Cation Selectivity of Acetylcholine-Activated Ionic Channel of Frog Endplate

SHIGENORI WATANABE and TOSHIO NARAHASHI

From the Department of Pharmacology, Northwestern University Medical School, Chicago, Illinois 60611. Dr. Watanabe's present address is Department of Pharmacology, Faculty of Pharmaceutical Sciences, Kyushu University, Fukuoka, Japan

ABSTRACT Ionic selectivity of the acetylcholine-activated ionic channel of frog endplate membranes to various organic cations has been studied. The ratio of test cation permeability ($P_x$) to sodium permeability ($P_{Na}$) was estimated by two methods, one based on the measurements in test cation solutions of the amplitude of transient depolarization induced by iontophoretic application of acetylcholine, and the other on the measurements of the reversal potential for the membrane current induced by iontophoretic application of acetylcholine under voltage-clamp conditions. The endplate channel is relatively nonselective to various test cations. The permeabilities relative to Na are ammonium (1.71), formamidine (1.49), methylamine (1.39), hydrazine (1.35), and Li (0.76), as measured from the reversal potential for acetylcholine currents, and guanidine (0.74), aminoguanidine (0.20), methylguanidine (0), and choline (0) as measured from the amplitude of acetylcholine potential. Methylguanidine and aminoguanidine block the endplate channel with the apparent dissociation constants of 0.5 and 15 mM, respectively. Based on these data, the dimensions of selectivity filter of acetylcholine-activated channel appear to be slightly larger than those of the sodium channel of frog nodes and smaller than those of the epithelial membrane of gallbladder of frogs and rabbits.

INTRODUCTION

Ionic channels of endplate membranes appear to be relatively nonselective to cations (Furukawa and Furukawa, 1959; Takeuchi and Takeuchi, 1960; Anwyl, 1977 b; Guy et al., 1977; Maeno et al., 1977; Huang et al., 1978), as compared with sodium and potassium channels of axonal membranes which show a high degree of selectivity to certain cations during activity (Chandler and Meves, 1965; Hille, 1971, 1972, 1973; Hironaka and Narahashi, 1977). Among several differences between the ionic channels of axonal membranes and those of endplate membranes, the cation selectivity represents an important feature in understanding the differences between the ionic channels of these two types of the membranes. In spite of a wealth of information accumulated for the ionic selectivity of nerve membrane ionic channels, our knowledge about the selectivity of endplate membranes remains sketchy and qualitative. Recent studies of the cation selectivity of the acetylcholine (ACh)-
activated channel by Maeno et al. (1977) and Guy et al. (1977) cover a fairly large number of organic cations, but the measurement was made by the moving surface electrode technique (Fatt, 1950), the accuracy of which in measuring the permeability ratio remains to be seen. The present study has been undertaken to obtain more quantitative information about the endplate ionic selectivity using iontophoretic application of ACh and voltage-clamp techniques.

Part of the present study has been reported (Watanabe and Narahashi, 1977; Watanabe et al., 1978).

METHODS

The sciatic nerve-sartorius muscle preparation of the frog, *Rana pipiens* or *Rana temporaria*, was used as material.

The membrane potential was measured by means of a glass capillary microelectrode filled with 3 M KCl solution and having a resistance of 5–15 MΩ. A large Ag-AgCl wire was used as the external reference electrode, and changes in junction potential caused by test cation solutions, which ranged from 0–1.2 mV, were corrected in measurement of membrane potential. Acetylcholine was applied iontophoretically to the endplate membrane through a glass capillary microelectrode filled with 1 M ACh solution and having a resistance of 40–150 MΩ. A small braking current was applied to stop diffusion of ACh from the tip of microelectrode. In some experiments, 1 M carbachol was used in place of ACh.

The input resistance of the muscle fiber was measured from the steady-state potential change induced by a step hyperpolarizing current pulse. Both current delivery and potential recording microelectrodes were filled with 3 M KCl solution and had a resistance of 4–7 MΩ. They were inserted into the muscle with an interelectrode distance of <150 μm.

The membrane current associated with iontophoretic application of ACh was measured by means of the conventional two-microelectrode voltage-clamp method (Takeuchi and Takeuchi 1959; Deguchi and Narahashi, 1970). Two glass capillary microelectrodes filled with 3 M KCl solution were inserted to the endplate region, one being used to deliver currents and the other to record the membrane potential. The electric resistances of the current delivery and potential recording microelectrodes were ~2 and 5–15 MΩ, respectively. ACh was applied to the endplate iontophoretically through a third, ACh-filled electrode. ACh currents were recorded at various holding potentials.

The ionic permeability ratio of endplate channels was estimated by two methods, one based on the measurement of the iontophoretically induced ACh potential and the other on the measurement of the reversal potential for ACh current under voltage-clamp conditions. In the ACh-potential method, the ratio of the peak amplitude of ACh potential in a solution in which a test cation (X) was substituted for sodium to that in normal Ringer's solution \((V_X/V_Na)\) was taken as a measure of the permeability ratio \(P_X/P_Na\). However, the amplitude of ACh potential could be affected by a few factors. First of all, it is related to the permeability to test cation. A larger test cation permeability relative to sodium permeability would cause an increase in ACh potential provided that no change occurs in other factors such as the internal concentrations of sodium and potassium and the input resistance of the muscle fiber. Secondly, an increase in input resistance would increase ACh potential.

The reversal potential \((E_r)\) for ACh current in normal Ringer's solution is given by the constant field equation (Goldman, 1943; Hodgkin and Katz, 1949):
\[ E_r = \frac{RT}{F} \ln \frac{P_K[K]_o + P_{Na}[Na]_o}{P_K[K]_i + P_{Na}[Na]_i}, \]

where \( P_K \) and \( P_{Na} \) refer to potassium and sodium permeabilities, respectively, \([ \_ ]_o\) and \([ \_ ]_i\) are concentrations (more strictly activities) of an ion outside and inside, respectively, and \( R, T, \) and \( F \) are the gas constant, the absolute temperature and the Faraday constant, respectively. When a test cation (X) is substituted for sodium, the reversal potential may take a new value \( E_r' \).

Thus we have

\[ E_r' - E_r = \frac{RT}{F} \ln \frac{P_K[K]_o + P_X[X]_o}{P_K[K]_i + [Na]_o}, \]

where \( P_X \) and \([X]_o\) refer to test cation permeability and its outside concentration, respectively. The ratio \( P_K/P_{Na} \) was calculated from Eq. 1 for each group of experiments assuming internal concentrations of sodium and potassium ions as 15.5 and 126 mM, respectively (Boyle and Conway, 1941). Then the ratio \( P_X/P_{Na} \) was calculated from Eq. 2.

The use of the constant field equation rather than the conductance equation (Takeuchi and Takeuchi, 1960) deserves some comments. Although the conductance equations fit the observed reversal potentials for endplate current well (Takeuchi and Takeuchi, 1960), there have been controversies as to which equation had more sound theoretical basis and which fit the observations better. Since the constant field equation (Goldman, 1943) is originally derived from the Nernst-Planck flux equation, it has a solid physical and chemical basis for describing the membrane potential, and has indeed been applied to the endplate membrane by several investigators (Ginsborg, 1973; Rang, 1975; Ritchie and Fambrough, 1975; Anwyl, 1977 a;). In the present study, the conductance equation cannot be used inasmuch as the reversal potentials for individual ions are not defined when sodium substitutes are applied to only one side of the membrane.

The Mg\(^{2+}\)-containing Ringer’s solution had the following composition (millimolar): Na\(^+\) 99, K\(^+\) 2.3, Ca\(^{2+}\) 1.8, Mg\(^{2+}\) 12, Cl\(^-\) 127, and HEPES 2. The final pH was adjusted to 7.3 using 1 N HCl solution. For the study of a test cation, sodium ions in Ringer’s solution were replaced by the equimolar concentration of the test cation. Since nitrate salt was used for aminoguanidine, NaNO\(_3\) was substituted for NaCl in the control solution. The sources of test compounds were as follows: ammonium chloride (J. T. Baker Chemical Co., Phillipsburg, N. J.), methylamine hydrochloride (Baker), hydrazine hydrochloride (ICN, K & K Laboratories, Inc., Plainview, N. Y.), formamidine hydrochloride (Sigma Chemical Co., St. Louis, Mo.), guanidine hydrochloride (Aldrich Chemical Co., Milwaukee, Wis.), aminoguanidine nitrate (Eastman Kodak Co., Rochester, N. Y.), methylguanidine hydrochloride (Sigma), hydroxylamine hydrochloride (K & K), choline chloride (Sigma), and lithium chloride (Baker). The chemical structures of some of the test compounds are shown in Fig. 1.

In order to avoid muscle contractions associated with large depolarizations in the voltage clamp experiments, the formamide method originally developed by del Castillo and de Motta (1977) was used. The muscle was soaked in a Ringer’s solution added with 2 M formamide for 10–15 min, brought back to normal Ringer’s solution, and kept in Ringer’s for 1–1.5 h before starting experiments. Nerve stimulation evoked
the normal action potential in the muscle without contraction. The resting membrane potential was maintained in the vicinity of -70 to -90 mV for a period of several hours. Thus the formamide method is superior to the glycerol method (Eisenberg and Gage, 1967; Gage and Eisenberg, 1967) and the ethylene glycol method (Sevcik and Narahashi, 1972) in maintaining the near normal resting potential for a long period of time.

Experiments were conducted at a room temperature of 23°C.

RESULTS

Acetylcholine Potential

Changes in Acetylcholine Potential by Test Cations

When sodium ions in the bathing medium were substituted by a test cation on the equimolar basis, the amplitude of iontophoretically induced ACh potential underwent changes. The steady-state effect was reached within 10 min after application of a test solution. Examples of the increase in ACh potential by three test cations are illustrated in Fig. 2. Each row of the figure represents a series of measurements of ACh potential before and during application of a test cation and after washing with normal Ringer’s solution. Ammonium (record B), methylamine (record E), and hydrazine (record H) increased the ACh potential. Fig. 3 illustrates three examples of the ions which decrease the ACh potential. Lithium suppressed the ACh potential slightly (record B), methylguanidine suppressed it completely (record E), and aminoguanidine suppressed it to a large extent (record H). All of these effects were completely reversed after washing with normal Ringer’s solution.

The mean amplitudes of ACh potentials in various test cations are given as the percentage of the control in Fig. 4. Formamidine, ammonium, methylamine, and hydrazine increased the amplitude of ACh potential, lithium and guanidine suppressed it slightly, aminoguanidine suppressed it to a large extent, and methylguanidine and choline blocked it completely.
Ammonium caused a 5-20 mV depolarization of the muscle membrane. Choline also caused a depolarization of ~20 mV. The changes in resting membrane potential in the other test cation solutions were either negligible or absent.

Exposure of the muscle to hydroxylamine solution appears to cause an irreversible damage. The effect on the ACh potential was variable from experiment to experiment, and could not be restored completely after washing with normal Ringer's solution. However, the ACh potential, though variable in amplitude, was always observed in hydroxylamine, indicating that the endplate channel is permeable to hydroxylamine.

The ratio of ACh potential in a test cation (X) solution to that in normal Ringer's solution ($V_x/V_{Na}$) is related to the permeability ratio $P_x/P_{Na}$, and is given in the last column of Table I. Several factors must be taken into consideration to relate the ratio $V_x/V_{Na}$ to $P_x/P_{Na}$ as described in the following sections.

**Input Resistance** If the input resistance of muscle membrane were increased by a test cation, this effect alone would increase ACh potential. In order to clarify this point, the input resistance was measured from the steady-state values of hyperpolarizations as induced by step hyperpolarizing currents.
Figure 3. Decreases or block of iontophoretically induced acetylcholine potentials by substitution of sodium with lithium, methylguanidine, and amino-
guanidine. The resting membrane potentials were (millivolts): (A) -85; (B) 
-85; (C) -84; (D) -86; (E) -86; (F) -86; (G) -83; (H) -83; and (I) -81.

Figure 4. The peak amplitude of iontophoretically induced acetylcholine 
potential in solutions in which test cations are substituted for sodium. The data 
are given as the mean ± SEM (4-10 experiments each) as percentages of the 
control value obtained in normal sodium-containing Ringer's solution.
The ratios of the input resistance in test solutions to that in normal sodium Ringer's are (mean of three experiments): guanidine (0.95), Li (1.00), ammonium (1.04), formamidine (1.10), aminoguanidine (1.28), methylguanidine (1.30), hydrazine (1.90), and methylamine (2.00). Although changes in the specific membrane resistance several-fold beyond the normal value of ~4,000 Ωcm² alter the amplitude of miniature endplate potential only by a few percent (Gage and McBurney, 1973), some influences of membrane resistance changes on ACh potential are expected because of its relatively slow time course. Therefore, the ratios $V_X/V_{Na}$ for hydrazine and methylamine may include some contributions from the increases in the input resistance.

### TABLE I

| Cation           | $E_r$  | $E'_r$ | $E'_r - E_r$ | $P_X/P_{Na}$ (from $E'_r$) | $V_X/V_{Na}$ |
|------------------|--------|--------|-------------|-----------------------------|--------------|
|                  | mV     | mV     | mV          |                             |              |
| Ammonium         | -9.0   | +4.4   | 13.4        | 1.02                        | 1.71 (9)     |
| Methylamine      | -9.1   | -0.8   | 8.3         | 1.02                        | 1.39 (5)     |
| Hydrazine        | -5.8   | +1.8   | 7.6         | 0.88                        | 1.35 (5)     |
| Formamidine      | -6.5   | +3.5   | 10.0        | 0.91                        | 1.49 (3)     |
| Lithium          | -6.7   | -13.5  | -6.8        | 0.92                        | 0.76 (5)     |
| Guanidine        |        |        |             |                             | 0.74 (4)     |
| Aminoguanidine   |        |        |             |                             | 0.20 (5)     |
| Methylguanidine  |        |        |             |                             | ~0 (5)       |
| Choline          |        |        |             |                             | ~0 (5)       |

Permeability ratios $P_X/P_{Na}$ and $P_X/P_{Na}$ were calculated from the mean reversal potential for iontophoretically induced acetylcholine current in normal sodium Ringer's solution ($E_r$) and in solutions in which various test cations ($X$) were substituted for sodium ($E'_r$). $V_X$ and $V_{Na}$ are the peak amplitudes of acetylcholine potentials in $X$ and $Na$, respectively. The number of each experiment is given in parentheses.

**Carbachol Potential.** The increase in ACh potential observed in a test cation solution could be produced by inhibition of acetylcholinesterase (AChE). This possibility would be ruled out if the carbachol potential changed in the same way as the ACh potential in response to test cations used. This was actually the case for formamidine, methylamine, ammonium, and hydrazine. Fig. 5 shows examples of such experiments with ammonium and hydrazine, both of which increased the carbachol potential as they did the ACh potential (see Fig. 2). Thus, the possibility that the observed changes in ACh potential caused by test cations are due to inhibition of AChE can be ruled out.

**Suppression of Acetylcholine Potential.** Methylguanidine and aminoguanidine, substituted for sodium, suppressed the ACh potential (Fig. 3). It was found that these guanidine derivatives exerted blocking actions on the
endplate ACh potential even at low concentrations. The dose-response relationships in inhibiting the ACh potential are shown in Fig. 6. The apparent dissociation constants were estimated to be 0.5 and 15 mM for methylguanidine and aminoguanidine, respectively.

**Ionic Permeability Ratio**

Ionic permeability ratio can be directly calculated from the shift of the reversal potential for iontophoretically induced ACh current under voltage-clamp conditions. Because of the difficulties associated with maintaining the ACh pipette at a fixed position while changing the membrane potential to various levels in both control and test solutions, the number of test cations examined in this way was limited. Despite this, five test cations could be studied by this technique.

In Fig. 7 illustrates the families of iontophoretically induced ACh currents at various holding membrane potentials before and during application of ammonium (records A and B) and methylamine (records C and D). In agreement with the ACh potential experiment (Fig. 2), methylamine prolonged the decay of ACh current. The peak ACh currents are plotted as a function of the membrane potential in Fig. 8. Graph A represents the experiments for ammonium. The reversal potential in the control solution is estimated to be −9 mV, and it is shifted to +2 mV in ammonium solution. Shifts in the same direction occur in methylamine (graph B) and hydrazine (graph C). In
Figure 6. Percentage of inhibition of iontophoretically induced acetylcholine potential by various concentrations of methylguanidine and aminoguanidine. For aminoguanidine experiments, sodium ions were replaced by the equimolar concentration of the test cation. Average of five (methylguanidine) and three (aminoguanidine) experiments.

Figure 7. Families of iontophoretically induced acetylcholine currents recorded at various holding membrane potentials before and during substitution of sodium with ammonium and methylamine. A RC-coupled high-gain amplifier was used to maintain the stable base line causing some distortion of the time-course toward the end of the record.
contrast, a shift in the opposite direction is observed in lithium solution (graph D). The current-voltage relation is almost linear in both normal and test solutions in the membrane potentials ranging from -90 to +50 mV.

The reversal potentials for ACh current in normal Ringer’s solution ($E_r$) and in test solutions ($E'_r$) and the shift of the reversal potential ($E'_r - E_r$) are given in Table I (second, third, and fourth columns). The ratio $P_K/P_{Na}$ was first calculated by Eq. 1 (fifth column, Table I), and using that ratio, the ratio $P_X/P_{Na}$ was then calculated by Eq. 2 (sixth column).

![Figure 8. Current-voltage relations for iontophoretically induced acetylcholine currents under voltage-clamp conditions before and during substitution of sodium with ammonium (A), methylamine (B), hydrazine (C), and lithium (D).](image)

The overall averages of the reversal potential for ACh current and the ratio $P_K/P_{Na}$ in normal Ringer’s solution are calculated as -7.4 mV and 0.95, respectively. Ammonium, methylamine, hydrazine, and formamidine are more permeant than sodium, whereas lithium is less permeant than sodium. It is striking to find that the ACh potential ratio $V_X/V_{Na}$ give almost the same values as the $P_X/P_{Na}$ ratios (Table I).
The present experiments clearly show that the ratio of the amplitude of iontophoretically induced ACh potential \((V_X/V_{Na})\) is in reasonably good agreement with the permeability ratio \(P_X/P_{Na}\) calculated from the shift of the reversal potential for iontophoretically induced ACh current. The latter method should give more accurate data, although it involves greater technical difficulties to such an extent as to make it difficult to compare a large number of cations.

The results of the present experiments clearly indicate that the endplate ionic channels are relatively nonselective to a variety of cations. The permeability ratio \(P_X/P_{Na}\) as calculated from the reversal potentials for iontophoretically induced ACh current ranges from 1.35 to 1.71 for ammonium, methylamine, hydrazine, and formamidine, and is 0.76 for lithium (Table I). Guanidine is also fairly permeant relative to sodium judging from the \(V_X/V_{Na}\) value of 0.74. Among the cations examined, only aminoguanidine, methylguanidine, and choline are poorly permeant (Table I). Decamethonium has also been found to penetrate the endplate membrane (Creese and Maclagan, 1970).

Relatively nonselective cation permeability has also been found in the cultured chick muscle (Huang et al., 1978), in the endplate of locust muscle (Anwyl, 1977b), in the postjunctional membrane of eel electroplaque (Lassignal and Martin, 1977), and in the gallbladder epithelia of frog and rabbit (Moreno and Diamond, 1975). On the contrary, the membranes of frog node of Ranvier, squid giant axon, and frog skeletal muscle exhibit a high cation selectivity during peak transient current (Hille, 1971, 1972; Campbell, 1976; Hironaka and Narahashi, 1977).

Cation selectivity of the ACh activated ionic channels has been studied with frog endplate membranes (Maeno et al., 1977) and chronically denervated frog muscle membranes (Guy et al., 1977). However, inasmuch as these measurements were made using the moving surface electrode technique (Fatt, 1950), the data cannot rigorously be taken as quantitative but rather represent a rough guide. Granting this situation, the present results are in general agreement with those of the two studies for ammonium, guanidine, and methylamine, all of which are permeant to the ACh-activated channels. The data on methylguanidine are controversial because it was almost impermeant to the channel in the present study, whereas it was permeant to some extent in the study by Guy et al. (1977). They found strong depression of ACh-induced depolarization using the methylguanidine hydrochloride sample obtained from Sigma Chemical Co., which was also used in the present study.

The low permeability of lithium compared with sodium with the ratio \(P_{Li}/P_{Na}\) of 0.76 agrees with the data of the recent noise analysis of single-channel conductance. Van Helden et al. (1977) and Gage and Van Helden (1979) have found that lithium causes a reduction in single-channel conductance with a conductance \((g)\) ratio \(g_{Li}/g_{Na}\) of 0.66 and a prolongation of mean open time of individual channels by 30%. However, the shift of the reversal potential was smaller in their experiments \((-3.2 \text{ mV})\) than that in the present experiments \((-6.8 \text{ mV})\).
In an attempt to estimate the dimensions of the ACh-activated ionic channels of frog endplate based on the present data, several factors must be taken into consideration. First of all, steric restriction is obviously an important factor for an ion to be able to penetrate the channel. Secondly, certain molecules, even when they are slightly larger than the channel, can pass through the channel provided that they form hydrogen bonds with the proton acceptors in the channel, thereby reducing the effective size of the cation. This contracting effect of hydrogen bonding (Pauling, 1960) was assumed to play a critical role for certain organic cations to pass through ionic channels (Hille, 1971; Moreno and Diamond, 1975; Huang et al., 1978). The third factor to be considered is the free energy change in transferring the cation from water to the binding sites in the membrane. The fourth factor is the field strength in the membrane. Partial negative charges on cation binding site in the membrane affects the electrostatic forces between the cation and the site, thereby influencing the cation selectivity of the channel (Eisenman, 1962, 1969).

We can estimate the size of the ionic channel of endplate membranes from the comparison of ionic radii and permeability ratios. If we assume that a cation can pass through the channel with its longest dimension oriented in the axis of channel, then the present permeability data are compatible with 3.8 × 4.8 Å rectangular dimension for the selectivity filter of the channel based on the Pauling (1960) dimensions. However, more data on other cations with various dimensions are necessary to draw a conclusion about the size of the channel. The selectivity filter of the nerve membrane sodium channel was estimated to have a dimension of 3.1 × 5.1 Å (Hille, 1971). The channel radii of the gallbladder epithelium of frog and rabbit were estimated to be 8.1 and 4.4 Å, respectively (Moreno and Diamond, 1975). Thus the endplate channels appear to have a dimension somewhere between the nerve and gallbladder channels.

The relative permeability measured in the present study does not necessarily represent the permeability of individual ionic channels. It depends on the ionic current per channel and the number of channels conducting. Thus, if a test cation had a partial blocking action to decrease the number of channels that are open upon receptor activation, the permeability to the test cation could be underestimated. Recent study by Dwyer et al. (1979) reports a considerably high permeability to methylguanidine with the \( \frac{P_X}{P_{Na}} \) ratio of 0.8. The discrepancy between their data and the present data on the methylguanidine permeability might be due to the difference in the rate at which it penetrates the channel and that at which it blocks the channel. Further study is needed to clarify this point.

Behavior of guanidine derivatives deserves special comments. Although guanidine itself is permeant to the channel to a lesser extent than sodium, addition of a certain group makes the molecule much less permeant. Aminoguanidine appears to be only slightly permeant, and methylguanidine is almost impermeant. In addition, these guanidine derivatives exert blocking action on the ionic channel at millimolar concentrations. Steric hindrance may be a factor responsible for the block. This notion is supported by the
finding that the methylguanidine block is at least in part current-dependent, being enhanced by an inward current and relieved by an outward current (Farley et al., 1979).

The authors wish to thank LaVerne Brown and Caroline Myss for secretarial assistance. This study was supported by grant NS-14145 from the National Institutes of Health.

Received for publication 2 October 1978.

REFERENCES

ANWYRY, R. 1977 a. Permeability of the post-synaptic membrane of an excitatory glutamate synapse to sodium and potassium. J. Physiol. (Lond.). 237:367–388.

ANWYRY, R. 1977 b. The effect of foreign cations, pH and pharmacological agents on the ionic permeability of an excitatory glutamate synapse. J. Physiol. (Lond.). 273:389–404.

BOYLE, P. J., and E. J. CONWAY. 1941. Potassium accumulation in muscle and associated changes. J. Physiol. (Lond.). 100:1–63.

CAMPBELL, D. T. 1976. Ionic selectivity of the sodium channel of frog skeletal muscle. J. Gen. Physiol. 67:293–307.

CHANDLER, W. K., and H. MEVES. 1965. Voltage clamp experiments on internally perfused giant axons. J. Physiol. (Lond.). 180:788–820.

CREESE, R., and J. MACLAGAN. 1970. Entry of decamethonium in rat muscle studied by autoradiography. J. Physiol. (Lond.). 210:363–386.

DEGUCHI, T., and T. NARAHASHI. 1970. Effects of procaine on ionic conductances of end-plate membranes. J. Pharmacol. Exp. Ther. 176:423–433.

del CASTILLO, J., and G. E. DE Motta. 1977. Influence of succinic anhydride on the decay of endplate currents evoked by high frequency stimulation. Soc. Neurosci. Abstr. 3:371.

DWYER, T. M., D. J. ADAMS, and B. HILLE. 1979. Ionic selectivity of end-plate channels. Biophys. J. 25(2, Pt. 2):67a. (Abstr.)

EISENBERG, R. S., and P. W. GAGE. 1967. Frog skeletal muscle fibers: changes in electrical properties after disruption of transverse tubular system. Science (Wash. D.C.). 158:1700–1701.

EISENMAN, G. 1962. Cation selective glass electrodes and their mode of operation. Biophys. J. (2, Pt. 2):259–323.

EISENMAN, G. 1969. Theory of membrane electrode potentials: an examination of the parameters determining the selectivity of solid and liquid ion exchangers and of neutral ion-sequestering molecules. In Ion-Selective Electrodes. R. A. DURST, editor. Washington, D.C. National Bureau of Standards Special Publication No. 314. 1–56.

FARLEY, J. M., S. WATANABE, J. Z. YEH, and T. NARAHASHI. 1979. Mechanism of end-plate channel block by N-alkyl guanidine derivatives. Biophys. J. 25 (2, Pt. 2):16a.

FATT, P. 1950. The electromotive action of acetylcholine at the motor endplate. J. Physiol. (Lond.). 111:408–422.

FURUKAWA, T., and A. FURUKAWA. 1959. Effects of methyl- and ethyl-derivatives of NH₄ on the neuromuscular junction. Jpn. J. Physiol. 9:130–142.

GAGE, P. W., and R. S. EISENBERG. 1967. Action potentials without contraction in frog skeletal muscle fibers with disrupted transverse tubules. Science (Wash. D.C.). 158:1702–1703.

GAGE, P. W., and R. N. MCBURNEY. 1973. An analysis of the relationship between the current and potential generated by a quantum of acetylcholine in muscle fibers without transverse tubules. J. Membr. Biol. 12:247–272.
GAGE, P. W., and D. VAN HELDEN. 1979. Effects of permeant monovalent cations on end-plate channels. *J. Physiol. (Lond.)* 288:509–528.

GINSBORG, B. L. 1973. Electrical changes in the membrane in junctional transmission. *Biochim. Biophys. Acta.* 300:289–317.

GOLDMAN, D. E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* 27:37–60.

GUY, H. R., M. S. DEKIN, and R. MORELLO. 1977. Effects of denervation on the permeability of acetylcholine-activated channels to organic cations. *J. Neurobiol.* 8:491–506.

HILLE, B. 1971. The permeability of the sodium channel to organic cations in myelinated nerve. *J. Gen. Physiol.* 58:599–619.

HILLE, B. 1972. The permeability of the sodium channel to metal cations in myelinated nerve. *J. Gen. Physiol.* 59:637–658.

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

HIRONAKA, T., and T. NARAHASHI. 1977. Cation permeability ratios of sodium channels in normal and grayanotoxin-treated squid axon membranes. *J. Membr. Biol.* 31:359–381.

HODGKIN, A. L., and B. KATZ. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.)* 108:37–77.

HUANG, L-Y. M., W. A. CATTERALL, and G. EHRENSTEIN. 1978. Selectivity of cations and nonelectrolytes for acetylcholine-activated channels in cultured muscle cells. *J. Gen. Physiol.* 71:397–410.

LASSIGNAL, N. L., and A. R. MARTIN. 1977. Effect of acetylcholine on postjunctional membrane permeability in eel electroplaque. *J. Gen. Physiol.* 70:23–36.

MAENO, T., C. EDWARDS, and M. ANRAKU. 1977. Permeability of end-plate membrane activated by acetylcholine to some organic cations. *J. Neurobiol.* 8:173–184.

MORENO, J. H., and J. M. DIAMOND. 1975. Nitrogenous cations as probes of permeation channels. *J. Membr. Biol.* 21:197–259.

PAULING, L. 1960. The Nature of the Chemical Bond. Cornell University Press, Ithaca, N.Y.

RANG, H. P. 1975. Acetylcholine receptors. *Q. Rev. Biophys.* 7:283–399.

RITCHIE, A. K., and D. M. FAMBROUGH. 1975. Ionic properties of the acetylcholine receptor in cultured rat myotubes. *J. Gen. Physiol.* 65:751–767.

SEVCIK, C., and T. NARAHASHI. 1972. Electrical properties and excitation-contraction coupling in skeletal muscle treated with ethylene glycol. *J. Gen. Physiol.* 60:221–236.

TAKEUCHI, A., and N. TAKEUCHI. 1959. Active phase of frog's end-plate potential. *J. Neurophysiol.* 22:395–411.

TAKEUCHI, A., and N. TAKEUCHI. 1960. On the permeability of end-plate membrane during the action of transmitter. *J. Physiol. (Lond.)* 154:52–67.

VAN HELDEN, D., O. P. HAMIL, and P. W. GAGE. 1977. Permeant cations alter end-plate channel characteristics. *Nature (Lond.)* 269:711–713.

WATANABE, S., and T. NARAHASHI. 1977. Permeability of frog end-plate membranes to organic and inorganic cations. 7th Annual Meeting. *Soc. Neurosci. Abstr.* 3:378.

WATANABE, S., J. FARLEY, and T. NARAHASHI. 1978. End-plate block by guanidine derivatives. 8th Annual Meeting. *Soc. Neurosci. Abstr.* 4:375.