Acetylcholine-Induced Current in Perfused Rat Myoballs

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ABSTRACT Spherical "myoballs" were grown under tissue culture conditions from striated muscle of neonatal rat thighs. The myoballs were examined electrophysiologically with a suction pipette which was used to pass current and perfuse internally. A microelectrode was used to record membrane potential. Experiments were performed with approximately symmetrical (intracellular and extracellular) sodium aspartate solutions. The resting potential, acetylcholine (ACh) reversal potential, and sodium channel reversal potential were all ~0 mV. ACh-induced currents were examined by use of both voltage jumps and voltage ramps in the presence of iontophoretically applied agonist. The voltage-jump relaxations had a single exponential time-course. The time constant, \( \tau \), was exponentially related to membrane potential, increasing e-fold for 81 mV hyperpolarization. The equilibrium current-voltage relationship was also approximately exponential, from -120 to + 81 mV, increasing e-fold for 104 mV hyperpolarization. The data are consistent with a first-order gating process in which the channel opening rate constant is slightly voltage dependent. The instantaneous current-voltage relationship was sublinear in the hyperpolarizing direction. Several models are discussed which can account for the nonlinearity. Evidence is presented that the "selectivity filter" for the ACh channel is located near the intracellular membrane surface.

INTRODUCTION

Acetylcholine (ACh) acts on the endplate of striated muscle by opening a large, water-filled pore which is selectively permeable to cations (Maeno et al., 1977; Huang et al., 1978; Dwyer et al., 1979). Attempts to understand the details of permeation have been hampered by the inability to control or even determine the ionic composition of the intracellular milieu. For example, measurement of ACh currents at very depolarized potentials is made difficult by the presence of large voltage-activated K currents. The K channel cannot be blocked simply with extracellular tetraethylammonium (TEA) because TEA also interacts strongly with the ACh channel (Adler et al., 1979). If the intracellular K could be replaced by a cation which is impermeable through the K channel, this difficulty would be alleviated. Another difficulty that is encountered in traditional studies of the motor endplate is the inability to test...
quantitative theories of ion permeation. For example, ion permeation through the ACh channel may be depicted as the movement across two Eyring-type energy barriers with a low energy site (well) between them (Adams, 1979; Lewis and Stevens, 1979; Marchais and Marty, 1979). However, the details of the inner barrier must remain obscure until permeation is studied during systematic variations of the intracellular ionic composition.

We have developed a method for internal perfusion of muscle by using a suction pipette (Lee et al., 1978) on spherical myoballs (Fischbach and Lass, 1978) grown from cultured rat myotubes. We have employed a voltage clamp using a separate microelectrode to record membrane potential while passing current through the suction pipette. The use of the microelectrode reduces series resistance artifacts. In the experiments reported here we have examined ACh-induced currents in approximately symmetrical Na aspartate solutions using both voltage jumps and voltage ramps. A preliminary report has appeared (Horn et al., 1979).

MATERIALS AND METHODS

Tissue Preparation

The thigh muscles were removed from 1–3-d-old Sprague-Dawley white rats. The tissue was minced with scalpels, then dissociated at room temperature in a spinner flask containing 0.025 percent trypsin in divalent-free minimal attachment medium (Klebe, 1974). The supernate was removed at 15–20-min intervals and replaced with fresh trypsin solution until the tissue was completely dissociated. The cells were then centrifuged for 10 min at 3,000 rpm, resuspended in DV-10 (Dulbecco’s medium enriched with 10% fetal calf serum, 1 mM proline, 0.5% chick embryo extract, 600 mg% glucose, 100 U/liter penicillin and 100 µg/ml streptomycin), and plated at a density of 1–4 × 10^6 cells per 60-mm Lux plastic tissue culture dish. The plates were then incubated at 37°C in 10% CO₂.

After 2–3 d myoblasts began fusing to form multinucleate myotubes. At this time, most of the plates were treated with 10^-5 M araC (cytosine arabinoside, Sigma Chemical Co., St. Louis, Mo.) for 2 d to prevent further growth of fibroblasts. The araC had no apparent effect on the electrophysiological properties of the tissue.

In some plates, spherical myoballs formed spontaneously from myotubes. These were occasionally used in experiments, but usually the myotubes were treated for 1–3 d with 10^-6 M vinblastine (Velban, Eli Lilly & Co., Indianapolis, Ind.) which induced myoball formation (Sachs and Lecar, 1977). The myoballs used for experiments were typically 70–120 µm in diameter. Both myotubes and myoballs twitched spontaneously in DV-10.

Solutions

The external solution was ½ Na Ringer containing 85 mM Na aspartate, 150 mM sucrose, 5 mM HEPES buffer, 1.5 mM CaCl₂, pH adjusted to 7.4 with NaOH. The internal solution was ½ Na SIS (standard internal solution) which consisted of 85 mM Na aspartate, 155 mM sucrose, 5 mM HEPES, 2 mM EGTA, 1 mM NaCl, pH 7.3. The activity of Na in each of these solutions was 63 ± 2 mM, as determined by a glass Na electrode (MI – 420, Microelectrodes, Inc., Londonderry, N. H.). A free Ca concentration of 1.47 mM in ½ Na Ringer was calculated from the critical stability constants for aspartate (Martell and Smith, 1974). In a few experiments 10^-6 M
Tetrodotoxin and 0.2 mM CdCl₂ were added to ½ Na Ringer in order to block currents in the voltage-activated Na and Ca channels, respectively. However, the Na channel block was incomplete, and all the experiments shown below did not make use of these blocking agents.

Voltage Clamp

Due to a significant series resistance in the tip of the suction pipette (see below), the transmembrane potential was assumed to correspond to the potential of the microelectrode ($V_m$, Fig. 1) with respect to circuit ground. Therefore, the voltage clamp circuitry was designed to clamp the microelectrode potential. The amplitude of the

![Figure 1. Experimental setup. Diagram shows myoball attached to suction pipette. Internal solution entered the inlet continuously. The suction was applied at the outlet. The potential recorded in the pipette by a chlorided silver wire was $V_p$. $V_m$ was the potential recorded by the microelectrode. Either $V_p$ or $V_m$ could be voltage-clamped to the command voltage $V_{com}$. Current was passed into the cell by means of a 75 μm Pt wire wrapped around the polyethylene perfusion tubing, and led from the bath by a current-voltage converter with a 1 MΩ feedback resistor. ACh was applied iontophoretically with another microelectrode. $A_{1}$, W-P Instruments, Inc., model 750 electrometer (New Haven, Conn.); $A_{2}$, Tektronix, Inc., AM 502 differential amplifier (Beaverton, Ore.); $A_{3}$, Tektronix AM 501 operational amplifier (gain = 10); $A_{4}$, LF 356 operational amplifier (National Semiconductor Corporation, Santa Clara, Calif.).

Net transfer function of the myoball, electrodes, and voltage follower on the microelectrode had a zero frequency asymptote of −6 dB and simple poles at $10^3$ Hz, and $5 	imes 10^6$ Hz. In order to clamp at frequencies on the order of $10^4$ Hz, the clamping amplifiers were designed to achieve a gain of $>60$ dB at $10^4$ Hz. The electrical arrangement is shown in Fig. 1. The rise time of the voltage in response to a step command was usually less than 300 μs. The current signal was filtered at $10^5$ Hz to remove high frequency noise.
Data Analysis

Data were displayed either on a Nicolet 1090 digital oscilloscope or on a Nicolet 1170 signal averager (Nicolet Instrument Corp., Madison, Wis.). Data were then stored either on a Kennedy digital tape recorder (model 9700, Kennedy Co., Altadena, Calif.) or on an Omnigraphie 2000 X-Y plotter (Houston Instruments, Austin, Texas). For voltage jump experiments, the sampling rate was usually 100-200 μs/point. For voltage ramps, the data were sampled every millisecond. 12-bit resolution was used in all cases.

For voltage jump experiments, control traces in the absence of ACh were subtracted digitally from experimental traces in the presence of ACh by use of a PDP 11-70 computer and a program kindly provided by Dr. Douglas C. Eaton. Additional analysis was performed on a PDP 11-20 computer (Digital Equipment Corp., Marlboro, Mass.).

Experimental Procedure

The experiments were performed in the tissue culture dishes after replacing the DV-10 with 89 Na Ringer. The experiments reported here were done at reduced room temperature (18°C).

A myoball was selected and sucked onto the tip of a broken and polished glass pipette (Fig. 1). The method is a modification of the technique described by Kostyuk and Krishtal (1977) and Lee et al. (1978). The inner diameter of the glass tip was 10–20 μm and had a resistance in the usual experimental solutions of ~700 kΩ. When the myoball was sucked onto the tip of the pipette, the membrane usually ruptured within a few seconds, as evidenced by a sudden drop in pipette potential (Vp, Fig. 1) and a sudden decrease in resistance to a hyperpolarizing current pulse. Most cells exhibited anodal-break spikes at the end of the current pulse. The steady-state resistance, using this method, was 7–15 MΩ in a healthy cell. The cell was then impaled with a 5–25 MΩ microelectrode (3 M KCl) for recording membrane potential. The cell could then be voltage-clamped, using either the potential recorded by a chlorided silver wire in the pipette, Vp, or that recorded by the microelectrode,Vm (Fig. 1). Current was passed into the pipette from a 75 μm platinum wire, and was led from the bath with a 3 M KCl agar bridge through a chlorided silver wire to a current-to-voltage converter with a 1 MΩ feedback resistor. The tip of the suction pipette was continuously perfused with ½ Na SIS at a rate of ~1 ml/min.

Acetylcholine (ACh, Sigma Chemical Co.) was applied iontophoretically by a pipette placed within 100 μm of the membrane surface. The pipette contained ~3 M ACh. The backing current was usually < 20 nA.

After impaling a myoball and observing an ACh response, i.e., an increased conductance in response to iontophoretic application of ACh, the cell was usually allowed to dialyze for 15–20 min to permit the exchange of intracellular ions. During this period, the steady-state membrane resistance, Vm/Im (see Fig. 1) increased from ~4 to ~5 MΩ (Table I), presumably due to both (a) an increased sealing resistance between the membrane and electrodes, and (b) a replacement of intracellular K for Na. After recording the resting potential with the microelectrode, the cell was voltage-clamped, using Vm as a measurement of membrane potential. The ACh reversal potential was determined. Then a series of voltage steps were applied both in the presence and in the absence of ACh from a holding potential of ~−40 mV. In some experiments, the command voltage was an ~200 mV, 1 Hz triangular wave.

RESULTS

The first part of this section describes and evaluates the voltage clamp and the internal perfusion. The remainder of the section describes voltage jump
and voltage ramp experiments. The purpose of these experiments was to
examine the voltage dependence of both the gating and the permeation of the
ACh channel in this preparation.

Evaluation of the Voltage Clamp

The ability to voltage-clamp the myoball membrane accurately is limited by
several factors. We will discuss three of these factors individually. They are
the shunt resistance, \( R_{sh} \), the pipette resistance, \( R_p \), and the series resistance,
\( R_s \).

The shunt resistance, \( R_{sh} \), is the sealing resistance between the suction
pipette and the membrane. A low value of \( R_{sh} \) decreases the total impedance
of the combined pipette and myoball. It can degrade the voltage clamp in
two ways. It increases the output current load on the clamping amplifiers,
and it increases the relative series resistance error. The latter effect may be
especially severe, because it limits the capacity to determine and control the
membrane potential while passing current.

| Table I |
|------------------|------------------|------------------|------------------|
|                  |                  |                  |
| RESTING POTENTIAL, ACh REVERSAL POTENTIAL, Na |
| CHANNEL REVERSAL POTENTIAL, AND MEMBRANE |
| RESISTANCE OF MYOBALLS |
| Mean ± SEM (number of observations) |
| Resting potential   | -1.4 ± 1.4 mV (19) |
| ACh reversal potential | -0.1 ± 0.9 mV (25) |
| Na channel reversal potential | +3.5 ± 3.4 mV (2) |
| Membrane resistance  | 5.2 ± 0.4 MΩ (19) |

Data were obtained 20 min after impalement with the microelectrode. The
external solution was \( \frac{1}{2} \) Na Ringer, the internal was \( \frac{1}{3} \) Na SIS. Membrane
resistance was the steady-state response, \( V_m \), divided by the stimulating
current, \( I_m \), a 3 nA hyperpolarizing step.

We have only indirect evidence that \( R_{sh} \) is high. The steady-state leakage
resistance in chick myoballs impaled by two microelectrodes is \( \sim 4 \  MΩ \)
(Fischbach and Lass, 1978). In our experiments \( R_m \) was \( \sim 5 \  MΩ \) (Table I). A
finite value of \( R_{sh} \) would decrease the apparent value of \( R_m \). Without having
done the comparable experiment on rat myoballs, we can only suggest that
\( R_{sh} \) is much greater than 5 MΩ. In any case our measured value of \( R_m \) is at
least two orders of magnitude greater than \( R_s \) (see below).

The pipette resistance, \( R_p \), can be determined by a comparison of the
microelectrode voltage, \( V_m \), with the suction pipette voltage, \( V_p \), while passing
current (see Fig. 1). A current step produced a larger voltage response in the
suction pipette than the voltage response in microelectrode. This is illustrated
in Fig. 2 A. The upper trace shows the \( V_m \) and the lower trace shows the \( V_p \)
response to a 6 nA hyperpolarizing current step. The steady-state response
was \( \sim 1.4 \) times larger in the suction pipette. In seven experiments \( V_p \) was 1.6
± 0.3 (SD) times as large as \( V_m \) in response to a 3 nA current step. A difference
between $V_m$ and $V_p$ was not reported in similar experiments using snail neurons (Lee et al., 1978). In the remainder of this paper the membrane resistance, $R_m$, was measured from the steady-state change of $V_m$ in response to a 3 nA hyperpolarizing current step. The difference between $V_m$ and $V_p$ is due to the pipette resistance, $R_p$, which is in series with the membrane resistance. This resistance was usually $\sim 3 \, \text{M}\Omega$, which is larger than the $\sim 700 \, \text{k}\Omega$ resistance of the suction pipette in the bath. Apparently the tip of the pipette becomes partly occluded by cytoplasmic material or the myoball membrane.

The pipette and cell membrane may be represented as $R_p$ in series with the

![Diagram](image-url)
parallel $R_m$ and $C_m$ of the membrane. If $V_p$ is voltage-clamped, a command voltage step, $V_c$, will produce an exponential response in $V_m$.

$$V_m = A \cdot V_c \cdot (1 - e^{-t/\tau})$$

$$A = \frac{R_m}{R_m + R_p} \quad \tau = A \cdot R_p \cdot C_m$$ (1)

The values $R_m$, $R_p$, and $C_m$ can be determined in the current injection mode by the response of $I_m$ and $V_p$ to a hyperpolarizing current step. These values then can be used to predict the time constant $\tau$ during the voltage clamp of $V_p$.

The voltage response ($V_m$) to a hyperpolarizing step of current was a single exponential charging curve. This is in contrast with results obtained from adult rat skeletal muscle where two passive time constants were observed (Duval and Léoty, 1978). However, Ritchie and Fambrough (1975) also observed a single time constant in rat myotubes. We estimated the area specific capacitance in three myoballs by dividing the measured capacitance by an apparent area (assuming that two-thirds of the spherical membrane area was intact after sucking up the cell onto the suction pipette). The average value was 13.6 $\mu$F/cm$^2$. Because of potentially large errors in estimating surface area, this value may be in error by as much as 50%. Nevertheless, it suggests that membrane infolding occurs, but without an extensive, narrow, T tubule system (Falk and Fatt, 1964). A preliminary electron microscope examination of rat myoballs showed this to be the case.

Fig. 2 B shows the effect of voltage-clamping $V_p$ in a myoball. In this cell $R_m = 4.1 \text{ M}\Omega$, $R_p = 1.24 \text{ M}\Omega$, and $C_m = 3.4 \text{ nF}$. The predicted value of $\tau$ (see above) is then 3.2 ms. $V_p$ closely approximated the command pulse, $V_c$, and produced an exponential response of $V_m$ (Fig. 2 B1). The measured time constant was 3.9 ms, which is in reasonable agreement with the predicted value. It is clear that $V_p$ is a poor representation of $V_m$, both in its absolute magnitude and in its time-course.

The insufficiency of using $V_p$ for voltage-clamping the membrane is especially apparent in Fig. 2 B2 from the same cell. The lower trace shows $I_m$ (see Fig. 1). The holding potential (measured by the microelectrode) was −30 mV. $V_p$ (not shown) was a 75 ms, 40 mV hyperpolarizing voltage pulse. The steady-state response of $V_m$ during the pulse was 30 mV. At the end of the pulse the cell responded with an anodal break inward current. Although this current was not accompanied by a voltage response of $V_p$, the microelectrode recorded a small action potential. In the presence of ACh the membrane resistance decreased as much as 12-fold at hyperpolarizing potentials. Under these conditions $R_p > R_m$, and attempts to compensate electronically for $R_p$ were unsuccessful. Therefore, the remaining experiments discussed in this paper employed a voltage clamp of the microelectrode potential, $V_m$.

Fig. 3 shows another experiment in which the microelectrode potential, $V_m$, was voltage-clamped. $V_0$ is the holding potential (−40 mV in all experiments), and $V_1$ is the potential to which $V_m$ was pulsed. In Fig. 3, $V_1 = −120 \text{ mV}$. The

1 Krauhs, J., R. Horn, and M. Brodwick. Unpublished observation.
dotted line represents zero current. Fig. 3 A shows the voltage jump current in the absence of ACh. The measured membrane resistance for this voltage jump was 2.1 MΩ. Note the anodal break Na current at the end of the voltage jump. In the presence of ACh (Fig. 3 B), the negative holding current

\[ \text{VOLTAGE CLAMP, } V_m \]

\[ \text{A CONTROL} \]

\[ \text{B ACH} \]

\[ \text{C ACH MINUS CONTROL} \]

**Figure 3.** Voltage clamp of \( V_m \) and the effect of ACh. Holding potential, \( V_0 \), was \(-40 \text{ mV}\). In each record the dotted line represents zero current. Each record shows the effect of a 130 ms voltage jump to \( V_1 \), in this case, \(-120 \text{ mV}\). (A) The control response. Note the small anodal break inward current, during which \( V_m \) was constant. (B) The same voltage jump recorded in the presence of iontophotically applied ACh. Note that the negative holding current increased in ACh and that the current relaxed to an equilibrium value after jumping to \(-120 \text{ mV}\). The magnitude and time-course of \( V_m \) was not affected by ACh, indicating that the gain of the clamping amplifiers was sufficiently high. (C) The effect of digitally subtracting \( I_m \) recorded in A from that recorded in B. \( I_{m,\theta} \) is the ACh-induced current at \( V_0 \). \( I_{m,1} \) is the instantaneous current induced at the beginning of the voltage jump. \( \tau_i \) is the relaxation time constant at \( V_1 \), in this case, 21.1 ms. The steady-state current at \( V_1 \) is \( I_{m,1} \), and the instantaneous current at the end of the jump is \( I_{m,\theta} \). Note that the current through the Na channel was not completely subtracted at the end of the jump (see text).

increased, and the voltage jump produced a large inward current. The steady-state resistance during this voltage jump was 276 kΩ. Both in the presence and in the absence of ACh, the rise time of \( V_m \) was \(-300 \mu\text{sec}\). Fig. 3 C shows
the result of digitally subtracting the control current from the current in the presence of ACh. The subtracted current represents the ACh-induced current. The capacity transient at the beginning of the voltage jump has been completely removed by the subtraction. Note, however, that the anodal break Na current was not subtracted. This is probably due to a series resistance error, as discussed below. The voltage jump current at $V_1$ was a single exponential with a time constant $\tau_1 = 21.1$ ms.

**Evaluation of Series Resistance, $R_s$**

The lumped membrane impedance is in series with both $R_m$ and $R_s$. We used several criteria to estimate both the magnitude of $R_s$ and its effect on ACh current. These criteria included (a) the voltage discontinuity in response to a current step, (b) the current capacity transient in response to a voltage step, (c) the difference in the current capacity transients in the presence or absence of ACh, (d) the time-course of the voltage jump relaxation, (e) the steepness of the $\tau$ vs. $V_m$ relationship for voltage jump relaxations, and (f) the effect of current magnitude on the shape of the instantaneous current-voltage ($I-V$) relationship.

(a) In most experiments we did not observe a pronounced discontinuity of $V_m$ in response to a step of current. This may be due to a finite capacitance to ground at the input of the voltage follower. This was usually not compensated.

(b) The current capacity transient in response to a voltage clamp step was usually both small in amplitude and so rapid that it could not be measured reliably. In many experiments the capacity transient occurred predominantly during the $\sim 300$-ms rise time of the voltage step. This sets an approximate upper limit for $R_s$ of $\sim 200 \, \text{k}\Omega$.

(c) A change in current capacity transient due to a reduction of $R_m$ (e.g., in the presence of ACh), if measurable, could provide an estimate of $R_s$ (see Eq. 1). However, even a large value of $R_s$, e.g., $200 \, \text{k}\Omega$, and a large change of $R_m$, e.g., by a factor of 10, leads to a $<200$-ms difference in the time constant. This could not be detected in our experiments. It was encouraging to note, however, that capacity transients were in all cases effectively subtracted, as seen in Fig. 3 C and especially in Fig. 5 A for a voltage jump to the reversal potential.

(d) The voltage jump relaxations were in all experiments well described by a single exponential, even at the most negative potentials where the amplitude of the relaxations were large (see Figs. 3 and 5). In Fig. 5 A the voltage jump to $-120$ mV produced an ohmic jump, then a relaxation with a magnitude of 130 nA. Fig. 5 B shows that the current was well fitted with a single time constant. If $R_s$ were as much as $200 \, \text{k}\Omega$, 130 nA would cause a 26 mV change in the actual membrane potential during the increasing current. From the normal voltage dependence of $\tau$ (see below), this would produce a nearly 10-ms difference in $\tau$ from the beginning to the end of the pulse. This was clearly not observed.

(e) A significant series resistance error would tend to decrease the apparent voltage dependence of $\tau$. However, our $\tau$ vs. $V_m$ relationship (see below) agrees well with published data on vertebrate endplate (for reference, see Steinbach and Stevens, 1976; Adams and Sakmann, 1978). A large $R_s$ error would also
produce a deviation at negative potentials from the exponential relationship of \( \tau \) and \( V_m \). This was not observed.

(9) The instantaneous \( I-V \) relationship (below) was obtained with as much as four-fold differences in current magnitude in different experiments. However, the scaled instantaneous \( I-V \) curves superimposed (Fig. 7 A). We were able to discriminate \( \sim 10 \) mV shifts in these curves. From the relative current magnitudes we could set an upper limit of \( \sim 30 \) k\( \Omega \) for \( R_\Lambda \). In the worst cases this could produce an \( \sim 10 \) mV error in recorded voltage. This error would be most apparent at negative potentials because of the large driving force for the current and the increased channel lifetime (see below). The effects of such an error could produce shifts along the steady-state inactivation curve for the Na channel (Duval and Léoty, 1978). Such shifts could be responsible for the incomplete subtraction of anodal break Na currents (Figs. 3 and 5). However, such shifts would usually produce a \(<10\%\) error in the conductance-voltage relationship of the ACh channel, and only at the most negative potentials.

**Evaluation of the Internal Perfusion**

Since the internal and external solutions were approximately symmetrical, we expected a 0 mV reversal potential for (a) the resting membrane, (b) the ACh-induced current, and (c) the Na channel current. The ACh reversal potential was determined directly within 1 mV (Fig. 4 a). Table I summarizes the results and suggests that the cells were adequately perfused. The possible effects of ion accumulation within the myoball during maximum ACh current were calculated from the apparent cell volume. The predicted changes in intracellular Na concentration during 30-s applications of ACh were \(<1\) mM.

**Voltage Jump Currents**

For voltage jump experiments a 20–30-sec ACh pulse was delivered iontophotically (Fig. 4 b). When the current reached a nearly steady-state level, four voltage jumps were given, usually in the following order: \( V_1 = -80, 0, +40, -120 \) mV. Fig. 5 shows typical voltage jump currents at these potentials. The jump to 0 mV shows only an off-relaxation at the holding potential. The jump to +40 mV produced an outward current with a rapid inward-directed relaxation. Because of the contamination of Na currents observed during the off-response with negative voltage jumps, the relaxations at \(-40\) mV were determined during the off-response following positive voltage jumps. The off-relaxation time constants measured after jumps to 0 mV and to +40 mV always agreed within 5 percent. Fig. 5 B shows that the relaxations had exponential time-courses at all potentials.

**Theory**

The theoretical framework we have adopted has been formulated previously (Neher and Sakmann, 1975; Ascher et al., 1978). The \textquotedblleft instantaneous\textquotedblright current immediately following a voltage jump to \( V_1 \) (Fig. 3 C) is given by

\[
I_{n,1} = N\gamma_1 p_0 V_1,
\]
where $N$ is the total number of channels, $\gamma_1$ is the single channel conductance at $V_1$, and $p_0$ is the equilibrium probability of a channel being open at $V_0$. Because the reversal potential is close to 0 mV in all experiments, the driving force for the ACh current at potential $V_1$ is $V_1$. The steady-state current at $V_1$ is given by

$$I_{m,1} = N\gamma_1 p_1 V_1,$$

where $p_1$ is the equilibrium probability of a channel being open at $V_1$. The value for $I_{m,0}$ and $I_{m,0}$ have corresponding formulae.

For this analysis the ACh channel is assumed to exist in two states, closed and open, with the following rate constants:

$$\alpha \overset{\beta^*}{\Rightarrow} \text{open}.$$

$\beta^*$ is a pseudo first-order rate constant which depends on ACh concentration and on the binding constants for ACh on the ACh receptor. When the voltage
is stepped from $V_0$ to $V_1$, the rate constants $\alpha$ and $\beta^*$ change instantaneously, and the current relaxes exponentially to its equilibrium value with a time constant,

$$\tau_1 = (\alpha_1 + \beta^*)^{-1}.$$  

The equilibrium probability of a channel being open at $V_i$ is given by

$$p_i = \beta^* \tau_i$$  for $i = 1, 2.$  

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.png}
\caption{Voltage jump currents. (A) The voltage jump currents induced by ACh after subtracting control records. Notice that a step to 0 mV, the reversal potential, shows no observable relaxation. However, a comparison of the ohmic jumps before and after this jump gives evidence of a decrease in channel lifetime at 0 mV. The step to +40 mV shows an inward relaxation superimposed on the outward current. (B) Relaxations recorded in A are single exponentials. The relaxation at -40 mV was the off response after a jump to +40 mV.}
\end{figure}

$\alpha$ is an exponential function of voltage (Magleby and Stevens, 1972) with the form $\alpha (V) = a \exp (kV)$, where $k$ is a constant representing the voltage sensitivity of $\alpha$, and $a$ is the closing rate constant at $V = 0$. In our experiments we used low iontophoretic doses of ACh in order to insure that $\beta^*$ was negligible compared with $\alpha$. This condition was checked in two ways. First,
the iontophoretic current was adjusted to a level that produced little desensitization (Fig. 4 b). Second, the relaxation time constants were determined at several iontophoretic current levels. An iontophoretic dosage was considered acceptable only if an increase of iontophoretic current by a factor of a third caused no change in \( \tau \) at all potentials. Since \( \beta^* \ll \alpha_i, 1/\tau_i \approx \alpha_i \).

If \( \beta^* \) is voltage independent (Neher and Sakmann, 1975; Adams, 1977; Ascher et al., 1978), then the equilibrium (i.e., steady state) voltage depen-

![Diagram](image)

**Figure 5**

dence of ACh current will be the same as the voltage dependence of \( \alpha \), as implied by Eqs. 3 and 4. Note, however, that \( I_{m_1} \) depends on \( N \) and \( \gamma_i \). It was necessary to correct the voltage jumps currents for both of these factors to get a good estimate of \( \mu_i \) as a function of membrane potential (see below). The equilibrium current-voltage relationship was obtained using both voltage jumps and voltage ramps.

The relaxation time constants were usually determined by fitting single exponentials to the current relaxations. In some cases, however, the relaxations were difficult or impossible to measure. At positive potentials, for example, the relaxations were often too small in amplitude and too rapid to measure accurately. At the reversal potential the channel relaxation could not be
measured at all. However, it was still possible to estimate the relaxations at these potentials by comparing the relative amplitude of the instantaneous current jumps at the beginning and end of the voltage pulse (Neher and Sakmann, 1975; Ascher et al., 1978; Marchais and Marty, 1979). The ratio of jump amplitudes equals the ratio \( p_0/p_1 \), without the necessity of correcting for \( \gamma_i \). If \( \alpha_i \gg \beta^* \) and \( \beta^* \) is voltage independent, then

\[
\alpha_1 = \alpha_0 p_0/p_1.
\]

(5)

This method was used to estimate \( \alpha_1 \) for \( V_1 \geq 0 \) mV.

**Voltage Dependence of \( \tau \)**

The relationship between \( \alpha \) and \( V_m \) was exponential from \(-120 \) mV to 0 mV (Fig. 6). The value of \( \alpha \) decreased slightly from the exponential relationship at \(+40 \) mV. This may have been due to inaccuracies of measurement at this potential, or to a slight voltage dependence of \( \beta^* \), since the calculated values of \( \alpha \) at \(+40 \) mV assume \( \beta^* \) is voltage independent (Eq. 5). However, a similar trend in \( \alpha(V) \) was observed at the endplate of *Rana temporaria* (Neher and

![Figure 6](image-url)

**Figure 6.** The relationship between alpha and membrane potential. \( \alpha \) is plotted as frequency \( [\alpha = (2\pi \tau)^{-1}] \) on a logarithmic scale. The numbers in parentheses give the number of experiments. Each data point shows mean \( \pm \) SEM. (○) Measured values (see Fig. 5 B). (□) Calculated values obtained by measuring the ratio of jump amplitudes before and after the voltage jump (see text). The straight line (drawn by eye) has the equation, 

\[
\alpha = 26.1 \exp (0.0123 V_m) \text{ Hz.}
\]
The assumption that $\alpha \gg \beta^*$ is given further support by the fact that the exponential relationship between $\alpha$ and $V$ was maintained for the most negative potentials, where $\alpha$ was small. The voltage dependence of $\alpha$ ($1/k = 81 \text{ mV/e-fold change}$) is similar to values previously reported for other preparations (Steinbach and Stevens, 1976; Adams and Sakmann, 1978). The value of the preexponential factor $a$ is smaller than that found at endplate (Steinbach and Stevens, 1976), but similar to values obtained with chick myoballs (Sachs and Lecar, 1977).

**Instantaneous $I$-$V$**

Fig. 4b shows that the mean ACh-induced current at the holding potential was not the same during each voltage jump. The time-dependent changes in mean current were assumed to be due to changes in $N$, the number of available ACh channels. Thus, the magnitude of $I_{in,1}$ for each voltage step was scaled in a way that made $N$ a constant. This was done by arbitrarily scaling the mean current at $-40 \text{ mV}$ to $-90 \text{ nA}$ for each voltage jump in each of four experiments. The value of $I_{in,1}$ was determined by extrapolating the exponentially relaxing current to the time of the change in voltage. For $V_i = 0$ or $+40 \text{ mV}$, $I_{in,1}$ was usually measured $400 \mu\text{s}$ after the beginning of the voltage jump. The pooled data for four experiments is shown in Fig. 7a. At most potentials the standard error bars were smaller than the size of the symbol. The instantaneous $I$-$V$ passed through the origin, as expected, and had a sublinear shape in the hyperpolarizing direction. The degree of sublinearity can be seen by comparing the chord conductance at $-120 \text{ mV}$ with that at $-40 \text{ mV}$. The former was about 83% of the latter. The difference in chord conductance is indicative of a voltage dependence of the single-channel conductance. Sublinear $I$-$Vs$ have been observed previously for both peak miniature endplate currents and single channel conductances (Dionne and Stevens, 1975; Gage and Van Helden, 1979).

A series resistance artifact could produce an apparent sublinearity from a linear instantaneous $I$-$V$. This is unlikely in our experiments, since the magnitude of such an error would increase with an increase in ACh current. However, the degree of sublinearity was similar in each of the four experiments, although the actual currents were as much as fourfold different.

The dotted line through the data points for the instantaneous $I$-$V$ (Fig. 7a) was derived from a saturating, two-barrier, one-site model (see Discussion). In such a model the sublinearity is achieved by making the outer barrier asymmetrically located near the outer membrane surface.

**Steady-State $I$-$V$**

When corrected for $N$ and $\gamma_i$ at each potential, the steady-state $I$-$V$ relationship provides an estimate of the equilibrium probability of a channel being open (see Eq. 3). $I_{in,1}$ was measured for each voltage jump in four experiments and corrected for $N$ as above. The current value at each $V_i$ was corrected for $\gamma_i$ by multiplying it by $\gamma_0/\gamma_i$. The ratio $\gamma_0/\gamma_i$ was obtained from the instantaneous $I$-$V$. The resulting steady-state $I$-$V$ is shown in Fig. 7b. The chord conductance was an exponential function of voltage for negative potentials. The voltage
dependence in these four cells \( (k = 0.0094 \text{ mV}^{-1}) \) is somewhat less than the voltage dependence of \( \alpha \) \( (k = 0.0123 \text{ mV}^{-1}) \). The aberrant data point at \( V = +40 \text{ mV} \) may be due to an overestimate of \( \gamma_0/\gamma_1 \). Because of the rapidity of the relaxation at \( +40 \text{ mV} \), the instantaneous current may have been underestimated, which would lead to such an error.

The steady-state \( I-V \) can also be measured by using ramp clamps (Lester et al., 1975; Adams and Sakmann, 1978). Fig. 8a shows the current in response to a 1 Hz triangular command voltage from \(-91 \text{ mV} \) to \(+81 \text{ mV} \). The control response to the triangular command voltage was nearly linear. When ACh was applied (note the arrow), both inward and outward current increased,

\[
I = I_{\text{m},1} = C \cdot V \cdot \exp (-k \cdot V)\]

where \( C = 8.58 \times 10^{-7} \Omega^{-1} \) and \( k = 0.0094 \text{ mV}^{-1} \).

although the inward rectification is obvious. 3 s after beginning ACh application, the current during a single hyperpolarizing ramp was analyzed in detail. After digitally subtracting the control current from the current recorded in the presence of ACh and correcting for the instantaneous \( I-V \), the chord conductance was plotted against membrane potential on a semilogarithmic scale (Fig. 8b). The conductance was an exponential function of voltage for the entire voltage range, from \(-91 \text{ to } +81 \text{ mV} \). The voltage dependence \( (k = 0.0098 \text{ mV}^{-1}) \) was similar to the steady-state value obtained by voltage jumps in the same cell \( (k = 0.0097 \text{ mV}^{-1}) \). In order to confirm that the conductance was at a steady-state level during the ramp clamp, we repeated the measurement. Figure 7. Instantaneous and steady-state \( I-V \) from voltage jumps. \((a)\) The instantaneous \( I-V \) recorded for the step from \( V_0 \) to \( V_1 \). The steady-state current at \(-40 \text{ mV} \), \( I_{\text{m},0} \), was arbitrarily set at \(-90 \text{ nA} \) for each voltage jump in four experiments and the currents, \( I_{\text{m},1} \), were scaled accordingly. The symbols show mean ± SEM for the four experiments. In most cases the standard error bars were smaller than the symbol. The dotted line is a theoretical fit of the data using the model and parameters shown in Fig. 9. \((b)\) The steady-state \( I-V \) for the same experiments. In this case the mean current at \( V_0 \) was arbitrarily set at \(-50 \text{ nA} \). The currents were corrected for instantaneous rectification (see text). The dotted line is given by \( I_{\text{m},1} = C \cdot V_1 \cdot \exp (-k \cdot V), \) where \( C = 8.58 \times 10^{-7} \Omega^{-1} \) and \( k = 0.0094 \text{ mV}^{-1} \).
the experiment using 5-Hz triangular waves. The voltage dependence was identical to that observed for 1-Hz waves, indicating that the voltage change was sufficiently slow for the conductance to reach a steady state.

The voltage dependence of $a$ was compared statistically with that of the steady-state $I-V$ in order to determine if $\beta^*$ had a significant voltage dependence. In six experiments the relationship between log $a$ and voltage between $-120$ and $-40$ mV had a regression coefficient of 0.0122 mV$^{-1}$. The corrected steady-state $I-V$ was determined in the same experiments by use of both voltage jumps and voltage ramps. Its regression coefficient was 0.00962 mV$^{-1}$. These values were found to be statistically different by use of Student’s $t$ test ($p < 0.001$; Steele and Torrie, 1960). Therefore $\beta^*$ had a small (~390 mV/e-
fold change), but significant voltage sensitivity. This value is similar to that observed by Dionne and Stevens (1975).

These experiments support the notion that $\beta^*$ has a slight voltage dependence, and extend previous observations to more positive potentials.

**DISCUSSION**

This paper presents the first study of ACh current in approximately symmetrical (external and internal) solutions. In general the data are consistent with results obtained under the usual asymmetric conditions, i.e., with Na as the predominant extracellular cation and K as the predominant intracellular cation. In particular, the voltage dependence of $\tau$ agrees well with previous reports (for references, see Steinbach and Stevens, 1976; Adams and Sakmann, 1978). The first part of Results deals with an evaluation of both the internal perfusion and the voltage clamp. The analysis can be summarized as follows: the adequacy of the internal perfusion was demonstrated by showing that the reversal potentials for (a) the resting membrane, (b) the ACh channel, and (c) the Na channel were all approximately 0 mV (Table I). The voltage clamp was considered to be adequate for the study of ACh current. The use of a microelectrode for recording membrane potential reduced the total series resistance from $\sim 3 \, M\Omega$ to $<3 \, k\Omega$. For the largest currents this could produce $\sim 10$-mV errors in recorded voltage, which might cause, at most, 10% errors in the $I-V$ relationship at very negative potentials. The voltage dependence of the ACh conductance could be underestimated by such an error. For potentials $< -100 \, mV$, in the worst cases the measured value of voltage dependence ($k$) would be about 90% of the actual value. In practice, however, there was no deviation of the value of $k$ at negative potentials (see Fig. 6). The series resistance error, however, could cause significant effects on the Na channel conductance, which has a greater voltage dependence than that of the ACh conductance (Duval and Léoty, 1978).

**Voltage-Dependent Gating**

Since we were careful to use sufficiently low iontophoretic doses of ACh (see above), the $I/\tau$ vs. $V_m$ relationship was equivalent to the $a$ vs. $V_m$ relationship. As in other endplate preparations, this was exponential over a range from $-120 \, mV$ to $0 \, mV$ (Fig. 6). A slight deviation from exponentiality was already noted at $+40 \, mV$.

Since the voltage dependence of the equilibrium (i.e., steady state) $I-V$ was similar to that of $a$ (Figs. 6–8), the two-state model suggests that the channel-opening rate constant, $\beta^*$, has only a small voltage sensitivity (Eq. 4). This confirms previous results (Neher and Sakmann, 1975; Adams, 1977; Ascher et al., 1978; Marchais and Marty, 1979). However, there was a consistent deviation between the voltage dependence of $\tau$ and that of the corrected steady-state $I-V$. The steady-state $I-V$ had a mean voltage dependence of $0.00962 \, mV^{-1}$ in six experiments using either voltage jumps or ramps. This value is significantly less than $k = 0.0122 \, mV^{-1}$ obtained for the $\tau$ vs. $V_m$ relationship in the same cells. The discrepancy was corrected by assuming a slight voltage sensitivity for $\beta^*$, $\sim 390 \, mV/e$-fold change. If the gating of the
ACh channel is accompanied by a change in effective dipole moment (Magleby and Stevens, 1972; Neher and Stevens, 1979), the channel closing corresponds to a change of about 75 debye while the channel opening corresponds to a change of only about 16 debye. This small voltage sensitivity of $\beta^*$ can explain the deviation in the $\alpha$ vs. $V_m$ relationship at +40 mV (Fig. 6). The mean value of $\alpha$ at +40 mV was determined, by use of Eqs. 4 and 5, to be 36 Hz, which falls below the straight line extrapolated from more negative potentials. By including the estimated voltage dependence of $\beta^*$ in the calculation, $\alpha(40)$ increases to 45 Hz which is close to the value of 43 Hz given by the straight line at +40 mV.

Although our equilibrium I-V was slightly less voltage dependent than $\alpha$, Adams and Sakmann (1978) recently found apparently steeper than normal equilibrium I-V curves. Their results are probably attributed to their use of slope rather than chord conductance. Our analysis shows that the equilibrium chord conductance, $G(V)$, is described by

$$G(V) = C e^{-kV},$$

where $C$ is a constant, and $k$ represents the voltage dependence of $G(V)$. The slope conductance, $S$, is

$$S(V) = \frac{\partial I_{m1}}{\partial V} = C \cdot (1 - kV) e^{-kV},$$

which in general is not an exponential function of $V$. However, when $kV$ is small,

$$S(V) \approx C \cdot e^{-2kV}$$

which has twice the voltage dependence of $G(V)$.

Another way of looking at the apparent discrepancy caused by use of slope conductance is by use of a correction factor, $H$. The steepness of the voltage dependence is obtained by examining relative conductances at different potentials. The relative slope conductance is

$$\frac{S(V)}{S(V + \Delta V)} = \frac{G(V)}{G(V + \Delta V)} \cdot H,$$

where $H = (1 - kV)/(1 - k(V + \Delta V))$ and $\Delta V$ is a positive increment of voltage. Since $k$ is positive, $H > 1$ for $kV < 1$. Therefore, for most cases where $V < +100$ mV, the relative slope conductance is steeper than the relative chord conductance. A trivial simulation shows that the equilibrium I-V of Adams and Sakmann (1978) is consistent with their $\tau$ vs. $V$ data without the necessity of invoking a voltage dependence of $\beta^*$ or other ad hoc assumptions.

**Instantaneous I-V**

The instantaneous I-V (Fig. 7a) was arbitrarily fitted with a saturating, two-barrier, one-site model (Adams, 1979; Lewis and Stevens, 1979; Marchais and Marty, 1979). This is a particularly simple model which satisfactorily describes
our data; however, further elaboration may well be required by the results of future experiments. Although the data are not sufficient to determine all of the free parameters in such a model, certain general principles can be formulated. It is important to indicate initially however, that the instantaneous $I-V$ is not consistent either with that predicted by a Goldman-Hodgkin-Katz model (for example, see Lewis, 1979) or that predicted by a long pore model with neutral sites (Barry et al., 1979). Both models predict a linear $I-V$ in symmetrical solutions, whereas our $I-V$ shows a definite curvature.

The lower part of Fig. 9 shows the kinetic model, which assumes that the rate constants for hopping over the barriers are exponential functions of both barrier height and fractional membrane potential. The site was arbitrarily
placed midway through the field. In accordance with Lewis and Stevens (1979) the energy level of the site was set at the solution level. If the barriers were symmetrically shaped and spaced (see Adams, 1979), the $I-V$ would be slightly supralinear. The sublinearity in our model was produced by placing the peaks of the barriers asymmetrically close to the membrane surface. In the simulation used for Fig. 7 a, the peak of the outer (i.e., extracellular) barrier was placed 11% through the membrane field from the outer membrane surface. The sublinearity arises because negative potential cannot supply ions to the site as effectively as it drives them out of the site.

The sublinearity in the instantaneous $I-V$ could be produced by other factors than the location of an outer barrier. For example, the Ca ions in the extracellular solution may not be negligible. In this case negative potentials could drive the Ca ions into the site, where they would reduce the apparent single channel conductance (Lewis, 1979; Lewis and Stevens, 1979). For example, if the dissociation constant for Ca ions at the mid-channel site is $\sim 25$ mM at $-70$ mV (Lewis, 1979), then the 1.5 mM Ca$^{2+}$ we used in the bath could significantly saturate the site at $-120$ mV, where the dissociation constant would be $\sim 3$ mM for a simple first-order reaction. This would effectively reduce the “instantaneous” conductance at the more hyperpolarized potential. This simple model predicts that the instantaneous conductance at $-120$ mV will be 68% of that at $-40$ mV. We actually saw a reduction to only 83% of that at $-40$ mV. However, this simplistic scheme does not take into account a finite flux of Ca ions over the inner barrier. This Ca permea-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{Two-barrier model. The upper part of the figure shows a hypothetical ACh receptor-channel complex (gray) in the lipid bilayer (cross-hatched). The outer dimensions are approximately drawn to scale from morphological evidence. A Na ion with the appropriate Stokes-Einstein radius is depicted in the site. The lower part of the figure shows the rate-limiting, free energy profile for ion movement through the channel. Free energy is plotted in $kT$ units against fractional electrical distance of an applied membrane potential. The rate constants are

\begin{align*}
F_1 &= A \cdot \exp (-G_i) \cdot \exp (-d_1 V F / RT) \\
B_1 &= B \cdot \exp (-G_i) \cdot \exp (d_2 V F / RT) \\
F_2 &= B \cdot \exp (-G_2) \cdot \exp (-d_3 V F / RT) \\
B_2 &= A \cdot \exp (-G_2) \cdot \exp (d_4 V F / RT),
\end{align*}

where $G_i$ and $G_2$ are the heights of the outer and inner barriers respectively, $A = 10^{11}$ s$^{-1}$ mol$^{-1}$, $B = 10^{13}$ s$^{-1}$, $V$ is applied membrane potential, and $F$, $R$, and $T$ have their usual meanings. The fractional electrical distances shown are $d_1 = d_4 = 0.11$ and $d_2 = d_3 = 0.39$. The net flux through the channel is given by

\begin{equation}
J = \frac{C \cdot (B_1 \cdot B_2 - F_1 \cdot F_2)}{C \cdot (B_2 + F_1) + B_1 + F_2'},
\end{equation}

where $C$ is the activity of Na ions. The single-channel current is given by

$I = e \cdot J$, where $e$ is the absolute value of the charge of an electron in coulombs.}
bility will increase the dissociation constant, depending on the relative barrier heights. Therefore our data are at least qualitatively consistent with a Ca blocking scheme. If Ca ions are responsible for the sublinearity in the instantaneous I-V, then the I-V should be sublinear only in the hyperpolarizing direction. Our data are not detailed enough to show this, but the instantaneous I-V recorded by Gage and Van Helden (1979) was approximately linear for outward currents and sublinear for inward currents, again consistent with a Ca blocking scheme.

Two other factors could produce the sublinearity. Both are related to the fact that the ionic strength of the solutions used in our experiments was lower than that of normal Ringer. The first possibility is that “diffusion polarization” causes ion depletion and/or accumulation near the mouth of the channel (Läuger, 1976). This increases the access resistance to the channel when the ionic strength is low. The second possibility is that multiple binding sites occur in the ACh channel. In some versions of multiple-site models, the shape of the I-V changes from supralinear to sublinear as the ionic strength is decreased (Hladky et al., 1979). These possibilities remain to be tested.

The instantaneous I-V was steeper in the negative than in the positive direction. This was achieved by making the inner barrier (G2) larger than the outer barrier (G1). Although G2 is only about one half a kT unit (where, in this case, k is Boltzmann’s constant and T is °K) larger than G1, this causes a twofold difference in the rate constants over each barrier. An equivalent way to fit the I-V was to make the barrier heights equal but include an extracellular surface potential of about −16 mV.

The model presented in Fig. 9 makes several predictions which are consistent with previously published data (for review, see Steinbach and Stevens, 1976). It predicts that, in normal Na Ringer, the single channel conductance will be about 25 pS at −80 mV. At this potential the open channel transports about 107 ions/s. The probability of the site being occupied is about 8 × 10⁻⁴. This value may be low because of the high values assumed for the attempt frequency for leaving the site. The accuracy and adequacy of the model is testable by systematic variations of ionic composition on both internal and external membrane surfaces.

The upper part of Fig. 9 is a schematic representation of a cross section through the ACh receptor-channel complex (gray) residing in the lipid bilayer (cross-hatched). The approximate external dimensions of the protein and its position in the membrane were determined by use of X-ray diffraction, negative staining transmission electron microscopy, and low angle neutron scattering of the ACh receptor from Torpedo californica (Ross et al., 1977; Klymkowsky and Stroud, 1979; Wise et al., 1979). The structure of the channel walls is not known. However, it is tempting to suppose that the 25Å depression observed in the center of the ACh receptor, when viewed from the external membrane surface, is the opening of the channel. There is suggestive evidence that the “selectivity filter” of the channel corresponds to the inner barrier (G2) of our proposed kinetic model:

(a) The selectivity filter has minimum dimensions of ~ 6.5 × 6.5Å (Maeno et al., 1977; Huang et al., 1978; Dwyer et al., 1979).
(b) Quaternary anesthetics, when applied extracellularly, probably bind to an intrachannel site which is well within the membrane field (Neher and Steinbach, 1978).

(c) The anesthetic QX-314 is incapable of reaching the binding site from the intracellular membrane surface (Horn et al., 1979).

(d) The dimensions of QX-314, determined from a Cory-Pauling-Koltun model (~8.2 × 6.0 × 12.8Å), are sufficiently large that it is unlikely that it can fit through the selectivity filter.

(e) Lewis and Stevens (1979) used a two-barrier, one-site model to fit reversal potentials and single-channel conductances observed with systematic changes of extracellular ion concentrations. They could fit their data only when the inner barrier height was equal to or greater than the outer barrier height.

(f) Our instantaneous I-V was best fit by assuming that the inner barrier was greater than the outer barrier.

It seems likely to us that both permeant ions and QX-314 are affected by the barrier asymmetry. In the latter case the inner barrier is insurmountably high. Although the outer barrier is depicted in Fig. 9 as a constriction in the channel, an image force barrier (Parsegian, 1975) may be sufficient to create the proposed energy barrier to ion movement.

The relative mobility of alkali metal cations is similar in water and in the ACh channel (Dwyer et al., 1979; Gage and Van Helden, 1979). This suggests that these ions exist in a hydrated form within the channel. Accordingly, the schematic drawing in Fig. 9 shows a scaled representation of a hydrated Na ion in the center of the channel.

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