LOCAL ANESTHETIC ALTERATION OF MINIATURE ENDPLATE CURRENTS AND ENDPLATE CURRENT FLUCTUATIONS

ROBERT L. RUFF

From the Department of Physiology and Biophysics, University of Washington School of Medicine, Seattle, Washington 98195

ABSTRACT The effect of the local anesthetic QX222 on the kinetics of miniature endplate currents and acetylcholine induced endplate current fluctuations was studied in voltage clamped cutaneous pectoris muscle of *Rana pipiens*. Both the endplate current fluctuation spectra and the miniature endplate current decay consisted of two or three components depending upon the holding potential and local anesthetic concentration. The cutoff frequency of each spectral component was equal to the decay rate of its corresponding constituent of the miniature endplate current. Comparison of the relative amplitudes of the spectral and miniature endplate components indicated that QX222 did not act by creating two kinetically distinct populations of acetylcholine receptors. QX222 action could be explained by alteration of the acetylcholine receptors such that they sequentially change conformation from one open state to another. A specific case in which QX222 binds to the open state of the acetylcholine receptor creating a blocked state, was found to account for the observed relationship between the relative amplitudes of the miniature endplate current and spectral components, as well as the previously observed voltage and concentration sensitivity of the decay rates of endplate current components.

Normally endplate currents (EPC) decay with a single exponential time course (1). However, the decay of EPC and miniature endplate currents (MEPC) becomes multiphasic in the presence of procaine (2–5) or lidocaine and its derivatives (6, 7). Usually the EPC consist of an initial component which decays faster than the EPC recorded in untreated control muscle followed by a prolonged component which decays slower than the control. Beam (7) has shown that the trimethyl derivative of lidocaine, QX222, produces triphasic EPC consisting of a fast, an intermediate, and a slow component. The intermediate component has the same decay rate as control EPC and is thought to represent a population of normal receptors (7). Hyperpolarization or increase in local anesthetic concentration diminishes the relative amplitude of the "normal" component of the EPC (7), and slows the decay of the slow phase of the EPC while hastening the decay of the fast component (3, 7). The parallel effect of increased local anesthetic concentration and hyperpolarization may imply that the effectiveness of local anesthetics is voltage dependent.

Several mechanisms may be proposed to account for local anesthetic alteration of

the EPC. Three kinetic schemes will be discussed, one only briefly. It will be shown that of the models tested only those in which the local anesthetic causes the acetylcholine receptor (AChR) to undergo a sequential series of steps from one open state to another were consistent with the experimental results.

Maeno (8) postulated that there exist separate channels for sodium and potassium and that local anesthetics alter the kinetics of sodium conductance. Kordaš (3, 9) demonstrated that the monotonic dependence of EPC decay on voltage and the presence of a clear reversal potential for EPC in the presence of local anesthetic were inconsistent with this hypothesis. Consequently, Deguchi and Narahashi (4) proposed that the sodium and potassium channels were voltage sensitive, local anesthetics affect both sodium and potassium channel kinetics, and the sodium channel conducts another unspecified ion.

The separate channel hypothesis may be represented by a number of different kinetic schemes. Initial analysis of conductance fluctuations as a function of membrane potential indicates that only kinetic schemes in which the sodium and potassium channels follow similar time courses will account for the behavior of EPC and EPC fluctuations (21), which are described below. Because the support for independent control of sodium and potassium conductance is slender, this hypothesis will not be further discussed.

Steinbach (10) and more recently Adams (11) proposed that local anesthetics alter EPC according to a kinetic scheme in which the local anesthetic molecule binds to the receptor-transmitter complex in its open configuration and produces a receptor-transmitter-local anesthetic complex having a much lower single channel conductance. For concentrations of local anesthetic and membrane depolarizations at which the EPC consists of only two components, a kinetic scheme which will account for the action of a local anesthetic, in this case QX222, may be represented as:

$$nT + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} T_n \cdot R \underset{A}{\overset{B}{\rightleftharpoons}} T_n \cdot R^* + Q \underset{F}{\overset{G}{\rightleftharpoons}} T_n \cdot R \cdot Q^*, \tag{1}$$

where T represents acetylcholine (ACh), R represents the AChR, Q represents the local anesthetic, n represents the number of transmitter molecules bound to the AChR, $T_n \cdot R$ represents the transmitter-receptor complex associated with a closed channel, $T_n \cdot R^*$ is the open conformation of this complex, and $T_n \cdot R \cdot Q^*$ is the transmitter-receptor-local anesthetic complex which is assumed to be nonconducting compared with $T_n \cdot R^*$; k_1, k_2, A, B, G, and F are the rate constants for the processes indicated. In accord with Magleby and Stevens (12) it is assumed that the conformation changes are rate limiting and that agonist concentration is far from a saturating level. This scheme predicts a double exponential decay of EPC with observed rate constants

$$\alpha_2 = (A + F + G \cdot Q + [(A + F + G \cdot Q)^2 - 4A \cdot F]^{1/2})/2$$

and $$\alpha_1 = (A + F + G \cdot Q - [(A + F + G \cdot Q)^2 - 4A \cdot F]^{1/2})/2,$$

where $\alpha_2 > \alpha_1$. If G increases with hyperpolarization, then α_1 and α_2 will have the

voltage and concentration dependence of the experimentally observed rate constants. The proposed voltage dependence of G could result from a voltage-sensitive conformation change following the binding of Q to $T_n \cdot R^*$ (12). Alternately, the local anesthetic may bind inside the endplate channel. In this case the voltage dependence of G would result from the voltage-dependent entry of Q into the endplate channel (13, 14).

Another kinetic scheme can be proposed based upon the observation of Keynes and Rojas (15) that procaine shortened the time course of sodium gating currents. They suggested that procaine might act by increasing membrane fluidity so that conformation changes associated with sodium channel opening and closure would be more rapid. It is possible that local anesthetics may alter EPC kinetics due to a dual effect on the AChR. Accordingly, the rapid decay phase would result from a population of AChR which is associated with a membrane of high fluidity. Direct binding of the local anesthetic to other receptors might slow their rate of channel closure, thus producing the slow phase. With the assumptions that local anesthetic binding to the AChR and to the surrounding membrane is voltage dependent, and that both the receptor and the surrounding lipid matrix have multiple binding capacity for local anesthetic, one could account for the observed voltage dependence and local anesthetic concentration dependence of the fast and slow phases of the EPC. For concentrations of QX222 and membrane potentials at which the EPC consists of only two components, the proposed dual action of QX222 may be represented as:

$$
\begin{aligned}
&nT + R \underset{k_2}{\overset{k_1}{\rightleftharpoons}} T_n \cdot R \underset{\alpha_2}{\overset{\beta_2}{\rightleftharpoons}} T_n \cdot R^* \\
&+ \underset{k_3}{mQ} \Big\Updownarrow {\scriptstyle k_4} \\
&nT + R \cdot Q_m \underset{k_6}{\overset{k_5}{\rightleftharpoons}} T_n \cdot R \cdot Q_m \underset{\alpha_1}{\overset{\beta_1}{\rightleftharpoons}} T_n \cdot R \cdot Q_m^*
\end{aligned}
\tag{2}
$$

where m is the number of local anesthetic molecule binding to the AChR; and $k_{1\text{-}6}$, $\alpha_{1\text{-}2}$, and $\beta_{1\text{-}2}$ are the rate constants for the indicated processes, each of which may vary with local anesthetic concentration as well as voltage.

The kinetic schemes outlined above can be easily tested by comparing the effect of a local anesthetic on MEPC and ACh produced conductance fluctuations recorded from the same endplate. For both schemes the spectrum of the ACh produced conductance fluctuations should have the same number of components as the MEPC, with the cutoff frequency of each component of the conductance fluctuation spectra equal to the rate constant of decay of the corresponding phase of the MEPC. For scheme 1 the relative amplitudes of the components of the spectrum should be predicted by the relative amplitudes of the MEPC components as follows:

$$A_2/A_1 = M_2/M_1 \cdot \alpha_1/\alpha_2, \tag{3}$$

while scheme 2 would predict

$$A_2/A_1 = M_2/M_1 \cdot \gamma_2/\gamma_1 \cdot (\alpha_1/\alpha_2)^2, \quad (4)$$

where the MEPC decay is described by $M_1 e^{-\alpha_1 t} + M_2 e^{-\alpha_2 t}$, the spectrum of conductance fluctuation is fit as $A_1/(1 + (2\pi f/\alpha_1)^2) + A_2/(1 + (2\pi f/\alpha_2)^2)$, and γ_2 and γ_1 are, respectively, the single channel conductances of $T_n \cdot R^*$ and $T_n \cdot R \cdot Q_m^*$ in scheme 2. γ_2/γ_1 cannot be directly measured. Consequently, in a given cell it may not be possible to distinguish between scheme 1 and 2 since γ_2/γ_1 may equal α_2/α_1. In scheme 2 the local anesthetic alters only the rates of conformation changes and not the conformations themselves. Anderson and Stevens (16) noted only slight variation in the single channel conductance of normal receptors, and found that single channel conductance did not vary with temperature or holding potential. Therefore, γ_2/γ_1 should not vary appreciably. By comparing the relative amplitudes of the spectral and MEPC components for cells with different values of α_2/α_1 one can determine whether scheme 1 or 2 best describes the data.

It is possible to propose other physical models to explain the effects of local anesthetics on EPC. In general, the kinetic schemes resulting from such models will fall into one of two categories. In the first category the local anesthetic interacts with the AChR to produce two parallel populations of receptors with different channel closing rates. The second category consists of kinetic schemes in which the local anesthetic alters the receptor so that the AChR undergoes sequential conformation changes from one open state to another. The kinetic predictions of the parallel models will be similar to those of kinetic scheme 2. The predictions of the sequential models will be similar to those of kinetic scheme 1, with Eq. 3 containing a multiplier term consisting of a combination of the rate constants and single channel conductances associated with the model.

The present study was conducted on muscle fibers from the cutaneous pectoris muscle of *Rana pipiens*. The muscle was dissected down to a single layer of fibers and visualized by Nomarski optics to allow accurate localization of the endplates (17, 18). Contraction was prevented by bathing the fibers in frog Ringer's containing 100 nM tetrodotoxin. QX222 (Astra) was added to the frog Ringer's in concentrations of 0.05–0.5 mM. The two-electrode voltage clamp used has been described (1, 18) and is similar to that introduced by Takeuchi and Takeuchi (19). To improve the signal to noise ratio several MEPCs were collected from a given endplate and averaged. The analysis of amplitudes and rate constants of the components of the averaged MEPC was carried out in a manner similar to that detailed by Beam (7). A computer generated exponential curve with variable amplitude and time constant was adjusted to fit the slowest component of the MEPC. The generated curve was subtracted from the MEPC and the process was repeated for the next component. Collection and analysis of EPC conductance fluctuations are detailed by Anderson and Stevens (16). The spectra were fit by a computer generated curve consisting of the sum of two or three lorentzians. The cutoff frequencies of the generated curve were determined by the decay rates of the corresponding components of the MEPC, and the amplitudes of the lorentzians were adjusted to fit the EPC spectra.

In the absence of local anesthetic, both the MEPC and conductance fluctuation spectra exhibited a single component with the decay constant of the MEPC equaling the cutoff frequency of the noise spectrum, as described by Anderson and Stevens (16). Following the application of QX222, both the MEPC and the noise spectra showed multiple components. Again the decay constants of the MEPC accurately predicted the cutoff frequencies of the spectra. For concentrations of QX222 of 0.1 mM or greater and membrane potentials more negative than −50 mV, the MEPC and spectra could both be accurately characterized by two components (see Fig. 1). Cells studied at 0.05 mM QX222 and −70 mV holding potential were characterized by three components, with the rate constant of the intermediate phase being equal to that of the drug-free control. The cutoff frequency of spectral component continued to equal the decay rate of the corresponding MEPC component after changes in holding potential. After a hyperpolarizing step the slow component of both the MEPC and spectrum became slower and the fast component of both became faster.

23 cells were studied at 0.1 mM and 0.5 mM QX222, at holding potentials of −50 mV to −100 mV, and at temperatures of 10–18.6°C. Several cells were studied at two or more holding potentials. For the entire population the relationship between the ratio of the amplitudes of the MEPC components M_2/M_1 and the ratio of the amplitudes of the spectral components A_2/A_1 was predicted by Eq. 3 to within 10% of the observed value. Fig. 1 illustrates a typical cell for which the relationship between the

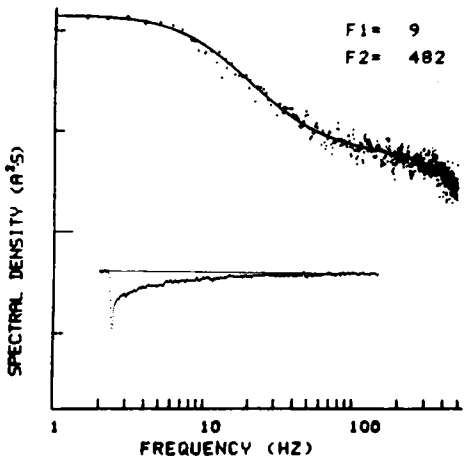

FIGURE 1 Spectrum of EPC fluctuations produced by a constant ACh iontophoretic application, with the average of 20 MEPC recorded from the same voltage clamped muscle endplate shown below. Cell studied at 0.1 mM QX222, −70 mV holding potential, and 18.6°C. The total time for the MEPC is 0.1024 s and the average MEPC amplitude was 0.9 nA. The ordinate is a logarithmic axis of spectral density in units of (ampere)^2 · second. The larger indicator on the ordinate signifies 10^{-23} A^2 · s, with each subsequent indicator signifying another power of 10. The continuous line through the spectral points is a theoretical two-component spectrum of the form $A_1/(1 + (f/F_1)^2) + A_2/(1 + (f/F_2)^2)$. The cutoff frequency, F_i, of each spectral component is determined from the decay rate, α_i, of the corresponding component of the MEPC, according to the relationship $F_i = \alpha_i/2\pi$. The spectral cutoff frequencies to the nearest hertz are shown in the upper right corner. $A_2/A_1 = 0.0260$. Eq. 3 predicted $A_2/A_1 = 0.0283$.

amplitudes of the components of the spectrum and MEPC was well predicted by Eq. 3. α_2/α_1 varied from 11.8 to 94.1 for all the cells studied. For 19 cells at 0.1 mM QX222 studied over the voltage and temperature ranges above and for six cells studied at 0.1 mM QX222, −70 mV holding potential, and 17.6 ± 0.8°C α_2/α_1 varied from 11.8 to 49.6. Consequently, kinetic scheme 2 and other members of the parallel class of models are ruled out if as argued earlier the ratio of the single channel conductances (γ_2/γ_1) is independent of voltage and drug concentration.

In conclusion it appears that the sequential action of local anesthetics on the AChR depicted in scheme 1 offers the best representation of the action of local anesthetics on EPC kinetics. Other members of the sequential class of models will also predict the data, but are not as economic because they would require arbitrary specification of the individual rate constants.

While this article was in review Katz and Miledi (20) published data on membrane voltage fluctuations and extracellularly recorded current fluctuations at the frog endplate in the presence of procaine. They found multiple component spectra and concluded, from comparison of the amplitudes of the components of the spectra and miniature endplate potentials, that procaine did not act by creating two kinetically distinct populations of acetylcholine receptors.

The author wishes to thank Dr. C. F. Stevens for the use of his equipment and his critical reading of this manuscript.

This work was sponsored by USPHS grant NS12961 to Dr. C. F. Stevens and National Institutes of General Medical Science Grant GM 02103 to Dr. R. L. Ruff.

Received for publication 2 September 1975 and in revised form 10 November 1975.

REFERENCES

1. MAGLEBY, K. L., and C. F. STEVENS. 1972. The effect of voltage on the time course of end-plate currents. *J. Physiol.* **223**:151.
2. GAGE, P. W., and C. M. ARMSTRONG. 1968. Miniature end-plate currents in voltage-clamped muscle fibres. *Nature (Lond.).* **218**:363.
3. KORDAŠ, M. 1970. The effect of procaine on neuromuscular transmission. *J. Physiol.* **209**:689.
4. DEGUCHI, T., and T. NARAHASHI. 1971. Effects of procaine on ionic conductances of end-plate membranes. *J. Pharmacol. Expl. Ther.* **176**:423.
5. MAENO, T., C. EDWARDS, and S. HASHIMURA. 1971. Difference in effects of end-plate potentials between procaine and lidocaine as revealed by voltage-clamp experiments. *J. Neurophysiol.* **34**:32.
6. STEINBACH, A. B. 1968. Alteration by Xylocaine (Lidocaine) and its derivatives of the time course of the end-plate potential. *J. Gen. Physiol.* **52**:144.
7. BEAM, K. G. 1975. A voltage clamp study of the effect of two lidocaine derivatives on the time course of endplate currents. *J. Physiol.* In press.
8. MAENO, T. 1966. Analysis of sodium and potassium conductances in the procaine end-plate potential. *J. Physiol.* **183**:592.
9. KORDAS, M. 1969. The effect of membrane polarization on the time course of the end-plate current in frog Sartorius muscle. *J. Physiol.* **204**:493.
10. STEINBACH, A. B. 1968. A kinetic model for the action of Xylocaine on receptors for acetylcholine. *J. Gen. Physiol.* **52**:162.
11. ADAMS, P. R. 1975. A model for procaine end-plate current. *J. Physiol.* **246**:618.

12. MAGLEBY, K. L., and C. F. STEVENS. 1972. A quantitative description of end-plate currents. *J. Physiol.* **223**:173.
13. ARMSTRONG, C. M. 1971. Interaction of tetraethylammonium ion derivatives with the potassium channel of giant axons. *J. Gen. Physiol.* **58**:413.
14. STRICHARTZ, G. R. 1973. The inhibition of sodium currents in myelinated nerve by quarternary derivatives of lidocaine. *J. Gen. Physiol.* **62**:37.
15. KEYNES, R. D., and E. ROJAS. 1974. Kinetics and steady-state properties of the charged system controlling sodium conductances in the squid giant axon. *J. Physiol.* **239**:393.
16. ANDERSON, C. R., and C. F. STEVENS. 1973. Voltage clamp analysis of acetylcholine produced endplate current fluctuations at frog neuromuscular junction. *J. Physiol.* **235**:655.
17. DREYER, F., and K. PEPER. 1974. A monolayer preparation of innervated skeletal muscle fibres of the M cutaneous pectoris of the frog. *Pfluegers Arch.* **348**:257.
18. DIONNE, V. E., and C. F. STEVENS. 1975. Voltage dependence of agonist effectiveness at the frog neuromuscular junction: resolution of a paradox. *J. Physiol.* **251**:245.
19. TAKEUCHI, A., and TAKEUCHI, N. 1959. Active phase of frog's end-plate potential. *J. Neurophysiol.* **22**:395.
20. KATZ, B., and R. MILEDI. 1975. The effect of procaine on the action of acetylcholine at the neuromuscular junction. *J. Physiol.* **249**:269.
21. DIONNE, V. E., and R. L. RUFF. 1976. Endplate current fluctuations reveal only one channel type at the frog neuromuscular junction. *Biophys. J.* **16**(2, pt. 2):212*a*. (Abstr.).