_i}{(1 - \Delta \sigma_t/\sigma_i)^2}\right). \quad (6a) \]

APPENDIX II

Derivation of Eq. 9 of Text

Consider Fig. 16 corresponding to our experimental situation: $[K^+]_{ct} = [K^+]_{trans}$, and $[QA^+]_{trans} = 0$. The presence of QA + on the cis side has introduced a positive surface potential there of $\psi_{st}$. (For simplicity, we have assumed that the surface potential is zero in the absence of QA +. We see, in fact, that the surface potential does not appear in the final
expression [Eq. 9].) Assuming that the fluxes of QA$^+$ and K$^+$ obey the constant field equation, we have (Hodgkin and Katz, 1949):

$$\Phi_{QA} = P_{QA} \frac{F V_m [QA^+]_{loc} - [QA^+]_{loc} e^{-F V_m/RT}}{(1 - e^{-F V_m/RT})}. \quad (1b)$$

$$\Phi_K = P_K \frac{F V_m [K^+]_{loc} - [K^+]_{trans} e^{-F V_m/RT}}{(1 - e^{-F V_m/RT})}. \quad (2b)$$

But,

$$[QA^+]_{loc} = [QA^+]_{trans} = 0;$$

$$[QA^+]_{loc} = [QA^+]_{loc} e^{-F V_m/RT};$$

$$[K^+]_{loc} = [K^+]_{trans} = [K^+]_{loc};$$

$$[K^+]_{trans} = [K^+]_{trans} e^{-F V_m/RT};$$

so that Eqs. 1b and 2b become

$$\Phi_{QA} = P_{QA} \frac{F V_m [QA^+]_{loc} e^{-F V_m/RT}}{(1 - e^{-F V_m/RT})}. \quad (3b)$$

$$\Phi_K = P_K \frac{F V_m [K^+]_{loc} (1 - e^{-F V_m/RT}) e^{-F V_m/RT}}{(1 - e^{-F V_m/RT})}. \quad (4b)$$

Since virtually all of the current is carried by K$^+$,

$$I = F \Phi_K, \quad (5b)$$

and

$$g = \frac{I}{V}. \quad (6b)$$

Substituting Eqs. 5b and 6b into 4b and then dividing this into Eq. 3b gives Eq. 9 of the text:

$$\frac{P_{QA}}{P_K} = \frac{[K^+]_{loc}}{[QA^+]_{loc}} \frac{F \Phi_{QA} (1 - e^{-F V_m/RT})}{g V}.$$

Although we have used the constant field equation to derive Eq. 9, the result is not

18 Even though the basis of inactivation is that [QA$^+$]$_{loc} \neq 0$, for virtually all of the data presented in this paper, [QA$^+$]$_{loc} < [QA^+]_{loc}$; therefore, [QA$^+$]$_{loc} = 0$. Alternatively, we can say that since [QA$^+$]$_{loc} e^{-F V_m/RT} < [QA^+]_{loc}$, the second term in the numerator of Eq. 1b can be set equal to zero.
critically dependent on this. This is seen most readily if we set \( V = 0 \), in which case Eq. 9 becomes:

\[
\frac{P_{QA}}{P_K} = \frac{[K^+]_{eq}}{[QA^+]_{eq}} \frac{\Phi_{QA}}{RTg/F^2}.
\]  

But \((RT/F^2)g\) equals the unidirectional flux of \( K^+ \) (assuming the Behn-Ussing-Teorell flux ratio relation holds). Eq. 7b, thus, is intuitively obvious. It simply states that the ratio of \( QA^+ \) and \( K^+ \) permeabilities equals the ratio of their fluxes normalized for their respective concentrations. The voltage term in Eq. 9 introduces a correction of about a factor of 2 at \( V = 40 \) mV, the voltage applied in the tracer flux experiment.

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