# Inactivation of Potassium Current in Squid Axon by a Variety of Quaternary Ammonium Ions

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ABSTRACT The characteristics of potassium channel block by a diverse group of quaternary ammonium (QA) ions was examined in squid axons. Altering the size and nature of the head and/or tail groups of the QA ions applied internally produced only quantitative differences in the potassium current block. Although their entry rate is diminished, compounds with head groups as large as 11 × 12 Å are capable of occluding the channel, whereas the smallest QA ions, with head groups approximately 5 × 6 Å, are not potent blockers. When one or three terminal hydrogens of the head group were replaced by hydroxyl moieties, the compound's blocking ability was diminished, suggesting that QA binding is not improved by hydrogen bonding at these positions. QA ions bound to their site within the potassium channel with 1:1 stoichiometry, and the site is perhaps 20% or more of the distance through the membrane electric field. Raising external potassium concentration did not alter the steady-state or kinetic features of the QA block of outward potassium currents; however, increasing temperature or adding $Ba^{2+}$ internally increased the rate of decay of the QA-blocked currents. From the structure-function analysis of the QA ions, projections concerning both the architecture of the potassium channel's inner mouth and the significance of various chemical constituents of the ions were made. The potassium channel may now be pictured as having a wider mouth (up to 11 × 12 Å) extending to the QA binding site and then narrowing quickly to the region of channel selectivity. Important alterations that improve the blocking ability of the compounds include: (*a*) lengthening the alkyl hydrocarbon tail group (up to 10 carbons), (*b*) lengthening a second hydrocarbon chain of the head group (e.g., decyldimethylphenylammonium bromide [$C_{10}DM\phi$]), and (*c*) adding a carbonyl moiety to the tail (e.g., ambutonium).

## INTRODUCTION

After depolarization, intracellular tetraethylammonium ion ($TEA^+$) and its derivatives with one alkyl chain extended have been shown to diffuse into open potassium channels, producing a time-dependent block of the outward potassium currents in squid axons (Armstrong, 1969, 1971, and 1975). From the interactions between these compounds and the potassium channel, Armstrong (1975) has described the intracellular mouth of the potassium channel

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as relatively large and nonselective (≃8 × 8 Å). Similar in radius, both the fully hydrated potassium ion and the blocking $TEA^+$ enter this region. Farther into the membrane, the channel narrows to form a more selective region, ~3 Å in diameter, where the permeant potassium ion partially dehydrates.

The potassium channel is thought to be blocked by $TEA^+$ and its derivatives as the ion diffuses into the open channel, directly impeding the permeation pathway of the potassium. In some respects the resulting time-dependent block of the potassium current resembles sodium-current inactivation. When the holding potential is reinstated after a depolarization, increased potassium-ion influx speeds the recovery from $TEA^+$ block (Armstrong, 1975).

Perhaps the most significant observation concerning potassium channel architecture arising from these experiments was the location of the gating apparatus on the intracellular side. After having entered the channel from the internal side of the membrane, $TEA^+$ and its derivatives were trapped inside the channel when the channel gates were rapidly closed by hyperpolarizing the membrane to −90 mV (Armstrong, 1971).

In this report, using internally perfused squid giant axons, I have fit the block by a broad group of quaternary ammonium (QA) ions by the model just described. The characteristics of the block by QA ions were not altered after modest chemical changes in the compounds; however, consideration of all the data produced both a more detailed map of the potassium channel and important information concerning the potency of QA ions. The potassium channel appears to have a wider mouth than previously thought, and the region surrounding the nitrogen binding site does not allow hydrogen bonding. Two general features of the compounds correlate with potency. First, the absolute size of the head group influences the block, inasmuch as both large and small head groups decrease potency. Second, the ability of the head group constituents to form hydrophobic, but not hydrogen, bonds is important for strong blocking ability.

## METHODS

Giant axons were isolated from the mantle of the squid *Loligo pealei* ob tained from the Marine Biological Laboratory, Woods Hole, Mass. The internal perfusion and voltage-clamp techniques were similar to those described by Bezanilla and Armstrong (1977). Linear ionic and capacitive currents were subtracted by the P/4 technique also as described by Bezanilla and Armstrong (1977), and the records were stored on the PDP-8E computer (Digital Equipment Corp., Marlboro, Mass.) described therein.

The standard external solution (Tris 50 + TTX) consisted of 480 mM Trizma 7.0 (Sigma Chemical Co., St. Louis, Mo.), 50 mM $CaCl_2$, and 200 nM tetrodotoxin. The internal perfusate (275 KFG) contained 50 mM KF, 225 mM potassium glutamate, 420 mM sucrose, and 10 mM Trizma. In all cases, the pH was adjusted to 7.0–7.1, and the osmolarity to 950–1,000 mosM.

The quaternary ammonium ions were either synthesized for Dr. C. M. Armstrong by Eastman Organic Chemicals Div., Eastman Kodak Co., Rochester, N. Y., or made in the laboratory. The addition of two parts tertiary amine to one part bromohydrocarbon in the presence of excess acetone with gentle refluxing for 12–18 h yielded the

desired quaternary ammonium compound. The compound was then precipitated in petroleum ether, dried, and tested for purity using thin-layer chromatography and staining techniques. Decanol and $C_{10}DM\phi$ were purchased from Eastman Organic Chemicals, and the local anesthetic QX314 was a gift from Dr. Bertil Takman of the Astra Pharmaceutical Co.

## RESULTS

Armstrong and co-workers (Armstrong and Binstock, 1965; Armstrong and Hille, 1972; Armstrong, 1969 and 1971) reported that $TEA^+$ and its derivatives diffuse into the open potassium channel and block the pore without affecting the channel's activation gating. They successfully described the blocking process using a sequential model for channel activation and block:

$$\text{CLOSED} \rightleftharpoons \text{OPEN} \underset{\text{UNBLOCK (L)}}{\overset{\text{BLOCK (K)}}{\rightleftharpoons}} \text{QA-BLOCKED} \quad (1)$$

### *Tests of the Conformity of New QA Ions to the Existing Model*

COMPUTER SIMULATIONS OF THE BLOCK The initial objective of these experiments was to test the applicability of the existing model to the block elicited by the new QA ions. With the exception of decanol, all the compounds tested were quaternary ammonium salts (QA) differing only in the exact constitution of the head or tail group (see Table I). When internally applied to perfused squid axons, all the compounds behaved qualitatively similarly, causing the potassium currents to "inactivate" without apparently altering the channels' activation kinetics.

Fig. 1 reiterates the fundamental features of potassium channel block by one of the new QA derivatives, $C_8$DEEtoh (see Table I for nomenclature). In Fig. 1 *A* potassium currents at two positive membrane potentials, before and after the addition of 30 $\mu$M $C_8$DEEtoh, are shown. As the model predicts, the earliest part of the rising phase of the currents is unaffected by the drug. After the channels have opened, entrance of $C_8$DEEtoh pulls channels into the nonconducting, QA-blocked state, causing the currents to appear to inactivate.

A more rigorous test of these features of the model was performed by fitting the time-course of the QA-blocked currents. The model assumes that channel-opening kinetics are unaffected by the presence of QA compounds. Thus, at potentials (e.g., $V_m \geq +60$ mV) and drug concentrations at which all the channels are activated before any are blocked, potassium currents in the presence of QA compounds may be described as follows:

$$\frac{dI_{QA}}{dt} = \frac{dI_{CONTROL}}{dt} - KI_{QA} + L\,(I_{CONTROL} - I_{QA}), \quad (2)$$

where $I_{QA}$ and $I_{CONTROL}$ are the potassium current in the presence and absence of the drug, respectively, $K$ is the blocking rate constant, and $L$ is the unblocking rate constant (Eq. 1). As shown in Fig. 1 *B*, this formalism adequately fits the $C_8$DEEtoh-treated potassium currents. In general, the action of all the compounds in Table I could be well fit with Eq. 2.

QA IONS BIND 1:1 TO THE SITE The blocking and unblocking process in the model (Eq. 1) may be more explicitly written,

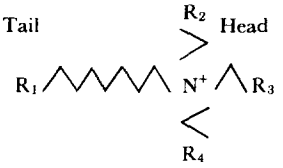
$$QA + S \underset{l}{\overset{b}{\rightleftharpoons}} QA - S, \tag{3}$$

where S is the blocking "site" in the open potassium channel, QA − S

TABLE I

GENERAL MOLECULAR STRUCTURES OF THE COMPOUNDS

General Structure




Triethylammonium Compounds ($R_2$, $R_3$, $R_4 = C_2H_5$)

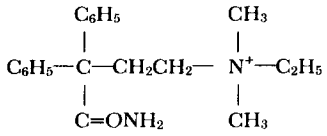
| | $R_1$ |
|---|---|
| ($C_7$TE) heptyltriethylammonium | $C_7H_{15}$– |
| ($C_8$TE) octyltriethylammonium | $C_8H_{17}$– |
| ($C_9$TE) nonyltriethylammonium | $C_9H_{19}$– |
| ($C_{10}$TE) decyltriethylammonium | $C_{10}H_{21}$– |
| ($\phi$PTE) phenylpropyltriethylammonium | $C_6H_5(C_3H_6)$– |
| QX314 | $(CH_3)_2C_6H_5NHCOCH_2$– |

Octyl- and Decylalkylammonium Compounds ($R_1 = C_8H_{17}$; $R_1 = C_{10}H_{21}$)

| | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|
| ($C_8$DMEtoh) octyldimethylethanolammonium | $-CH_3$ | $-CH_3$ | $-C_2H_4OH$ |
| ($C_8$DEEtoh) octyldiethylethanolammonium | $-C_2H_5$ | $-C_2H_5$ | $-C_2H_4OH$ |
| ($C_8$DEProh) octyldiethylpropanolammonium | $-C_2H_5$ | $-C_2H_5$ | $-C_3H_6OH$ |
| ($C_8$TP) octyltripropylammonium | $-C_3H_7$ | $-C_3H_7$ | $-C_3H_7$ |
| ($C_8$TEtoh) octyltriethanolammonium | $-C_2H_4OH$ | $-C_2H_4OH$ | $-C_2H_4OH$ |
| ($C_{10}$DM$\phi$) decyldimethylphenylammonium | $-CH_3$ | $-CH_3$ | $-C_6H_5$ |
| ($C_{10}$TP) decyltripropylammonium | $-C_3H_7$ | $-C_3H_7$ | $-C_3H_7$ |
| ($C_{10}$TEtoh) decyltriethanolammonium | $-C_2H_4OH$ | $-C_2H_4OH$ | $-C_2H_4OH$ |

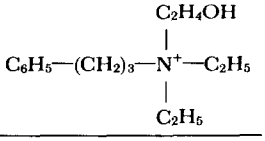
Other Compounds

Ambutonium




Decanol $C_{10}H_{20}OH$

($\phi$PDEEtoh) phenylpropyldiethylethanolammonium




represents the QA-blocked channel, $b$ is the rate of block, and $l$ is the rate of dissociation. As written, this scheme implies that one QA ion binds to one site inside each potassium channel. Fig. 2 illustrates two standard forms of evidence supporting 1:1 binding of QA ions to potassium channels. When dose and

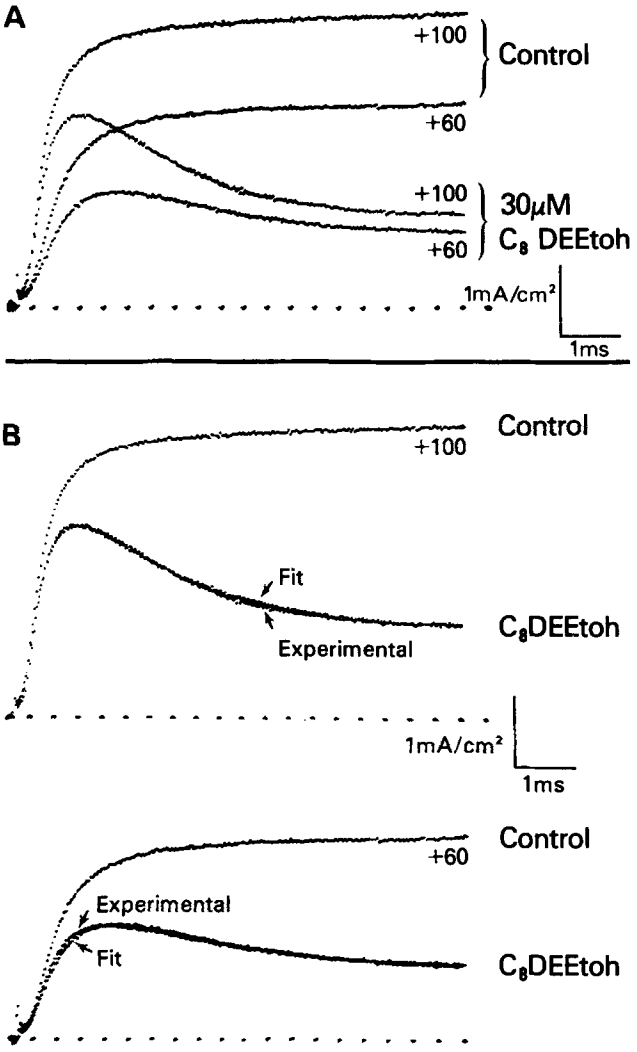



FIGURE 1. Block of the potassium current by 30 μM $C_8$DEEtoh. (*A*) Potassium currents before and during application of $C_8$DEEtoh at +60 and +100 mV. Axon AU159W. Tris 50 (−114 mM Tris) + TTX + 114 $K^+$//275 KFG. 8°C. (*B*) The *top panel* shows the fit (*Fit*) of the current in the presence of $C_8$DEEtoh (*Experimental*) with Eq. 2 at +100 mV. $K = 0.234$ ms$^{-1}$, $L = 0.112$ ms$^{-1}$. The *lower panel* is the same procedure at +60 mV; $K = 0.162$ ms$^{-1}$, $L = 0.118$ ms$^{-1}$.

response are plotted on a linear scale, 1 : 1 binding should produce a hyperbolic curve, whereas cooperative binding yields a sigmoid relation. Fig. 2 *A* illustrates that a hyperbolic curve does result when the block by $C_8$DEProh is analyzed in this way.

Fig. 2 *B* shows the same data plotted as a Hill plot. When a binding reaction with $n$ molecules takes place,

$$n\text{QA} + \text{S} \rightleftharpoons n\text{QA} - \text{S}, \tag{4}$$

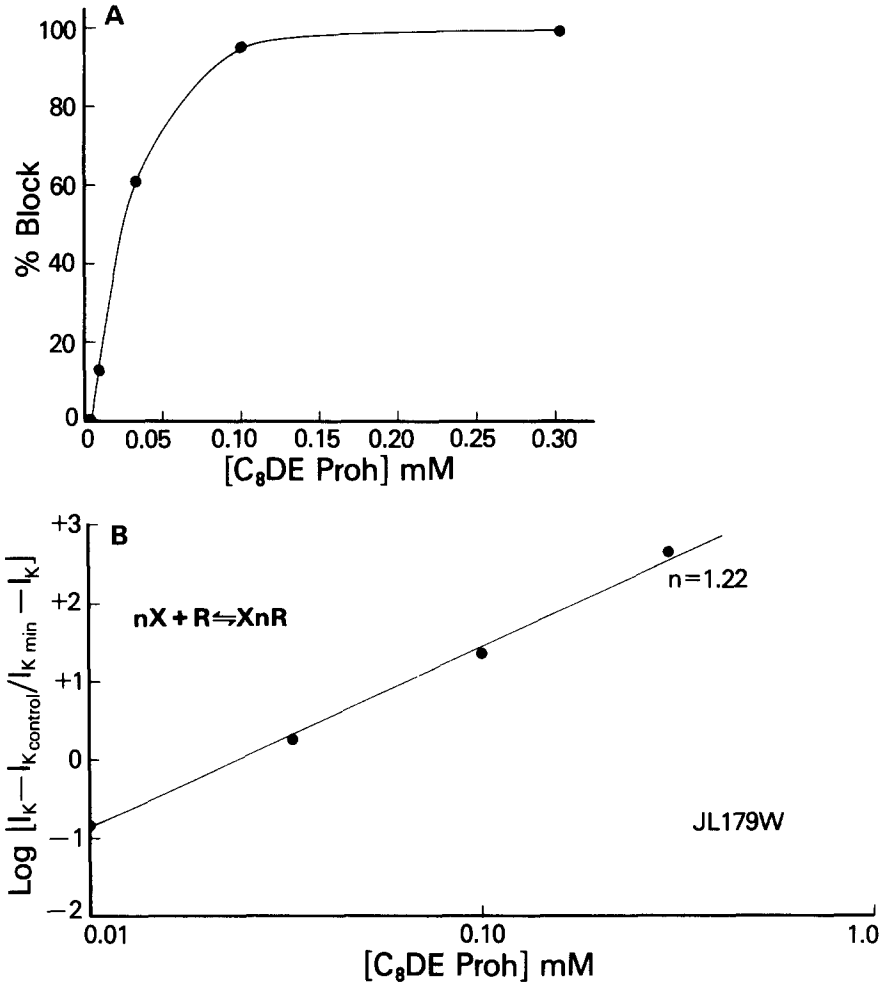



FIGURE 2. $C_8$DEProh blocks the potassium channel by binding with 1:1 stoichiometry. (*A*) The relationship between percent block at +100 mV and $C_8$DEProh concentration using linear axes. Axon JL179Z. Tris 50 + TTX//275 KFG. 8°C. (*B*) A Hill plot of the same data demonstrating that the points fall on a best fit straight line with a slope of 1.22 ($r$ = 0.99). Control potassium current, potassium current with $C_8$DEProh, and the potassium current with the greatest drug block are $I_{K_{CONTROL}}$, $I_K$, and $I_{K_{min}}$, respectively (y-axis).

the dose-response curve with the axes indicated is a straight line of slope $n$. The slope of the best fit linear regression line in Fig. 2 *B* was 1.22, consistent with 1:1 binding of the QA ion to the potassium channel.

QA BLOCKING RATE IS A LINEAR FUNCTION OF CONCENTRATION Another requisite of the model described in Eq. 3 is that the time constant of decay of the potassium current ($\tau_{decay}$) is inversely related to concentration of the QA

ion at any voltage at which all the channels are activated:

$$\tau_{decay} = \frac{1}{l + b\,[QA]}. \tag{5}$$

In Fig. 3 *A*, it is evident that the rate of decay of the current at +100 mV increased after the applied concentration of $C_8$DEProh was increased from 10

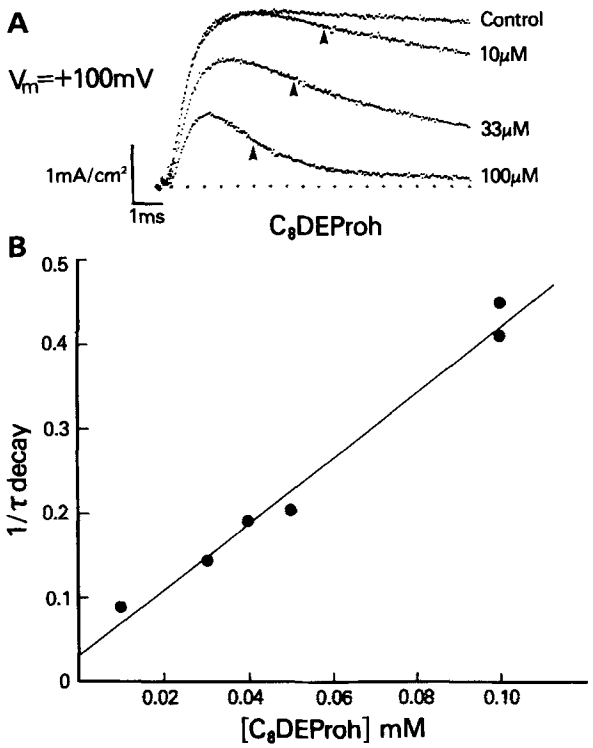



FIGURE 3. The time constant of decay of the $C_8$DEProh-blocked potassium currents is a linear function of concentration. (*A*) 10, 33, and 100 μM $C_8$DEProh are shown to inactivate the potassium current increasingly more rapidly at +100 mV. The exponential is fit from the point indicated by the arrowheads. Axon JL179W (same as Fig. 2). (*B*) The data from *A* and a number of other experiments are plotted. The straight line, $1/\tau_{decay} = b[C_8DEProh] + l$, was fit with $b = 4.0\ ms^{-1}\ mM^{-1}$, $l = 0.032\ ms^{-1}$ ($r = 0.98$).

to 100 μM. Fitting a single exponential to the declining potassium currents yielded $\tau_{decay}$ of 11.42, 6.88, and 2.21 ms for 10, 33, and 100 μM, respectively. When the inverse of $\tau_{decay}$ from several experiments with $C_8$ DEProh was plotted as a function of the applied concentration, a linear dependence was found (Fig. 3 *B*). The best fit linear regression line shown had a coefficient of determination ($r$) of 0.98. A similar dependence of $\tau_{decay}$ on concentration has been demonstrated by Armstrong (1971).

*Relative Potency of the QA Ions*

The relative potencies of all 17 compounds listed in Table I were measured from the steady-state block. In the steady state, at any voltage at which all the channels are open,

$$K_d = \frac{I_{QA(\infty)}}{I_{CONTROL(\infty)} - I_{QA(\infty)}}[QA] = \frac{l}{b}. \quad (6)$$

To ensure that $I_{QA}$ had reached steady state, a single exponential was fitted to the falling phase of the potassium current, and the exponential was extrapolated to time = $\infty$ to give $I_{QA(\infty)}$. Table II lists all the compounds tested in order of increasing $K_d$ at $V_m$ = +100 mV.

With the exceptions of the local anesthetic QX314 and $C_8$DMEtoh, all of

TABLE II

COMPOUNDS RANKED IN DECREASING ORDER OF POTENCY AT +100 mV

| | $K_d$ | $n$ |
|---|---|---|
| | $\mu M$ | |
| 1. ($C_{10}$TE) decyltriethylammonium | 3.7 | 3 |
| 2. ($C_9$TE) nonyltriethylammonium | 4.1 | 1 |
| 3. ($\phi$PTE) phenylpropyltriethylammonium | 7.0 | 1 |
| 4. ($C_8$DEProh) octyldiethylpropanolammonium | 7.0 | 7 |
| 5. ($C_8$TE) octyltriethylammonium | 7.3 | 2 |
| 6. ($\phi$PDEEtoh) phenylpropyldiethylethanolammonium | 7.7 | 1 |
| 7. ($C_8$DEEtoh) octyldiethylethanolammonium | 12.9 | 4 |
| 8. ($C_{10}$DM$\phi$) decyldimethylphenylammonium | 13.3 | 1 |
| 9. ($C_7$TE) heptyltriethylammonium | 17.6 | 1 |
| 10. ($C_8$TP) octyltripropylammonium | 28.3 | 2 |
| 11. Ambutonium | 56.3 | 2 |
| 12. ($C_{10}$TP) decyltripropylammonium | 92.0 | 2 |
| 13. ($C_{10}$TEtoh) decyltriethanolammonium | 132.3 | 4 |
| 14. Decanol | 937.5 | 1 |
| 15. QX314 | 961.0 | 1 |
| 16. ($C_8$TEtoh) octyltriethanolammonium | 1,112.0 | 3 |
| 17. ($C_8$DMEtoh) octyldimethylethanolammonium | >2,000.0 | 2 |

the compounds produced "inactivation" of the potassium current at +100 mV.

ADDING METHYLENE GROUPS TO THE TAIL ENHANCES BLOCK As previously proposed in potassium (Armstrong, 1969 and 1971) and sodium (Rojas and Rudy, 1976) channels, the addition of methylene groups to the tail of QA ions increases their potency in the manner expected from increased hydrophobic binding (n.b. $C_7 \rightarrow C_{10}$ TE; Table II). For $C_7$TE to $C_{10}$TE ions an increase of roughly 1,000 cal/mol/$CH_2$ group is calculated from the best fit straight line. This is higher than previously reported (525 and 560 cal/mol/$CH_2$, Armstrong [1969] and Rojas and Rudy [1976], respectively), suggesting that for the QA ions examined, all of the added $CH_2$ groups participate effectively in hydrophobic bonding, whereas for the shorter-chain compounds previously reported this is not the case.

The addition of one methylene group to $C_9TE$ to produce $C_{10}TE$ only slightly increased the potency in comparison to the approximate twofold enhancement between the other congeners. This is consistent with Armstrong's (1971) observation that $C_{12}TE$ is less potent than $C_9TE$, perhaps suggesting an optimum length of the tail group for beneficial hydrophobic binding or limiting aqueous solubility.

LARGE AND SMALL HEAD GROUPS DECREASE POTENCY Both increasing and decreasing the absolute size of the head group by a few angstroms (see Table I) relative to triethyl-N-R ($\simeq 8 \times 8$ Å) often decreases potency. The compounds with large head groups, tripropyl ($11 \times 12$ Å) and triethanol ($12 \times 10$ Å) have higher $K_d$s than their triethyl congeners. As may be seen in Table III, the high $K_d$ of these large compounds reflects their difficulty in reaching the QA blocking site (small forward rate constant), perhaps due to steric hinderance.

The compound with the smallest head group, $C_8$DMEtoh ($\simeq 5 \times 6$ Å), was the least potent blocker tested. Diminished blocking capacity of QA ions with a small head group has been previously noted for $C_8TM$ (Armstrong, 1971)

TABLE III

RATE CONSTANTS* OF BLOCK ($b$) AND UNBLOCK ($l$) AT +100 mV GROUPED ACCORDING TO HEAD GROUP TYPE‡

| Head groups | $n$ | $b$ ($ms^{-1}$ $mM^{-1}$) | $l$ ($ms^{-1}$) |
|---|---|---|---|
| -TE | 3 | 5.6 | 0.32 |
| -DEEtoh | 3 | 5.6 | 0.73 |
| -TP | 3 | 0.98 | 0.30 |
| -TEtoh | 4 | 0.60 | 1.22 |

* Calculated as in Table II.

‡ Each group is comprised of the following compounds: (-TP) $C_{10}TP$ and $C_8TP$; (-TEtoh) $C_{10}TEtoh$ and $C_8TEtoh$; (-TE) $C_{10}TE$, $C_8TE$, and $\phi PTE$; (-DEEtoh) $C_8DEEtoh$ and $\phi DEEtoh$.

and for $C_9DME$ (unpublished observation). Although this decreased potency requires further inspection, it probably reflects an inability to find a stable binding site not a hindered rate of entry.

HYDROXYL GROUPS DIMINISH POTENCY Hydroxyl-substituted QA ions can enter and block the potassium channels, the interior of which is generally considered to be hydrophilic. One would expect that the addition of hydroxyl groups should allow for hydrogen bonding between the terminal hydroxyl groups and any available electronegative groups near the QA binding site, resulting in a more stable bond and increased potency. Despite the potency of the hydroxyl-containing QA ions (n.b. Table II), there was no indication of an additional blocking capacity derived from the formation of hydrogen bonds.

The replacement of a single hydrogen by a hydroxyl (i.e., $C_8TE$ vs. $C_8DEEtoh$ or ØPTE vs. ØPDEEtoh) slightly decreased the compounds' potency (see Table II). As can be seen in Table III, the addition of one hydroxyl group (-DEEtoh from -TE) had no effect on the rate of block ($b$)

but more than doubled the rate of dissociation ($l$). Small changes in the values of $l$, however, must be treated cautiously (see below).

When all three termini of the head group were given a hydroxyl group, a more dramatic decrease in potency was manifested (compared $C_8$TE with $C_8$TEtoh or $C_{10}$TE with $C_8$TEtoh in Table II). Inasmuch as these triethanol-containing compounds have diminished blocking rate due to their large head group size, it is more appropriate to compare their kinetics with the tripropyl congeners with a similar head group size. Table III demonstrates that compared with the -TP group the rate of block ($b$) of the -TEtoh group is slightly slower, but rate of unblock ($l$) is increased by a factor of 4. The addition of three terminal -OH groups apparently limits the stability of the QA bond at the blocking site. These observations are consistent with the hypothesis that the termini of the head groups may participate in hydrophobic binding and that replacement of hydrogens by hydroxyl groups destabilizes the binding.

It seems that the region surrounding the binding site is probably not surrounded by electronegative groups to which the hydroxyl groups could hydrogen bond. If the potassium-channel selectivity occurs at a site surrounded by electronegative oxygen molecules (Bezanilla and Armstrong, 1972; Hille, 1973 and 1975), the QA receptor is most likely not at the site of ion dehydration (the "selectivity filter").

HYDROPHOBIC BONDS BY THE HEAD GROUP MAY BE IMPORTANT TO BLOCK Data in the last section suggested that some hydrophobic binding by the arms of the head group may be required to stabilize the QA ion at its site. $C_8$DMEtoh and $C_{10}$TM (Armstrong, 1971) may have a head group too small to form any hydrophobic bonds and therefore have limited blocking capacity. $C_{10}$DMØ, whose $K_d$ is less than would be anticipated from its dimethyl constituent, must derive this enhancement from the extended arm (Ø) in the head group. The addition of one methylene group to $C_8$DEEtoh to make $C_8$DEProh improved its blocking ability; the $K_d$ decreased from 12.7 to 7.0 $\mu$M. Further evidence supporting the proposition that QA ions may be stabilized at their blocking site through hydrophobic bonds is presented in Table IV. The rate constant of unblock ($l$) decreased (i.e. slower exit) when methylene groups were added to the head group, as exemplified by comparing $C_{10}$TE with $C_{10}$TP, $C_8$TE with $C_8$TP, and $C_8$DEEtoh with $C_8$DEProh. And finally, Shoukimas and French (1979) reported that the $K_d$s of symmetric QA ions decreased with increased methylene groups according to an increase in hydrophobic binding.

The formation of hydrophobic bonds by head group moieties may explain numerous alterations in QA block: ($a$) limited block by small QA ions, ($b$) decreased $K_d$s when methylene groups are added, ($c$) slower off rates when methylene groups are added, and ($d$) diminished potency when -OHs are added. Apparently, the formation of hydrophobic bonds by both the head group and tail of QA ions is important to their blocking ability, and as long as entry rate is not impaired (-TP) increases potency.

### *New Observations on the Characteristics of QA Ion Block*

LARGE DEPOLARIZATIONS INTENSIFY QA BLOCK A strongly rectifying block of the potassium current by $TEA^+$ (i.e., block was strong when potassium current was outward) was described by Armstrong and Binstock (1965), but the degree to which the block was affected by voltage was not examined. When positively charged QA ions enter potassium channels, their movement might be directly influenced by potential. Shoukimas and French (1979) have suggested that symmetrical QA ions exhibited a slight increase in block at large depolarizations.

TABLE IV

RATE CONSTANTS* OF BLOCK ($b$) AND UNBLOCK ($l$) AS A FUNCTION OF MEMBRANE POTENTIAL

| Compound | $b$ ($ms^{-1}$ $mM^{-1}$) | | | | $l$ ($ms^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|
| | +60 | +80 | +100 | $n_b$ | +60 | +80 | +100 | $n_l$ |
| Hydroxyl-containing | | | | | | | | |
| $C_8$DEProh ($n = 2$) | 2.56 | 3.55 | 4.10 | 0.294 | 0.0236 | 0.0308 | 0.0262 | 0.066 |
| $C_8$DEEtoh (1) | 6.2 | 8.0 | 9.4 | 0.26 | 0.0108 | 0.0125 | 0.0136 | 0.144 |
| $\phi$PDEEtoh (1) | 3.4 | 3.9 | 4.3 | 0.147 | 0.024 | 0.037 | 0.033 | 0.199 |
| $C_{10}$TEtoh (3) | 0.43 | 0.55 | 0.65 | 0.258 | 0.084 | 0.097 | 0.110 | 0.169 |
| mean ± SE | | | $\bar{n}_b = 0.240 \pm 0.03$ | | | | $\bar{n}_l = 0.145 \pm 0.03$ | |
| Triethyl-containing | | | | | | | | |
| $C_7$TE (1) | 2.8 | 3.4 | 3.6 | 0.157 | 0.071 | 0.072 | 0.063 | 0.075 |
| $C_8$TE (1) | 5.1 | 5.5 | 5.9 | 0.091 | 0.038 | 0.038 | 0.043 | 0.077 |
| $C_9$TE (1) | 5.5 | 6.1 | 7.3 | 0.177 | 0.030 | 0.028 | 0.033 | 0.060 |
| $C_{10}$TE (1) | 5.2 | 6.0 | 6.7 | 0.158 | 0.019 | 0.019 | 0.023 | 0.120 |
| $\phi$PTE (1) | 2.5 | 2.7 | 3.5 | 0.210 | 0.015 | 0.013 | 0.025 | 0.32 |
| mean ± SE | | | $\bar{n}_b = 0.159 \pm 0.02$ | | | | $\bar{n}_l = 0.10 \pm 0.06$ | |
| Tripropyl-containing | | | | | | | | |
| $C_{10}$TP (1) | 0.13 | 0.15 | 0.17 | 0.168 | 0.015 | 0.016 | 0.015 | 0 |
| $C_8$TP (2) | 0.95 | 1.22 | 1.40 | 0.242 | 0.021 | 0.031 | 0.039 | 0.39 |
| mean ± SE | | | $\bar{n}_b = 0.205 \pm 0.04$ | | | | $\bar{n}_l = 0.20 \pm 0.19$ | |
| grand mean ± SE | | | $\bar{n}_b = 0.197 \pm 0.02$ | | | | $\bar{n}_l = 0.134 \pm 0.04$ | |

* The values of $b$ and $l$ were calculated from simultaneous solution of the equations, $K_d = l/b$ and $\tau_{decay} = \frac{1}{l + b\,[QA]}$. $n$ is a parameter reflecting the steepness of the voltage dependence (see Eq. 7) of block.

Table IV lists the rate constants of block ($b$) and unblock ($l$) calculated from Eqs. 5 and 6 at potentials sufficiently positive to ensure that all the potassium channels are opened. These values were fitted with single exponentials of the form,

$$b = b_0 e^{n_b ZFV_m/RT}$$

and (7)

$$l = l_0 e^{-n_l ZFV_m/RT},$$

where the constant $n$ represents the fractional effect of the electric field, $z$ =

$+1$, $F/RT = 41.33\ V^{-1}$ at 8°C, and $V_m$ is membrane potential. The values of $n_l$ and $n_b$ are listed in Table IV, with the mean of $n_l$ and $n_b$ for each group, and for all 11 compounds. The increase in $b$ with potential is consistent with the movement of the charged QA derivatives through, on the average, 20% of membrane field is crossing an unspecified energy barrier, i.e., $n_b \simeq 0.2$. This increase can not be attributed to an interaction with $K^+$ because, as is shown below, increasing $K_o^+$ has no influence on either the kinetics or the steady-state block. Rather surprisingly, $l$ increases somewhat with potential, as described below.

To further evaluate the influence of potential on the blocking and unblocking processes, two other methods were employed. The straight line describing the relationship between $1/\tau_{decay}$ and QA concentration shown in Fig. 3 *B* has slope of $b$ and a $y$ intercept of $l$. For the three compounds for which there is sufficient data to calculate $1/\tau_{decay}$ as a function of concentration at +60, +80, and +100 mV, $b$ increased with potential (Table IV). In all three cases, $l$ slightly decreased over this voltage range.

The rate constants governing block and unblock of the potassium channel may also be measured by fitting the currents in the presence of QA with Eq. 2. Again, the blocking rate constant consistently increased with potential ($n = 5$), while the rate constant governing unblock was unchanged in two cases, decreased in two cases, and increased slightly with potential on one occasion.

Of the three methods of measuring the rate constants, that used in Table II (calculating $l$ and $b$ from the observed values of $\tau_{decay}$ and $K_d$) is most sensitive to errors arising from nonlinear leakage. Small increases in outward nonlinear leakage current at large potentials will tend to cause an underestimate of steady-state block ($K_d$) and, therefore, an overestimate of $l$, whereas the other two methods do not rely on the steady-state level of block. Any voltage dependence of $l$ must be slight, and further experiments will be required to properly assess this question.

BLOCK AT LARGE DEPOLARIZATIONS IS INDEPENDENT OF $K_o^+$ At potentials equal to or more negative than the holding potential increasing $K_o^+$ significantly speeds the rate of recovery from QA block (Armstrong, 1969 and 1971; Armstrong and Hille, 1972). At large depolarizations, I find that sizeable changes in $K_o^+$ have no effect on block. In Fig. 4 *A*, the block exhibited by $C_8$TEtoh in 0 or 120 mM $K^+$ is illustrated. In 0 $K_o^+$, the steady-state block was 47% and $\tau_{decay}$ equaled 1.74 ms. When 120 mM $K_o^+$ was substituted, the steady-state block was 46% and $\tau_{decay}$ was 1.80 ms. Similarly, (Fig. 4 *B*) with another new compound, $C_8$TP, the steady-state block was 16% in 0 $K_o^+$ and 18% in 120 mM $K_o^+$, while $\tau_{decay}$ was 2.08 and 1.89 ms, respectively. These small changes are not significant, and changing $K_o^+$ does not affect the block at these potentials.

INCREASING TEMPERATURE DRAMATICALLY SPEEDS BLOCK Fig. 5 shows an example of the influence of increased temperature on the rate of inactivation and steady-state block by 30 μM $C_{10}$TE at +100 mV. At 6°C the potassium current rises slowly and decays very slowly; the compound enters and blocks the channels slowly. In contrast, at 16°C, the current peaks quickly and

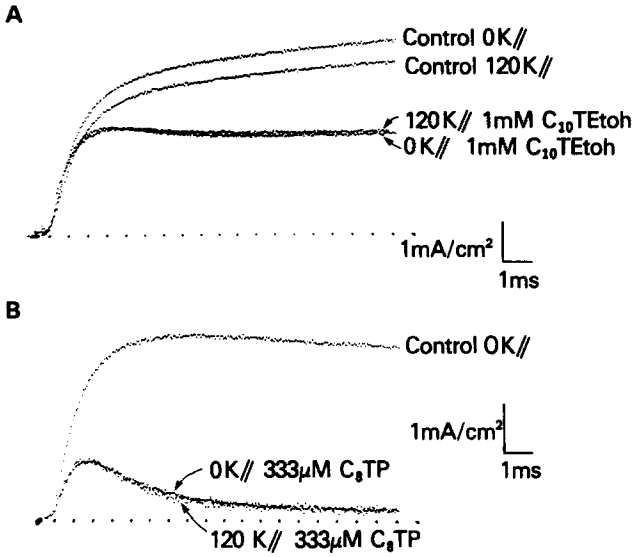



FIGURE 4. The effect of increased extracellular potassium on the QA block at +100 mV. (*A*) Two sets of potassium currents before and during application of 1 mM $C_{10}$TEtoh in 0 K// and 120 K//. The current in the presence of 1 mM $C_{10}$TEtoh and 120 K// was scaled by the difference in the magnitude of the controls (to account for the difference in driving force) to facilitate comparison of the kinetics of the block in 0 K//. Axon AU279Z. Tris 50 + TTX//275 KFG or Tris 50 (−120 mM Tris) + TTX + 120 $K^+$//275 KFG. 8°C. (*B*) Comparison of the block by 333 μM $C_8$TP in 0 K// and 120 K//. The trace in 333 μM $C_8$TP and 120 K// was scaled to account for the difference in driving force (as in *A*). Axon AU219Z. Solutions as in *A*. 8°C.

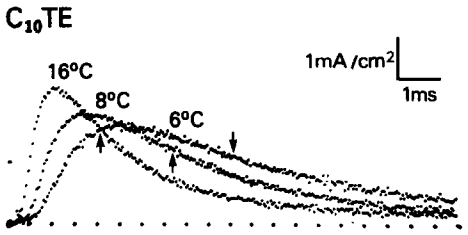



FIGURE 5. Comparison of the block by 30 μM $C_{10}$TE at 6°, 8°, and 16°C at +100 mV. The rate of decay of the potassium current is increased as the temperature is increased with a $Q_{10}$ = 2.8. Exponential fitted from the point indicated by the arrows. Axon JL249Z. Tris 50 + TTX//275 KFG.

subsequently decays quickly as $C_{10}$TE blocks. The current at 8°C illustrates an intermediate case. As the temperature was increased from 6° to 8°C, and finally to 16°C, $\tau_{decay}$ decreased from 5.78 to 3.83 to 2.06 ms, respectively. This change was logarithmic with temperature and had a $Q_{10}$ of 2.81. In other experiments, $Q_{10}$s with other compounds were as high as 5.0.

In free solution, diffusion of symmetrical QA ions exhibits $Q_{10}$s ranging from 1.12 to 1.19 (Robinson and Stokes, 1970), suggesting that this is not the significant mechanism speeding block as temperature increases. After diffusing into the channel, the QA ions bind, and it is probably this step in the blocking sequence that is highly temperature dependent.

QA IONS AND $Ba^{2+}$ BLOCK SIMULTANEOUSLY If QA ions do in fact enter and block the open potassium pore, then blocking cations, e.g., $Cs^+$ (Bezanilla and Armstrong, 1972) or $Ba^{2+}$ (Armstrong and Taylor, 1980), should compete with QA ions for the open potassium channels. Experiments with internal $Cs^+$ ion were not fruitful, because concentrations of $Cs^+$ sufficient to block the channel (≃50 mM) signifiantly slowed the kinetics of channel activation. Apparent competition for the open channel was observed with 2 mM $Ba^{2+}$ and 30 μM $C_8$DEEtoh internally (Fig. 6).

In the simplest case, in which only one blocking ion is allowed to enter each

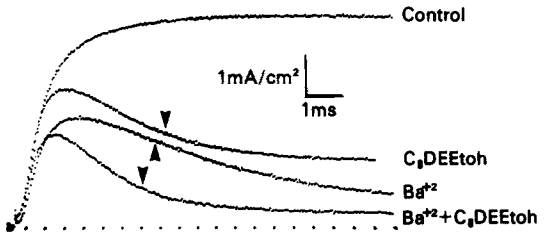



FIGURE 6. Simultaneous block of the open potassium channel by 2 mM $Ba^{2+}$ and 30 μM $C_8$DEEtoh at +100 mV. The steady-state block was 68, 88, and 95% by $C_8$DEEtoh, $Ba^{2+}$, and both together, respectively. The exponential is fit from the point indicated by the arrowheads. Axon AU159W. Tris 50 (−114 mM Tris) + TTX + 114 K// 275 KFG. 8°C.

potassium channel, a noncompetitive block by the two agents is described by independent parallel pathways

$$\begin{aligned} &\text{REST} \rightleftharpoons \rightleftharpoons \rightleftharpoons \text{OPEN} \xleftrightarrow{\text{QA}} \text{QA-BLOCKED} \\ &\text{REST} \rightleftharpoons \rightleftharpoons \rightleftharpoons \text{OPEN} \xleftrightarrow{\text{Ba}^{+2}} \text{BA}^{+2}\text{-BLOCKED}. \end{aligned} \tag{8}$$

This case is easy to test experimentally, because the rate of decay of the potassium current in the presence of both blockers should be equal to the sum of their independent rates (i.e., $1/\tau = 1/\tau_{QA} + 1/\tau_{Ba^{+2}}$). The block in the presence of $Ba^{+2}$ and $C_8$DEEtoh was slower than predicted by this scheme. $\tau$ equaled 1.92 ms, while the time constant measured in $Ba^{+2}$ (3.40 ms) and $C_8$DEEtoh (2.39 ms) separately predicted a smaller time constant, 1.40 ms. The result is consistent with the idea that both blockers compete for the open channel and, by so doing, alter rates of block as compared with the isolated cases; however, this is not a unique explanation.

## DISCUSSION

### *Potassium Channel Architecture*

Through the use of blocking cations two discrete barriers to cation permeation through the potassium channel have been described (Bezanilla and Armstrong, 1972). The channel possesses a relatively wide and nonselective inner mouth capable of accepting a variety of cations of modest dimensions, including $Li^+$, $Na^+$, $Rb^+$, $Cs^+$, and $TEA^+$ and other QA ions. Deeper within the membrane the channel tapers to form a more selective barrier through which only $K^+$, $Rb^+$, $NH_4^+$, and $Tl^+$ seem to readily permeate in squid axons. Given these considerations Bezanilla and Armstrong (1972) "tentatively" suggested that the inner mouth of the potassium channel was 8–9 Å in diameter.

Among the QA ions tested in the present study, a number possess lateral head group dimensions larger than that of $TEA^+$ (8 × 8 Å), most notably the tripropyl- and triethanol-containing compounds. The question thus arises as to the actual size of the large inner mouth of the channel. In the simplest case these dimensions may be estimated from the head group of the largest blocking compound. The lateral dimensions of the tripropyl head group are roughly 11 × 12 Å as measured from Corey-Pauling-Koltun space-filling models. As discussed in Results, portions of the head group of some of the compounds may form hydrophobic bonds with the channel, leaving only a portion of the compound's head group within the aqueous pore. The tripropyl-containing compounds apparently form such bonds, because their unblocking rate is slower than that of comparable triethyl-containing ions (Table III). In contrast, the triethanol head group cannot form these bonds, and, therefore, its head group should reside entirely within the aqueous pore. The relatively slow entry of the triethanol compounds as compared with that of their triethyl congeners suggests that they may be sterically hindered from entering the channel. If this is true, the head group of the triethanol-containing compounds may have nearly the same dimensions as the mouth of the pore, $\simeq 10 \times 12$ Å. The slight voltage dependence of the block suggests that this relatively nonselective region of the pore may be as deep as 20% of the distance through the membrane field; i.e., the charged nitrogen may move 20% of the distance through the field.

The wall of the channel around the QA binding site seems not to be surrounded by electronegative groups to which hydroxyl-containing QA ions would be expected to bind more tightly than their unhydroxylated congeners. In fact, the addition of hydroxyl groups diminished potency (Table II) and speeded unblocking (Table III) in a manner suggesting a loss of bonds, probably hydrophobic. The following evidence also supports the idea that the region surrounding the QA binding site has extensive hydrophobic regions: the hydrophobic binding of the tail of the QA ions, the increased potency of QA ions with one arm extended (i.e., $C_{10}DM\phi$, $C_8DEProh$), and the block by large symmetrical QA ions (French and Shoukimas, 1981). The exact geometry of the regions is uncertain, but it is intriguing to consider the channel as

constructed of an array of longitudinal α-helices similar to that hypothesized for the alamethicin channel (Baumann and Mueller, 1974).

In such a model, the QA molecule could be envisioned as extending portions of the head and tail group between the barrel staves to the hydrophobic regions of the lipid, and the small QA ions that fail to block effectively might be unable to extend arms into these hydrophobic areas.

### *The Potassium Channel and the QA Binding Site*

In summary, the following statements concerning the architecture of the large inner mouth of the potassium channel have been projected:

1) The potassium channel has a large inner mouth with lateral dimensions perhaps as large as 10 × 12 Å.

2) The charge on the nitrogen moves at least 20% of the distance through the membrane field to the QA binding site between the point of channel selectivity and the inner membrane surface.

3) The region around the QA binding site is probably not "lined" with oxygen groups but does have hydrophobic regions in close proximity.

### *Factors Improving Block*

One of the important propositions of this study is that through the survey of a diverse group of QA ions information allowing synthesis of a better blocking compound might be obtained. A number of criteria toward this end can be suggested. The ideal compound apparently should have: (*a*) a tail group no longer than $C_{10}$, and (*b*) head groups smaller than 12 × 10 Å ($C_{10}$TEtoh). Other factors that seem to enhance potency include two long tails, as found with $C_{10}$DMϕ, whose $K_d$ was higher than expected given the dimethyl component of the head group. The addition of one methylene group to the head group of $C_8$DEEtoh to make $C_8$DEProh also enhanced binding. Finally, ambutonium and $C_8$DEProh were more potent than anticipated, which may suggest that an electronegative oxygen three or more carbons away from the nitrogen may improve the blocking ability of QA ions.

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## REFERENCES

ARMSTRONG, C. M. 1969. Inactivation of the potassium conductance and related phenomena caused by quaternary ammonium ion injection in squid axons. *J. Gen. Physiol.* **54**:553–575.

ARMSTRONG, C. M. 1971. Interaction of tetraethylammonium ion derivatives with potassium channels of giant axons. *J. Gen. Physiol.* **58**:413–437.

ARMSTRONG, C. M. 1975. Ionic pores, gates and gating currents. *Q. Rev. Biophys.* **7**:179–210.

ARMSTRONG, C. M., and L. BINSTOCK. 1965. Anomalous rectification in the squid giant axon injected with tetraethylammonium chloride. *J. Gen. Physiol.* **48**:859–872.

ARMSTRONG, C. M. and B. HILLE. 1972. The inner quaternary ammonium ion receptor in potassium channels in the node of Ranvier. *J. Gen. Physiol.* **59**:388–400.

ARMSTRONG, C. M. and S. TAYLOR. 1980. Interaction of barium ions with potassium channels in squid giant axons. *Biophys. J.* **30**:473–488.

BAUMANN, G., and P. MUELLER. 1974. A molecular model of membrane excitability. *J. Supramol. Struct.* **2**:538–549.

BEZANILLA, F., and C. M. ARMSTRONG. 1972. Negative conductance caused by entry of sodium and cesium ions into the potassium channels of squid axons. *J. Gen. Physiol.* **60**:588–602.

BEZANILLA, F., and C. M. ARMSTRONG. 1977. Inactivation of the sodium channel. I. Sodium current experiments. *J. Gen. Physiol.* **70**:549–566.

FRENCH, R. J., and J. J. SHOUKIMAS. 1981. Blockage of squid axon potassium conductance by internal tetra-*n*-alkylammonium ions of various sizes. *Biophys. J.* In press.

HILLE, B. 1973. Potassium channels in myelinated nerve. Selective permeability to small cations. *J. Gen. Physiol.* **61**:669–686.

HILLE, B. 1975. Ionic selectivity of Na and K channels of nerve membranes. *In* Membranes, A Series of Advances. Vol. 3. G. Eisenman, editor. Marcel Dekker, Inc. New York. 255–323.

ROJAS, E., and B. RUDY. 1976. Destruction of sodium conductance inactivation by specific protease in perfused nerve fibers from *Loligo*. *J. Physiol. (Lond.)*. **262**:501–531.

ROBINSON, R. A., and R. H. STOKES. 1970. Electrolyte Solutions. Butterworth & Co., Ltd., London.

SHOUKIMAS, J. J., and R. J. FRENCH. 1979. The effects of symmetrical quaternary ammonium ions of varying size on K-channel conductance. *Biophys. J.* **25**:307*a*. (Abstr.).