Alteration by Xylocaine (Lidocaine) and Its Derivatives of the Time Course of the End Plate Potential

A. B. STEINBACH

From The Rockefeller University, New York 10021. Dr. Steinbach's present address is the Department of Biophysics, University College, London W. C. 1, England. After 1 September 1968 his address will be Department of Anatomy, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York 10461

ABSTRACT Xylocaine and its derivatives act specifically at the neuromuscular junction within the concentration range 0.05 to 2.0 mM. The charged form is the active form of the drugs. There is no correlation between "local anesthetic" activity and effect at the junction. Like d-tubocurarine, these drugs have little or no effect on quantum content, acetylcholinesterase activity, or the passive impedance of the muscle fiber. Yet they produce end plate potentials characterized by a brief, early component and a late, greatly prolonged component, as does procaine. Analysis of these changes in time course suggests that the drugs have little or no effect before receptors are activated by acetylcholine, but cause a decreased and often greatly prolonged response. Clear structure-activity relations indicate that the receptor to which the drugs bind to produce the prolonged response can be the receptor for acetylcholine. Comparison of the effects of the drugs on the end plate potential and on the response to iontophoretically applied acetylcholine also shows that the effects of Xylocaine depend on the time course of receptor activation and are quite different from the effects of d-tubocurarine.

The experiments reported here were prompted by previous studies on the effects of the local anesthetic procaine at the neuromuscular junction of frog skeletal muscle (4, 10, 20, 21). Procaine has been found to depress the amplitude of the response of the postsynaptic receptors to acetylcholine (ACh) (4). But unlike d-tubocurarine (d-TC), which depresses the amplitude of the end plate potential (e.p.p.) without greatly altering its time course (rise time of 1–2 msec and half-time of fall 2–8 msec [9]) procaine produces e.p.p.'s that have an initial rapid transient (rise time 0.5–1 msec, half-time of fall 1 msec or less) followed by a greatly prolonged late falling phase (10). Maeno (20, 21) has shown that this prolonged phase arises from a prolonged flow.
of end plate current, and he concluded that procaine acted on the post-synaptic receptors themselves. Although procaine does not activate receptors, it can apparently change the time course of the activation produced by ACh.

To search for an explanation of the change in the time course of end plate response, I have studied the action of Xylocaine (lidocaine, lignocaine) and several derivatives at the neuromuscular junction. Xylocaine's molecular structure is similar to that of procaine, but it has a lower pKa (7.85 as opposed to 8.92 [19]), which enables one to determine, by changes in external pH, whether the charged or uncharged form of the molecule is active.

**Table 1**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>pKa</th>
<th>pH</th>
<th>Arel</th>
<th>Cm(tot)</th>
<th>Cm(f.b.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10166</td>
<td>7.67</td>
<td>6.45</td>
<td>0.70</td>
<td>0.77</td>
<td>0.22</td>
</tr>
<tr>
<td>6603</td>
<td>9.80</td>
<td>6.45</td>
<td>0.70</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>QX-314</td>
<td>—</td>
<td>6.45</td>
<td>0.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylocaine</td>
<td>7.85</td>
<td>7.80</td>
<td>1.30</td>
<td>3.79</td>
<td>0.82</td>
</tr>
<tr>
<td>L-30</td>
<td>8.96</td>
<td>6.45</td>
<td>0.82</td>
<td>36.00</td>
<td>0.76</td>
</tr>
<tr>
<td>10666</td>
<td>8.21</td>
<td>6.45</td>
<td>1.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QX-222</td>
<td>—</td>
<td>6.45</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>QX-222</td>
<td>—</td>
<td>7.80</td>
<td>1.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14465</td>
<td>7.35</td>
<td>6.45</td>
<td>1.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14465</td>
<td>7.35</td>
<td>7.80</td>
<td>2.30</td>
<td>11.1</td>
<td>5.14</td>
</tr>
<tr>
<td>10766</td>
<td>9.5</td>
<td></td>
<td>6.45</td>
<td>2.40</td>
<td>25.4</td>
</tr>
<tr>
<td>Procaine</td>
<td>8.92</td>
<td>7.45</td>
<td>0.46</td>
<td>14.4</td>
<td>0.26</td>
</tr>
<tr>
<td>Xylocholine</td>
<td>—</td>
<td>6.45</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Arel, concentration necessary to reduce early amplitude of the end plate potential to 5 mv divided by the concentration of QX-314 at pH 6.45 required for the same reduction. Typical absolute concentration of QX-314 = 0.3 mM.
† Cm(tot), extrapolated minimum concentration (mM) required to block propagation in the nerve. Data from Ehrenberg (8).
§ Cm(f.b.), calculated minimum concentration of free base. Data from Ehrenberg (8). Measurements carried out at pH 7.78 on sheathed nerve.
|| pKa determined only as 9.0 to 9.5.

Xylocaine was synthesized by N. Löfgren (19) and is manufactured by Astra Pharmaceutical Products Inc., Worcester, Mass. I am indebted to this company for gifts of Xylocaine and derivatives. Other drugs were purchased commercially.
Methods

Two muscle preparations with attached motor nerve supply were used in this study; the sartorius (9) and the extensor digitorum longus IV (EDL IV) (16) of the frog Rana pipiens.

An intracellular micropipette was used to record voltage across the end plate membrane (9). In some experiments, an extracellular micropipette was used to record external miniature end plate potentials (e.m.e.p.'s) which provided an indication of the end plate current through that spot (2, 3, 17, 18). An indifferent calomel/agar-Ringer electrode was used in the bath.

E.p.p.'s were evoked by stimulating the motor nerve. Iontophoretic pulses of ACh lasting longer than those released by the nerve terminal were applied directly to the end plate (4-6), using a micropipette filled with 3 M ACh connected through a large resistance to a DC source (for applying a "braking" current) in parallel with a pulse generator. In a few experiments double or triple barreled micropipettes were used, one of the barrels always being filled with ACh or carbachol (for techniques, see references 4-6).

The pH's of all solutions were adjusted within the range 6 to 8 with 10 mM glycyl-glycine-piperazine buffer (Documenta Geigy Scientific Tables, 1962, page 314). To ascertain that this buffer did not produce unwanted side effects, all results were checked with phosphate-buffered solutions. The standard Ringer contained 111.3 mM NaCl, 2.1 mM KCl, and 1.8 mM CaCl₂. The drugs used other than carbachol, ACh, and d-TC are listed in Table I, and henceforth the term "drugs" used without qualification will refer to those listed. In most experiments, these drugs were applied by replacing the bath solution with the drug solution in Ringer's. Changes of the bath solution could be completed within 20 sec.

Results

The Effects of Xylocaine on the End Plate Potential

Xylocaine and its derivatives reduced the amplitude and modified the time course of the e.p.p. (Figs. 1 and 2). (All e.p.p.'s shown are reduced in amplitude relative to the e.p.p. in Ringer's which has an amplitude in excess of 30 mv [9, 26].) The effects of any one drug at a given concentration were reproducible and could be reversed in less than 3 min by washing with Ringer's. In different fibers the amplitude of the e.p.p. during treatment with any drug at a given concentration varied over a fourfold range. This variation was not clearly linked to variations in resting potential or in the passive electrical properties of the muscle fibers. The e.p.p.'s illustrated in Figs. 1 and 2 were obtained from a single muscle fiber by intracellular recording. Each record is a photographic superposition of three successive e.p.p.'s. Between tests with each drug, the preparation was washed with Ringer's, and then treated with a standard concentration of d-TC, allowing the same equilibration time in each case. Only one of these control responses
FIGURE 1. Comparison of the effects of Xylocaine derivatives on the e.p.p. in a single EDL IV fiber, resting potential -89 mv. Each chemical was applied, the resulting e.p.p. was recorded for several successive nerve stimulations, plus a base line trace without stimulation, the chemical was washed out with Ringer's, and the response in the presence of d-TC was reexamined before applying the next chemical. A, 0.004 mM d-TC. B, 0.56 mM Xylocaine. C, 0.39 mM L-30. D, 0.34 mM 6603. E, 1.02 mM 14465. F, 1.6 mM QX-222 (note different voltage scale). G, 0.96 mM 10666. H, 1.07 mM 10766. pH 6.8. Calibration pulses at the beginning of each trace, 1 mv, 1 msec. The rapidly rising phase of the e.p.p. in (F) is not clearly resolved but could be seen at higher sweep speed (e.g., Fig. 4 C, right).
Figure 2. Comparison of the effects of various Xylocaine derivatives (same experiment as that in Fig. 1, but at 5 times higher sweep speed). A, 0.005 mM d-TC. B, 0.37 mM Xylocaine. C, 0.40 mM L-30. D, 0.34 mM 6603. E, 0.32 mM QX-314. The e.p.p. shown in E was very similar to those produced (in the same preparation) during treatment with 0.4 mM FB-1, xylocholine, or 0.7 mM 10166. pH 6.8. Calibration pulses on each trace 1 mv, 1 msec.
is shown in each figure but they remained identical through the series of solution changes.

To facilitate discussion of the effects of the drugs, it is useful to specify the various phases of the e.p.p. in greater detail, using Fig. 1 A and B as paradigms. The *early brief component* of the e.p.p. refers to the initial rapid rise (depolarization) and early fall; this component is curtailed by the drugs. The *maximum early amplitude* is defined as the maximum amplitude of the e.p.p., or the amplitude at which the initial rapid rise changes abruptly to a slow rise (e.g., Fig. 4 C, right). The term *prolonged component* refers to a prolongation of depolarization seen during treatment with Xylocaine and some of its derivatives, but not during treatment with d-TC, where the duration of the falling phase of the e.p.p. primarily reflects the passive impedance of the muscle fiber membrane (9, 26).

**Figure 3.** Tracings of e.p.p.'s (EDL IV) showing effects of increasing concentrations of d-TC (dashed lines) and Xylocaine (solid lines). The upper curves were produced by 0.001 mM d-TC and 0.08 mM Xylocaine, respectively; the lower ones by 0.005 mM d-TC and 0.24 mM Xylocaine. The solution pH was 6.8.

**THE EARLY BRIEF COMPONENT** All the drugs reduce the early amplitude of the e.p.p. in proportion to their concentration, with slight differences in relative effectiveness (Table 1, column 4). They have qualitatively similar effects on the early time course of the e.p.p., and there are no obvious differences that might be correlated with molecular structure. During treatment with the drugs, recorded e.p.p.'s typically have a shorter time of initial rise than during treatment with d-TC (average 52% of the time of rise seen in d-TC, range 38 to 71%, 15 experiments tabulated). d-TC reduces the amplitude of the e.p.p. in proportion to the concentration applied, but produces little change in time course (Fig. 3). Thus the time to the peak is not greatly changed, and the absolute rate of rise of the e.p.p. is reduced in proportion to the concentration of d-TC. Xylocaine and its derivatives also reduce the
amplitude of the e.p.p., but reduce only slightly the absolute rate of rise (Fig. 3), and thus shorten the rise time, especially relative to an e.p.p. of equal amplitude during treatment with d-TC.

In instances in which the e.p.p. has no prolonged component, for example, in a muscle treated with drug 10766 (Fig. 1 H), the early rapid fall of the e.p.p. is very fast. In other cases (e.g., Fig. 1 E and F), the early fall is barely noticeable or obscured altogether by the prolonged component.

![Graph](image_url)

**Figure 4.** Effect of change in solution pH on the action of Xylocaine and two of its derivatives, A, Xylocaine, total concentration at both values of pH, 0.6 mm. B, QX-314, total concentration 0.53 mm. C, 14465, total concentration 1.0 mm. The waveforms shown in (A) and (B) were taken from a single preparation of EDL IV; calibration bars 1 mv and 5 msec. Those in (C) were taken from a different preparation, calibration pulses 1 msec and 1 mv.

**THE PROLONGED COMPONENT OF THE END PLATE POTENTIAL.** The relative amplitude of the prolonged component of the e.p.p. ($V_p$) will be defined as its maximum amplitude divided by the maximum early amplitude. Its presence and amplitude are definitely related to the molecular structure of the drugs. The value of $V_p$ produced by a given drug at a constant concentration varies slightly from preparation to preparation. However, the samples shown in Figs. 1 and 2 are typical and the following structure-activity relations were observed in all preparations. (a) $V_p$ depends critically on the substituents on the terminal nitrogen. The derivatives with three ethyl or two ethyl or two propyl (or butyl) groups produced no prolongation (Fig. 2 E). The derivative with two methyl groups and its quaternary analogue (drug
QX-222) produce a much larger $V_p$ than Xylocaine itself (Fig. 1 E and F).

(b) There appears to be an optimum distance of separation between the carbonyl group and terminal nitrogen, since the derivative with two carbon atoms (drug L-30) produces a larger $V_p$ than does Xylocaine or drug 6603 (Fig. 1 B, C, and D). This optimum is not related in any obvious way to differences in the ionization constant ($pK_a$) of the three drugs (Table I). (c) The carbonyl group of Xylocaine is essential for production of a prolonged component. Neither drug 10766 (Fig. 1 H) nor xylocholine (TM-10, Smith Kline and French Laboratories, Philadelphia, Pa.), Fig. 2 E) produces prolonged components of the e.p.p. (d) The substituents and electronic structure of the xylyl ring are significant in determining the action of Xylocaine. Drug 10666 (Fig. 1 G), lacking 2-, 6-substituents, produces a larger $V_p$ than does Xylocaine, while the derivative with propyl groups on the xylyl ring (drug FB-1, Fig. 2 E) and that with an additional methyl group in the 4-position (drug LL-31, Astra (not illustrated)) produce no prolonged component. (e) The amide linkage of Xylocaine apparently confers no special properties on the molecule, since procaine (which has an ester linkage) produces a $V_p$ equivalent to that produced by Xylocaine at pH 6.5 (see next section).

### Table II

<table>
<thead>
<tr>
<th>Chemical</th>
<th>$pK_a$</th>
<th>C</th>
<th>$\Delta E.A.$</th>
<th>$\Delta V_p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xylocaine</td>
<td>7.85</td>
<td>1.8</td>
<td>1/1.4–1/2.1</td>
<td>1.5–2.5</td>
</tr>
<tr>
<td>14465</td>
<td>7.35</td>
<td>3.3</td>
<td>1/2.1–1/3.0</td>
<td>2.6–3.3</td>
</tr>
<tr>
<td>L-30</td>
<td>8.96</td>
<td>1.1</td>
<td>1/1.1</td>
<td>—</td>
</tr>
<tr>
<td>Procaine</td>
<td>8.92</td>
<td>1.06</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Effects of pH

Lowering the pH of the bathing solution is known to decrease the permeability of the muscle membrane to chloride ions (14, 15), and to decrease the quantum content of the e.p.p. ([7] and unpublished personal observations).
In standard Ringer’s solution within the pH range 6 to 8, these competing direct effects of hydrogen ions balance each other, and the action of quaternary derivatives of Xylocaine (and that of d-TC) was not affected by changes in solution pH (Table I, Fig. 4 B).
The actions of Xylocaine and its partially charged derivatives were definitely influenced by pH. Decreases in external pH decreased the maximum early amplitude of the e.p.p. and increased \( V_p \) (Fig. 4). Both changes simulated changes produced by increasing the drug concentration, and were consistent with calculations based on the Henderson-Hasselbach equation if one postulates that only the charged form of the drugs is active (Table II).

The Effects of Xylocaine on the Miniature End Plate Potentials

Xylocaine and its derivatives decrease the amplitude of miniature end plate potentials (m.e.p.p.'s) and produce modifications of their time course similar to those observed in the e.p.p., in agreement with results reported for the effect of procaine (20) (Fig. 5). A drug concentration that reduced the amplitude of m.e.p.p.'s to about half (0.05–0.08 mM) reduced the time of rise of the waveform by about 30\% (seven experiments), though there was no indication that the absolute rate of rise was increased. The time course of e.m.e.p.p.'s was also altered (Fig. 6), but never showed a minimum separating the early transient component from the prolonged component, even during treatment with drugs that produced such a minimum in the e.p.p. (e.g., Fig. I E) (see [25]).

Deciding Where Xylocaine Acts

The known events governing the amplitude and time course of the e.p.p. may be separated under five main headings: the presynaptic nerve action potential, the presynaptic release of ACh, the reaction of ACh with postsynaptic receptors, the hydrolysis of ACh by acetylcholinesterase (AChE), and the charging of the membrane capacitance of the muscle by the end plate current. The following observations concern possible effects of the drugs other than those on the postsynaptic receptors and indicate that such actions probably do not significantly contribute to the effects of Xylocaine and its derivatives at the neuromuscular junction.
Table I shows that there is no correlation between local anesthetic activity (columns 5 and 6) and relative neuromuscular blocking effectiveness of the drugs (column 4). Block of the action potential in the nerve terminal produces a sudden and complete loss of the e.p.p., but this occurs for most of these drugs only at concentrations above 2 mM. Furthermore, some drugs that have effects on the e.p.p. have no detectable effects on electrical excitability. At 10 mM, neither QX-314 nor QX-222, both quaternary derivatives of Xylocaine, has any effect on the electrical excitability of muscle or on the propagation of the action potential in the desheathed nerve trunk. At 4 mM QX-314 has no effect on the ionic currents in isolated frog nodes of Ranvier (13), whereas 0.1 mM Xylocaine reduces the amplitudes of the sodium currents to about one-half in such preparations (12). The relations between molecular structure and the value of $V_p$ are not correlated with increasing local anesthetic activity (11, 23). The prolonged component is not the result of repetitive activity in the nerve terminal, since this produces repetitive e.p.p.'s. It might be produced by prolonged depolarization, but this would not account for prolongation of the m.e.p.p.'s.

Xylocaine and its derivatives have no significant effect on the quantum content of the e.p.p. (see reference 22 for discussion of quantum content). Concentrations of the drugs higher than 0.05 mM reduce the m.e.p.p. amplitude below the base line noise and do not produce the "quantally" fluctuating responses that occur during treatment with drugs which have a strong pre-
synaptic depressant action. Slight presynaptic effects masked by strong postsynaptic actions would not have been detected in the present experiments.

It is natural to suggest that the prolongation of the e.p.p. produced by Xylocaine and its derivatives might be the result of AChE inhibition. But not only are the prolongations produced of longer duration than the prolongation of the entire e.p.p. produced by known inhibitors of AChE in normal Ringer's (9, 26), but also the presence of either 0.1 mM neostigmine or diisopropylfluorophosphate (DFP) does not affect the change in waveform produced by Xylocaine (Fig. 7).
At concentrations up to 2 mM Xylocaine and its derivatives have no effect on the passive electrical properties of muscle fibers bathed in normal Ringer's. At any concentrations below 20 mM, none of the chemicals depolarizes the muscle when applied in the bathing solution.
These observations suggest that the modifications of the time course of the e.p.p. and of the m.e.p.p. by Xylocaine and its derivatives do not depend on changes in the presynaptic terminal, in the activity of AChE, or in the impedance of the muscle fiber. Thus the postsynaptic receptors seem to be the sites at which the drugs act.

**Effects of Xylocaine and Derivatives on the Response to Iontophoretically Applied Acetylcholine**

**EFFECTS OF DRUGS IN THE BATHING SOLUTION** Xylocaine itself and derivatives that produce relatively small amplitudes of $V_p$, significantly depress the response to iontophoretically applied ACh (Fig. 8). Drugs such as QX-222 and 14465 that produce large values of $V_p$ (i.e., large prolonged components of the e.p.p.) are much less effective in depressing the response to ACh (Fig. 8 A and C). The highest concentrations of such drugs used ($>6$ mM) failed to eliminate iontophoretically evoked responses to ACh. However, these drugs did depress the early rising phase and enhance the late falling phase, causing a general lengthening of the response (Fig. 8 B
and C). At very low concentrations drugs QX-222 and 14465 sometimes increased slightly and transiently the amplitude of iontophoretically evoked responses to ACh.

**EFFECTS OF BRIEF PULSES OF DRUGS** Using multiple barrelled pipettes, the effects of pulses of Xylocaine and QX-222 on the response to a test pulse of ACh or carbachol were examined in five preparations (Figs. 9 and 10). A pulse of QX-222 delivered before a pulse of ACh reduced the rate of rise and prolonged the time course of the test response, producing only slight depression in peak amplitude (Fig. 9 A). A pulse of Xylocaine delivered with similar timing produced depression with slight prolongation (Fig. 9 D).

The effects of increasing pulses of drug QX-222 applied at the same time as a standard pulse of ACh are shown in Fig. 9 B. The degree of prolongation, relative to depression of peak amplitudes, varied at different end plates in the same preparation, but was always much greater than in the case of Xylocaine (Fig. 9 D and E).

If a pulse of QX-222 was applied after a pulse of ACh or carbachol, the drug produced a characteristic effect as long as the response to ACh was visible. This consisted of an early phase of depression, and a later prolongation (Figs. 9 C and 10 B and C). Xylocaine produced the depression, but only slight prolongation (not shown).

The effect of a drug pulse appeared to last only slightly longer than the depolarization produced by a brief pulse of ACh, and it was considerably briefer than the depression following a pulse of d-TC. The drugs produced no depolarization when applied alone. All these effects were observed in preparations treated with 0.1 mM neostigmine, and similar effects were observed when carbachol was substituted for ACh (Fig. 10). Even with the briefest responses observed (rise time 5–8 msec) there was no sign that either Xylocaine or QX-222 produced an early brief component similar to that seen in the e.p.p. This was due to the relatively slow time course of application of ACh, compared with that released from the nerve terminal; e.p.p.'s with two maxima occur both because of the time-variant effects of Xylocaine, and because of the impedance properties of the muscle membrane (24, 25).

**DISCUSSION**

The action of Xylocaine at the neuromuscular junction seems unrelated to its action as a local anesthetic. Quaternary drugs that affect the neuromuscular junction are inactive as local anesthetics, and considering all derivatives tested, there is no correlation between local anesthetic activity and changes in amplitude or time course of the e.p.p. Although some drugs block presynaptic electrical activity at concentrations above 2.0 mM, the
A. B. STEINBACH  Alteration by Xylocaine of End Plate Potential Time Course

full range of effects noted (on e.p.p.'s, m.e.p.p.'s, and responses evoked by iontophoretically applied ACh) is unlikely to be the result of a presynaptic effect.

Although local anesthetics may inhibit AChE activity, and can modify the passive impedance of cell membranes, the experimental evidence presented suggests that neither of these possible actions is significant when considering the effects of the drugs at the neuromuscular junction. Taken alone, the range of effects of the drugs indicates an action on the postsynaptic receptors for ACh; the supplementary evidence suggests that this is the only significant locus of action.

The molecular properties of Xylocaine and its derivatives that appear to be important in causing a prolongation of the e.p.p. are: (a) a charged terminal nitrogen, preferably a methonium moiety; (b) a carbonyl group; (c) a distance of about 6 Å separating terminal nitrogen and carbonyl; and (d) a terminal ring of certain optimal properties. With the exception of (d) these properties are identical with the molecular properties of ACh known to be important in the activation of receptors (1) and different from the properties of good local anesthetics (11, 23). Since sites of action other than the postsynaptic receptors are not likely, the Xylocaine derivatives must be capable of blocking ACh receptors. Thus, although the two main effects of the drugs—a prolonged activation of end plate current, and a depression of receptor response causing the decrease in early peak amplitude—are very different, the available evidence suggests that they involve the binding of the drugs to the same, or very similar receptors. Furthermore, the two effects on the e.p.p. produced by the drugs cannot be separated by changes in concentration or in pH; a decrease in the amplitude of the early component is always accompanied by an increase in $V_p$, and vice versa. If two separate sites of action were involved, one would expect that such a separation could be obtained.

In experiments in which the e.p.p. was recorded, Xylocaine and its derivatives were tested at a constant bath concentration. Yet within the temporal sequence of the e.p.p. the drugs produce both depression and prolongation, suggesting that their action is dependent on the time course of receptor activation by ACh in a way that the action of d-TC is not. Such dependence could mean that the effects of the drugs on the iontophoretically evoked response might be qualitatively different from their effects on the e.p.p. To account for the effects of Xylocaine and its derivatives on the e.p.p., one must postulate that the depressant action producing the curtailment of the early brief component is supplemented within 1 to 2 msec by an action that enhances the late falling phase. Because of the relatively slow buildup of ACh near the receptors during iontophoretic application, it is not surprising that the only observed effect of the drugs on the early waveform of
iontophoretic responses is depression; the brief transient in the case of the
e.p.p. could be explained if Xylocaine, although present in constant con-
centration, does not become effective until receptors have been activated by
ACh. This suggestion fits well with the observation that none of the drugs
produces a significant depression of the initial rate of rise of the e.p.p. It
also would explain why both the drugs tested produce an early depression of
the response to iontophoretically applied ACh when applied in pulses after
the pulse of ACh (Figs. 9 and 10).

Drugs that produce a large prolonged component of the e.p.p. (large
$V_p$) also prolong the response to iontophoretically applied ACh after de-
pressing the early rate of rise of the response. Although the rate of buildup
of ACh is slow during iontophoretic application, the total amount of ACh
should become large within 5 to 10 msec, and any enhancing effect of the
drugs should then become evident. According to the hypothesis discussed in
detail in the following paper (25), the action of Xylocaine and its derivatives
that produces the curtailment of the early transient of the e.p.p., and the
early depression of the iontophoretically evoked response to ACh, also leads
to the production of some stable activated receptors. More of these are pro-
duced by QX-222, and thus this drug produces a relatively large prolonged
component of the e.p.p., and a great prolongation of the response to ion-
tophoretically applied ACh, while Xylocaine itself produces a lesser pro-
longed component (smaller $V_p$) and little prolongation of the iontopho-
retically evoked response. The two effects of any one drug can take place at
any time after ACh has begun to activate receptors, and this leads to the
effects of QX-222 when a pulse of the drug is applied after a pulse of ACh
(Fig. 10).

I am indebted to Dr. Alexander Mauro, my advisor during the predoctoral period in which I carried
out most of this work. I would also like to thank Drs. F. A. Dodge, Jr., and C. M. Connelly for their
advice, Bertil Takman and George Camougis for helpful discussion, and Enrico Stefani at University
College, London, for his help in some of the experiments using iontophoretic techniques.

Received for publication 12 December 1967.

BIBLIOGRAPHY

3. Castillo, J. Del, and B. Katz. 1956. Localization of active spots within the neuromuscular
4. Castillo, J. Del, and B. Katz. 1957. A study of curare action with an electrical micro-
5. Castillo, J. Del, and B. Katz. 1957. A comparison of acetylcholine and stable depolariz-
6. Castillo, J. Del, and B. Katz. 1957. Interaction at end-plate receptors between different
A. B. STEINBACH  Alteration by Xylocaine of End Plate Potential Time Course