CHARACTERIZATION OF DRUG IONTOPHORESIS WITH A FAST MICROASSAY TECHNIQUE

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ABSTRACT The iontophoretic release of drugs from micropipettes into free (Ringer's) solution was described using an ion-selective microelectrode assay method. This characterization, with a temporal resolution of 20 ms, showed that the equilibrium rate of drug transport was not linearly proportional to release current; the departure from linearity was increased by backing current and the result was demonstrated with analytically derived drug release functions. The general relation between the drug transport rate and release current was independent of the specific drug or pipette resistance; no functional relation was observed that might quantitatively predict this dependence without prior use of the assay. The diffusion coefficients at 25°C in frog Ringer's of the drugs used in this study, all neuromuscular agonists, were determined: all values $\times 10^6$ cm^2/s; acetylcholine 6.11 $\pm$ 0.30; carbamylcholine 7.44 $\pm$ 0.34; 3-(*m*-hydroxyphenyl) propyltrimethyl ammonium 5.79 $\pm$ 0.13.

INTRODUCTION

The iontophoretic application of drugs is widely used in physiological and pharmacological research, its attraction being that both location and duration of drug release can be carefully controlled. The technique was first described by Nastuk (1951, 1953) and more quantitatively by del Castillo and Katz (1955). Commonly a glass micropipette is filled with concentrated solution of the drug to be applied. A voltage of correct polarity placed between the micropipette interior and an external bath will cause ions (including drug ions) to flow through the pipette and be ejected at the tip, the iontophoretic current. Voltages of opposite polarity are applied to reduce drug leakage, the backing voltage. Control of iontophoretic current allows the duration and magnitude of drug ejection to be varied while careful positioning localizes the application.

Possibly because the iontophoretic current can be easily and accurately measured, iontophoretic application has always offered a promise of ready quantitation. However, technical difficulties have compromised all experimental attempts to resolve the relationship, the major problem being the drug assay. Consequently, it is commonly assumed that the rate of drug release is proportional to iontophoretic current. Thus the transfer number, the fraction of current carried by drug molecules, is treated as a

constant. Reports have appeared which tend to support this assumption (Kusano et al., 1975; Zieglgänsberger et al., 1969; Krnjević et al., 1963; see, however, Clarke et al., 1973).

In this report a new assay technique for quaternary amines will be described. The method involves the detection of amine-ion activity with an ion-selective microelectrode and has proved useful in studies of acetylcholine receptor kinetics at the neuromuscular junction. The technique is accurate, sensitive, nondestructive, and relatively easy to apply in contrast to conventional chemical (Hanin, 1973) and bioassay (Kuffler and Yoshikami, 1975) methods. In addition measurements can be made rapidly, allowing concentration to be monitored during application.

To demonstrate the speed and sensitivity of the assay, studies designed to characterize iontophoresis will be described. Measurement of concentration changes following drug release by iontophoresis has allowed the time course of drug release itself to be determined and factors which affect release to be identified.

METHODS

The ion-selective microelectrode used here for the assay of quaternary amine concentration has been described by others (Walker, 1971; Dionne and Stevens, 1975). It is constructed of a glass micropipette containing a bit of organic resin in its tip and filled with an aqueous reference solution. The resin which constitutes the liquid membrane is the Corning code 477317 K^+ exchanger. Functionally identical microelectrodes have been employed to measure K^+ activity in several physiological studies (Cornwall et al., 1970; Kunze and Brown, 1971; Khuri et al., 1972; Neher and Lux, 1973; Kříž et al., 1975). Detailed construction methods for the microelectrodes can be found in Walker (1971).

Although Baum (1970) indicated a nonideal response to quaternary amines for this exchanger, this can be corrected by tailoring the reference solution for the amine being assayed. In general, reference solutions were composed of 1–2 M KCl and 1–2 M quaternary-ion-of-interest. For example, acetylcholine assay electrodes contained a reference solution of 1.5 M KCl with 1.2 M acetylcholine · Cl, while those for carbamylcholine measurements contained 1.4 M KCl with 1 M carbamylcholine · Cl. Electrodes in which the reference solution contains a quaternary ion different from the one being assayed do respond but not in an ideal fashion nor with maximum sensitivity.

Following use the individual pipettes were calibrated by exposure to Ringer's baths containing known drug concentrations. Both the electrode sensitivity and the useful linear range of the sensitivity relation (Eq. 2) were determined.

The ion-selective microelectrodes have a nominal resistance of 10^9 Ω, necessitating a very high input impedance amplifier. Electrical connection to the reference solution was made by a Ag-AgCl wire which led directly to a MOSFET preamplifier with capacity compensation.

Iontophoretic micropipettes were selected to represent a broad impedance range, from 10 to 150 MΩ, although most ranged 20–60 MΩ. Three neuromuscular agonists were used: ACh (acetylcholine chloride, Sigma Chemical Co., St. Louis, Mo.), CCh (carbamylcholine chloride, Sigma Chemical Co.), and HPTMA (3-(*m*-hydroxyphenyl) propyltrimethyl ammonium, supplied by D. Colquhoun, St. George's Hospital Medical School, London, England).

During studies involving iontophoretic release the iontophoresis micropipette was positioned near the assay microelectrode, their tip separation much less than the distances to container wall or test solution surface. Separations from 2 to 80 μm were measured (±1 μm) through a compound microscope with a water immersion objective (total 400×) using a calibrated grati-

cule. Sampling was done with an 11-bit A-D converter operated by a small computer; its internal crystal clock error was < 1%. The iontophoretic micropipette was controlled with a constant current device which also allowed the tip capacity to be compensated. Although the assay microelectrode was always compensated, unless specifically indicated in the text the iontophoretic micropipette was not.

All measurements were made in a phosphate-buffered frog Ringer's solution. The normal salt composition was (millimolar): NaCl 117.2; KCl 2.5; CaCl 1.8; Na_2HPO_4 2.16; NaH_2PO_4 0.85; pH 7. Bath temperature was not controlled, but was normally room temperature, 24–26°C with extremes 21° and 28°C.

RESULTS

Characteristics of the Ion-Selective Electrode Response

The ion-selective electrode develops a voltage in response to membrane permeable ions driven by gradients in their electrochemical potentials. Desirable exchanger properties have been discussed by Walker (1971), and a theoretical description of liquid membrane ion-selective electrode behavior was given by Sandblom et al. (1967).

Dependence on Concentration. When a membrane permeable ion is present in unequal activities in the reference and test solutions a voltage will develop opposing the chemical potential of the ions. The expression relating electrical potential E and test solution ion activities derives from equilibrium of the electrochemical potential across the membrane.

$$E - E_r = (RT/F)\ln(A_a + K_iA_i). \tag{1}$$

Here E_r is a reference potential which depends on reference solution composition; A_a is the activity of quaternary amine cation "a", A_i that of an interfering cation "i" possessing relative membrane selectivity K_i, and RT/F is the product of the ideal gas constant and the absolute temperature divided by the Faraday. For simplicity, Eq. 1 assumes that only one interfering ion is present. At sufficiently high amine concentration voltage is developed proportional to logarithmic changes in amine activity, exhibiting an ideal sensitivity of 59 mV/decade activity for monovalent cations at 25°C. At low concentrations the response is limited by the interfering cations.

Consider the behavior of Eq. 1 when $A_a \ll K_iA_i$. Since $\ln(1 + y) = y - y^2/2 + y^3/3 - \cdots$, Eq. 1 can be rewritten to first order as

$$E - E_r - (RT/F)\ln(K_iA_i) = (RT/FK_iA_i)A_a = (RT/FK_iA_i)\gamma_aC_a,$$

where C_a is the amine ion concentration and γ_a its activity coefficient. Then, if E_0 is the electrode potential relative to the case that no amine is present,

$$E_0 = pC_a. \tag{2}$$

Thus induced potential is proportional to amine concentration under these circumstances. Notice that this relation depends on the presence of interfering ions, ions which have a small relative selectivity but make a non-negligible contribution to the electrode response. This is an advantage in physiological studies since the saline solu-

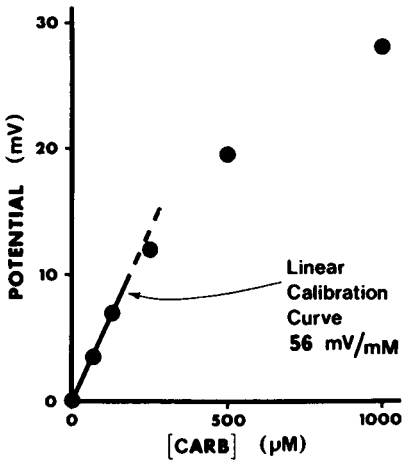

FIGURE 1 Carbamylcholine assay sensitivity. Both the linear response region below 10 mV and the extended nonlinear response of the assay microelectrode are shown by this calibration data for CCh. Similar curves having different linear mean sensitivities were obtained for ACh and HPTMA.

tions contain interfering ions, and quaternary amines may be used at relatively low concentration. In practice I have found that if electrode response to a quaternary ion in frog Ringer's is < 5–10 mV, Eq. 2 applies with negligible error. Responses > 10 mV behave nonlinearly as Eq. 1. This behavior is illustrated in Fig. 1.

The ion-selective electrode varies in its sensitivity to different quaternary amines; however, a comprehensive investigation of this has not been made. Rather, the ions studied reflect the laboratory use of the electrode as an assay device for neuromuscular agonists. Nominal values for agonist concentrations which produce a 5 mV electrode potential in Ringer's are given in Table I. These represent an approximate concentra-

TABLE I
ASSAY SENSITIVITY AND DIFFUSION COEFFICIENTS

Agonist	Assay sensitivity (nominal concentration for 5 mV response in Ringer's)	Diffusion coefficient $\times 10^6$ cm^2/s at 25°C ± SEM
	μM	
Acetylcholine chloride	50	6.11 ± 0.30 (11)
Carbamylcholine chloride	100	7.44 ± 0.34 (6)
3-(*m*-hydroxyphenyl)-propyltrimethylammonium	25	5.79 ± 0.13 (6)

The ion-selective assay microelectrode nominal sensitivity for the three drugs studied along with their diffusion coefficients in Ringer's are tabulated. Among individual microelectrodes sensitivity ranged at least two-fold from the numbers given. The diffusion coefficients have been obtained by averaging the mean value determined in the number of experiments shown in parentheses. Each experiment represents a different assay microelectrode and iontophoretic pipette. Data was not corrected for temperature.

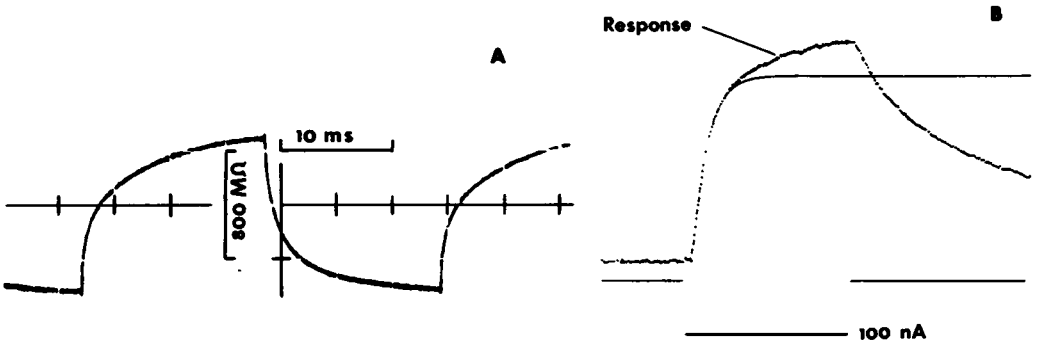

FIGURE 2 Estimation of the assay microelectrode time constant. (*A*) The follower output is shown when maximum capacity negation was applied to an 1,100 MΩ CCh-assay microelectrode with a triangle wave input. The time constant, estimated as time to 63% of maximum value, was 5 ms. (*B*) ACh was applied 2 μm from an assay microelectrode using a 100 nA, 100 ms iontophoretic pulse (lower trace). The concentration increased rapidly, saturating the assay response for several milliseconds. The initial saturated response was fit by Eq. 3 with $\tau = 8.75$ ms. When the rate of concentration increase slowed the electrode response was better described by Eq. 1, and deviated from the exponential as shown. Sample interval 0.5 ms.

tion limit below which the electrode response is linear. Using digital sampling and averaging, induced potentials < 0.5 mV can be accurately recorded in Ringer's, corresponding to a concentration as little as 5 μM for ACh.

Time Characteristics. The electrode time constant has been estimated with two methods giving similar results. The first method was applied to all 34 electrodes used for this study (16 ACh, 9 CCh, 9 HPTMA). A triangular waveform was injected through a 5 pF capacitor to the microelectrode to monitor both resistance and the degree of capacity compensation (Gesteland et al., 1959). The follower output to each ramp appeared as a step (see Fig. 2A) whose rise time was the charging time constant τ of the electrode. By compensating the tip capacity τ could be reduced from several seconds to typically <10 ms (e.g., Neher and Lux, 1973).

The second method of determine τ involved a saturating application of quaternary amine in the test solution. If drug could be presented to the assay microelectrode faster than it could faithfully respond, the induced potential would rise exponentially as

$$E_0 = A(1 - e^{-t/\tau}), \tag{3}$$

where A depended upon the rate of drug application. As the rate slowed the response became unsaturated and was described by Eq. 1. An example of this method, in which ACh was applied iontophoretically, is shown in Fig. 2B. Three electrodes were studied in this manner; all had response times <10 ms.

In the sections which follow the assay response will be mathematically analysed to determine the rate of drug release by iontophoresis. Presumably iontophoretic release may be controlled with great temporal accuracy, but its resolution will be limited in principle by the assay device time constant and in fact by the data-processing methods.

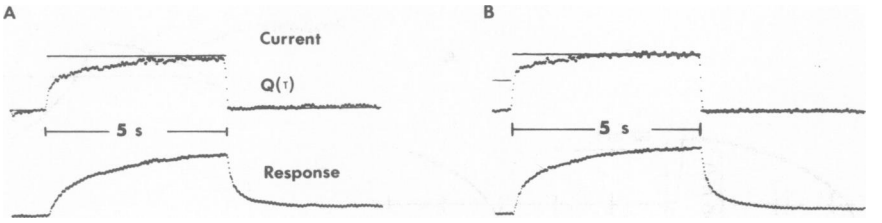

FIGURE 3 Transient changes in the rate of drug release. In two separate applications, *A* and *B*, CCh was iontophoretically released 13 μm from an assay microelectrode. The iontophoretic current and assay response were monitored, and the drug release function $Q(\tau)$ determined as described in the text. The current trace is composed of three parallel line segments, the initial backing, 5 s release and final backing currents. $Q(\tau)$ was scaled identically in both *A* and *B* and superimposed on the iontophoretic current final backing value. Below each trace is the assay response which is proportional to carbachol concentration. Response *B* differs from *A* because the initial backing current was less intense. *B* was measured within 2 min of *A*; the protocols for both could be repeated many times with no essential differences from the responses shown. Sample interval 20 ms. Iontophoretic pipette resistance 35 MΩ.

The Release of Quaternary Amines from Iontophoretic Pipettes

Observations. The fraction of iontophoretic current carried by drug is not constant following a step increase in current. Rather the transfer number increases with time to an equilibrium value characteristic of the particular iontophoretic current and pipette. Furthermore, the rate of approach to the equilibrium transfer number can be significantly altered by the prior iontophoretic current history. Large initial backing currents cause a more prolonged approach to equilibrium than do small backing currents.

The observation that the transfer number changes with time following a step increase in iontophoretic current is documented in Fig. 3A. If the transfer number were always proportional to iontophoretic current, the rise in assayed concentration caused by a release current step would be symmetric to (although inverted from) the fall in concentration following termination of release (see Quantitative Description below). In Fig. 3A this was clearly not the case. The initial backing current was −17.7 nA; detectable leakage occurred with −7 nA. Release was made with a −2.6 nA, 5 s current pulse after which current returned to −17.7 nA. CCh concentration rose more slowly than it decayed indicating that the CCh release rate was not constant during the pulse although iontophoretic current was.

The effect of backing current upon this transient change in the transfer number can be seen when Figs. 3A and 3B are compared. The protocol for CCh release in 3B differed from 3A only in the initial backing current, now −9.6 nA, which was closer to the value at which leakage was detected. The iontophoretic release pulse resulted in a CCh concentration which rose and decayed with greater symmetry than in 3A. That is, smaller backing currents cause less transient perturbation of the transfer number than larger backing currents do. When backing currents larger than −17.7 nA were employed the concentration rise was more prolonged than in 3A, and the release rate did not reach equilibrium during the 5 s pulse.

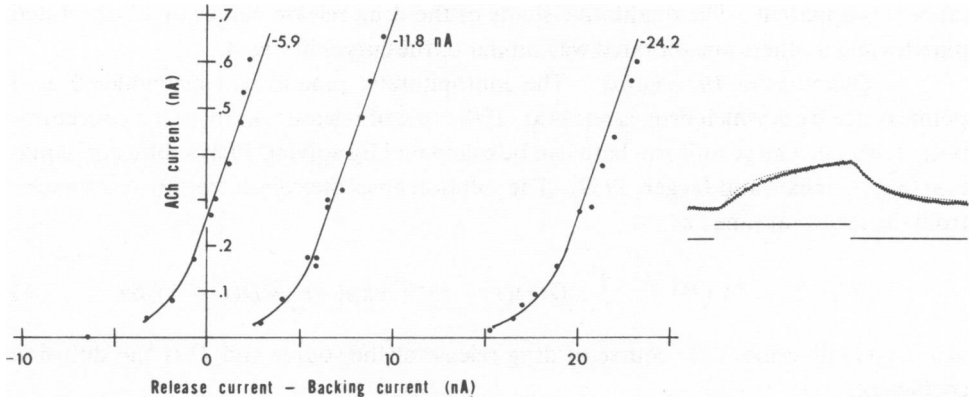

FIGURE 4 Estimation of the equilibrium transfer number. ACh concentration responses similar to the heavy upper trace shown in the inset (right) were obtained for various iontophoretic current pulse amplitudes. The inset lower trace shows the 5 s release current, while overlayed on the assay response is a theoretical curve fitted as described in the text. The ACh equilibrium current was plotted against the iontophoretic release current relative to backing to obtain the release curves shown (each labeled with the backing current). For this particular pipette, 1 nA of iontophoretic current carried 0.14 nA of ACh, essentially independent of backing current, at the larger release currents. The iontophoretic pipette was leaking ACh at −5.9 nA backing; that release curve was determined with both + and − current pulses. Iontophoretic pipette resistance 30 MΩ.

The transient change in the transfer number and the effect of backing current on it have been observed consistently. They have been detected in all cases studied for all the drugs used in this work, and qualitatively they appeared independent of the iontophoretic pipette resistance over the range 10–150 MΩ.

Although the transfer number increased with time following an iontophoretic release current, it also approached an equilibrium value which appeared to depend, for a given pipette, only on the iontophoretic current. Concentration responses to 5 s release currents with varying amplitude as in the inset of Fig. 4 were monitored and fit with theory using separately determined diffusion coefficients (below). The theory assumed a constant transfer number during release and was fitted at times before release began and near the end of the pulse when the transfer number had stabilized. This allowed the drug current at equilibrium to be determined and gave the equilibrium transfer number. In Fig. 4 ACh concentration response series were recorded for three different backing currents. Within the errors inherent in the method used to extract the ACh current values, the curves appear identical, shifted only by the backing current.

The release curves of Fig. 4 deviated from linearity at low ACh currents regardless of the backing current value, indicating that the equilibrium tranfer number was not linearly related to release current over the entire range. The maximum equilibrium transfer number shown, the slope of the linear portion of the fitted curves, was 0.14. Maximum equilibrium transfer number has been observed to vary; for 12 pipettes the range was 0.02–0.15 (most values <0.10). No correlation with drug or pipette resis-

tance was apparent. The qualitative shape of the drug release curves of 12 calibrated pipettes and 9 others uncalibrated was similar to the curves in Fig. 4.

Quantitative Description. The iontophoretic pipette can be modeled as a point source from which drug is released. If the rate of release is known, the concentration of drug in a large uniform bath can be calculated by solving Fick's diffusion equation (e.g., Carslaw and Jaeger, 1959). The solution gives the concentration at distance r from the source at time t as

$$C(r,t) = (4\pi D)^{-3/2} \int_{-\infty}^{t} Q(\tau)(t-\tau)^{-3/2} \exp(-r^2/4D(t-\tau))\,d\tau \qquad (4)$$

where $Q(\tau)$ describes time course of drug release at the source and D is the diffusion coefficient.

Two simple examples for the drug release function $Q(\tau)$ allow ready solution for $C(r,t)$. If drug is released at a constant rate q for $t \geq 0$,

$$C(r,t) = (q/4\pi Dr)\,\mathrm{erfc}\,(r[4Dt]^{-1/2}). \qquad (5)$$

Similarly, if drug is released at constant rate q for $t < 0$ and is abruptly stopped at $t = 0$,

$$C(r,t) = (q/4\pi Dr)\,\mathrm{erf}(r[4Dt]^{-1/2}). \qquad (6)$$

Here the complementary error function is defined as $\mathrm{erfc}(x) = 1 - \mathrm{erf}(x)$, where

$$\mathrm{erf}(x) = 2\pi^{-1/2} \int_{0}^{x} e^{-y^2}\,dy.$$

Eqs. 5 and 6 together would describe the rise and fall of concentration in Fig. 4 if the transfer number were constant during release. To write these solutions in closed form an approximation for the error function accurate to better than 0.01% was used (Hastings, 1955):

$$\mathrm{erf}(x) = 1 - (a_1 y + a_2 y^2 + a_3 y^3)\,e^{-x^2},$$

where $y = 1/(1 + px)$, $p = 0.47047$, $a_1 = 0.3480242$, $a_2 = -0.0958798$, and $a_3 = 0.7478556$.

A second simple solution of Eq. 4 results when Q_0 ions are released instantaneously at $t = 0$:

$$C(r,t) = Q_0(4\pi Dt)^{-3/2} \exp(-r^2/4Dt). \qquad (7)$$

After release, concentration at point r rises (with a diffusion delay) to a maximum then decays to zero.

Although knowledge of the drug release function allows one to utilize Eq. 4 to calculate $C(r,t)$ (this is how the equilibrium transfer number was estimated), the analysis can be reversed to reveal $Q(\tau)$ itself. Instead of using qualitative arguments about the lack of symmetry in the concentration assay response to show that drug release is not

constant, the actual time dependence of $Q(\tau)$ can be obtained from the data. Rewriting Eq. 4

$$C(r,t) = \int_{-\infty}^{t} Q(\tau)\, G(r, t - \tau)\, d\tau$$

allows $Q(\tau)$, solved by Fourier transformation, to be written as

$$Q(\tau) = \mathfrak{F}^{-1}\{\hat{C}(r,\omega)/\hat{G}(r,\omega)\}.$$

Here ω is the transform variable, $\mathfrak{F}^{-1}$ is the inverse Fourier operator and the symbol $\wedge$ indicates a Fourier transformed function. A computer algorithm was used to transform the functions and compute the digital drug release function $Q(\tau)$.

The concentration assay responses shown in Fig. 3 have been processed in this manner, and the functions $Q(\tau)$ are shown overlayed on the iontophoretic current traces. If the transfer number were independent of time these paired traces would be parallel; however, they were not. The drug release function changed significantly in the manner indicated by the earlier qualitative analysis. The qualitative effects of backing were confirmed by comparing $Q(\tau)$ in Figs. 3A and 3B. In 3B, with low initial backing current, $Q(\tau)$ reached equilibrium $2\frac{1}{2}$ s after release began; in 3A, with larger backing current, it appeared that $Q(\tau)$ may not have reached equilibrium even after 5 s. In both cases $Q(\tau)$ fell promptly and in a similar manner when release was terminated. More intense backing currents caused less initial drug release and prolonged its approach to equilibrium.

In the course of this work attempts were made to evaluate the use of capacity negation on the iontophoretic micropipette current controller. Dreyer and Peper (1974*a*) have argued that such compensation facilitates release from 100–200 MΩ pipettes instituted by brief intense iontophoretic currents. Circuitry similar to that described by Dreyer and Peper (1974*a*) was employed. The protocol for the release current is described in the legend of Fig. 5. The assay response was sampled at 5 ms intervals at distances between 5 and 25 μm through a 100 Hz filter and later blanked until the moment the iontophoretic pulse occurred; it was not otherwise processed. $Q(\tau)$ was then determined as described earlier. Although negative capacity circuitry was employed in the response of Fig. 5, responses obtained without capacity compensation were indistinguishable. Neither the rise nor the fall of the drug release function was materially different, nor was the function displaced in time with respect to the iontophoretic pulse, nor was its amplitude changed. These observations were made on pipettes in the range 20–60 MΩ. I have been unable to document any performance advantage of capacity negation when employed with the iontophoresis circuit using this assay.

Repeated attempts were made to determine if significant delays occurred in the initiation and termination of drug release by current steps. No evidence for either of these delays could be obtained within the temporal resolution of the method. Generally release pulses were applied over a backing current just sufficient to completely retard diffusional release, and turn-off was to this backing current value or a more

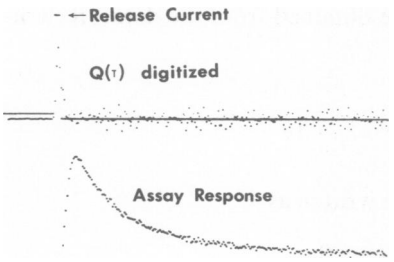

FIGURE 5

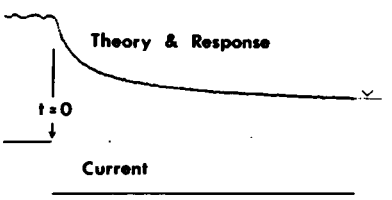

FIGURE 6

FIGURE 5 Capacity compensation of the iontophoretic current controller. 5 s before the release current pulse backing was reduced to an initial value (uppermost of three traces beginning at left) just below which detectable leakage occurred; following the 20 ms 95 nA release current backing was restored 10 nA more negative. The carbachol assay response sampled prior to the release pulse was zeroed before computing $Q(\tau)$. $Q(\tau)$, shown here as a rather noisy digital record, was smoothed three times and is displayed superimposed on the final iontophoretic backing current. Smoothing was operationally identical to the Hanning procedure commonly used with spectral data (Blackman and Tukey, 1959) to average out high frequency components. It was used here to present a more easily interpreted record. The slight skewing of $Q(\tau)$ relative to iontophoretic current may be attributed to data processing. No evidence that capacity compensation facilitated drug release could be obtained with or without smoothing. Maximum capacity compensation was applied to the iontophoretic pipette. 5 ms sample interval; 20 MΩ pipette.

FIGURE 6 Measurement of the diffusion coefficient. Digital records of the assay response (upper trace) and iontophoretic current (lower) for carbachol indicate how diffusion coefficient measurements were obtained. Steady drug release was terminated at $t = 0$ by suddenly reducing the iontophoretic current 95 nA, and the decay of the assay response was monitored and fit by Eq. 6. The theory was superimposed on the assay response beginning at $t = 0$ and can be seen separate from the response at the right (caret). Parameters: $r = 19\ \mu m$, 10 ms sample interval, $D = 6.88 \times 10^{-6}\ cm^2/s$, 24°C. Entire response time course is 2.56 s.

negative value. The best frequency response that could be obtained for $Q(\tau)$ in these studies was ~50 Hz limited by the data processing rather than the assay microelectrode. Although fitting the assay response itself offered better resolution than deconvolving the drug release function, independent knowledge of the drug release amplitude was necessary to make inferences of the release time course.

Evaluation of the Diffusion Coefficient

When the drug concentration at r relative to the iontophoretic source is measured as a function of time, Eq. 4 can be fit if D, the diffusion coefficient, and $Q(\tau)$ are known. This method allows D to be evaluated independently when $Q(\tau)$ is identically zero. Experiments were performed in which release current was adjusted on the iontophoretic pipette until a steady response from the assay microelectrode $\leq$ 5 mV was obtained. The assay was performed at varying source distances r from 15 to 80 μm. Following a steady response for 30–60 s the release current was turned off by applying a negative current step between 50 and 100 nA. This backing current was much more intense than critical backing and guaranteed that no detectable leakage occurred during its application. The response was sampled at 5-, 10-, or 20-ms intervals and fit by Eq. 6. An example of the method is shown in Fig. 5 where concentration samples have been

superimposed on theory. The theory, plotted beginning at $t = 0$, is distinguishable from the record only at the lower right where it emerges. Regardless of the sample length, theory fit experiment well, showing no characteristic deviations. In any one experiment 15–30 such measurements were made at different source distances and averaged. The averaged value from each such experiment was used to obtain the statistics in Table I.

DISCUSSION

This work has described a sensitive assay technique for neuromuscular agonists compatible with electrophysical methods. The assay microelectrode responds to quaternary amines. In addition to the drugs listed in Table I, responses to the following compounds have been observed: tetraethyl ammonium, hexamethonium, suberyldicholine, decamethonium, and 3-phenylpropyltrimethyl ammonium.

The performance and use of this assay have been illustrated with a study of how quaternary amines are released by iontophoresis. The results suggest the following model. The iontophoretic pipette shank is filled with a concentrated solution of the charged drug. To prevent drug leakage a backing current is applied. This current acts by diluting the drug concentration in the pipette tip both by moving non-drug ions into the tip and sweeping drug ions back. Backing is fully operative when the effective tip concentration is zero. When a current is passed to eject the drug, release will be proportional to the tip concentration. With a prolonged current, the tip concentration will gradually increase to a representative equilibrium value, and the rate of drug release will increase in parallel.

This picture suggests several factors which complicate the common assumption that drug release is proportional to iontophoretic current. Stepping to a constant release current should cause the rate of drug release to change with time as tip concentration changes. The initial rate of release following such a step, and hence its value at any time between start and equilibrium, will depend on the initial tip concentration, not the current. This dependence might be complex but the model suggests that increased backing lowers the initial rate and prolongs its approach to equilibrium. Finally, since the rate at which tip concentration approaches equilibrium may vary with the magnitude of a current step, it is possible that the release rate evaluated at a given time will not be linearly proportional to the current. All these factors relate to the initiation of drug release and are documented in Figs. 3 and 4.

Similar arguments can be made about the termination of drug release. Following a constant iontophoretic release current, the pipette tip contains an appreciable drug concentration which must relax back to the depleted condition representative of backing. It is possible that part of the excess drug in the tip diffuses into the external bath after application of the backing current. Such delayed release would be expected to be more significant after larger release currents, since the tip concentration should be higher. Within the resolution of the assay no absolute delays have been detected in the termination of drug release by iontophoretic backing currents. In addition no delayed release of drug by brief iontophoretic pulses could be resolved although the model sug-

gests that delays must be expected. Time constants for such processes must be < 20 ms, the best resolution for $Q(\tau)$ obtained.

These observations indicate the usefulness of the hypothetical picture of iontophoresis drawn here. In terms of this picture the backing effects demonstrated may be accounted for by dilution of drug in the pipette tip. Other behavior is suggested for which no documentation was attempted. Although for any given backing current, equilibrium should be attained more quickly with larger release currents this has not been demonstrated. The problem is that larger release currents also produce larger concentrations which can cause an assay response outside the linear region. I have not analyzed the nonlinear responses here. For similar reasons I have not analyzed the dependence of the initial value of $Q(\tau)$ on backing current. In addition the picture suggests that for different release currents, $Q(\tau)$ measured at a particular time will not be a linear function of current. This has been confirmed only for the equilibrium value $Q(\infty)$. Although this picture has been found useful for all the iontophoretic pipettes studied regardless of drug or resistance, no specific functional dependence on drug or resistance can be given to predict the actual release characteristics of any given pipette. Drug release must be assayed during individual iontophoretic applications if accurate quantitative values for concentration are required.

Although diffusion coefficients in Table I were obtained using an iontophoretic pipette as a point source of drug, they are independent of pipette characteristics. Estimates of the diffusion coefficient of ACh in Ringer's have been published by others. Del Castillo and Katz (1955) used 8×10^{-6} cm^2/s, apparently calculated from conductivity measurements. Krnjević and Mitchell (1960) obtained 9.8×10^{-6} cm^2/s at 20°C in an agar gel, while Dreyer and Peper (1974*b*) obtained 11.9×10^{-6} cm^2/s at 23°C in Ringer's using nerve-muscle endplate depolarization as a concentration assay. Comparison of the larger values with the diffusion coefficients of a variety of small molecules would suggest that they may be high. Longsworth (1953) measured free diffusion of 32 amino acids, peptides, and sugars using the Rayleigh interference method. Values at 25°C ranged from 4.339×10^{-6} cm^2/s (raffinose) to 10.554×10^{-6} cm^2/s (glycine). Estimating only from their relative molecular weight one would expect the drugs tested to fall in the middle of this range, as observed. The possibility exists that thermal convection in the test solution has systematically distorted the diffusion coefficient values. Although no specific measures were taken to prevent this, it is believed unlikely. Convection may have contributed significantly to the standard errors obtained.

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