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EFFECTS OF SOME ALIPHATIC ALCOHOLS ON THE CONDUCTANCE CHANGE CAUSED BY A QUANTUM OF ACETYLCHOLINE AT THE TOAD END-PLATE

By PETER W. GAGE, ROBERT N. McBURNEY* AND GAVAN T. SCHNEIDER†

From the School of Physiology and Pharmacology, University of New South Wales, Kensington, Australia

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SUMMARY

1. The post-synaptic effects of the aliphatic alcohols, ethanol to hexanol, were investigated at the neuromuscular junctions of toads, with particular emphasis on the effects of ethanol.

2. The alcohols increased the amplitude and duration of miniature end-plate potentials. It is shown that this effect was due to the prolongation of the decay phase of miniature end-plate currents (m.e.p.c.s). There was no effect of alcohols on the growth phase of m.e.p.c.s.

3. The prolonged decay of m.e.p.c.s in ethanol remained exponential and was normally sensitive to membrane potential. Prolonged m.e.p.c.s were associated with an equivalent prolongation of the mean duration of elementary events, as determined from power spectra of acetylcholine noise in 0·5 M ethanol.

4. The relationship between the time constant of decay of m.e.p.c.s (τ) and the concentration of an alcohol of carbon chain length N (C_N) was exponential, conforming to the equation $\tau = \tau_s \exp(B_N . C_N)$, in which τ_s is the decay time constant in standard solution and B_N is a constant, different for each alcohol.

5. There was also an exponential relationship between B_N and N, which closely followed the relationship between membrane-buffer partition coefficient and carbon chain length for the different alcohols, indicating that the alcohols are active in the lipid phase of the post-synaptic membrane.

6. It is suggested that the alcohols act by causing a change in the dielectric constant of the post-synaptic membrane which forms the

* N.H. and M.R.C. Senior Research Officer. Present address: Physiological Laboratory, University of Cambridge, England.

† N.H. and M.R.C. B.Sc. (Med.) Scholar.

environment of the rate-limiting reaction responsible for the decay of the end-plate conductance. On the assumption that this reaction involves dipoles, it is shown that the small changes in dielectric constant, calculated from the partition coefficients of the alcohols and by assuming an initial lipid dielectric constant of 3, would give an exponential relationship between the time constant of decay of m.e.p.c.s and alcohol concentration.

7. The results support the hypothesis that the decay (but not the onset) of acetylcholine-induced conductance changes is rate-limited by a first-order reaction which involves dipoles and occurs in the lipid environment of the post-synaptic membrane.

INTRODUCTION

A possible explanation for the observations that the decay phase of miniature end-plate currents (Gage & McBurney, 1972, 1974; Anderson & Stevens, 1973) and end-plate currents (Takeuchi & Takeuchi, 1959; Magleby & Stevens, 1972*a*; Kordas, 1972*a*) is exponential, is that the rate-limiting process is a first-order reaction. This is supported by the observation that the relationship between temperature and the time constant of decay of miniature end-plate currents (m.e.p.c.s) conforms to the Arrhenius equation (Gage & McBurney, 1974). Because the time constant of decay is affected by membrane potential (Takeuchi & Takeuchi, 1959; Gage & Armstrong, 1968; Kordas, 1969; Magleby & Stevens, 1972*a*; Gage & McBurney, 1972, 1974) it seems likely that the underlying rate-limiting process involves molecules with a dipole moment situated in the electric field of the membrane (Magleby & Stevens, 1972*b*; Gage & McBurney, 1974) although, of course, the explanation is not unique.

Clearly, any procedure which alters the time course of end-plate currents may provide information about the underlying rate-limiting reactions. The effects of temperature, membrane potential and neostigmine have been helpful in eliminating diffusion of acetylcholine, or its hydrolysis, as the processes normally controlling the decay phase (Magleby & Stevens, 1972*a*, *b*; Kordas, 1972*a*, *b*; Gage & McBurney, 1972, 1974). The possibilities which remain are that the rate of dissociation of acetylcholine from receptors, or the decay of some other process responsible for the increase in ionic conductance (such as a conformational change in a protein), controls the decay of end-plate currents. We have investigated the effects of aliphatic alcohols on m.e.p.c.s in the hope that the results might help to define the processes which determine the kinetics of the end-plate conductance change produced by acetylcholine.

It has been reported previously that some aliphatic alcohols can cause an increase in the amplitude of miniature end-plate potentials (m.e.p.p.s;

Gage, 1965; Inoue & Frank, 1967; Okada, 1967, 1970). This effect of alcohols on m.e.p.p.s will be shown to be due primarily to a prolongation of the decay phase of m.e.p.c.s. The alcohols could be acting to prolong the decay phase of m.e.p.c.s at a number of different steps in the reaction sequence described in the preceding paper: the 'buffered' diffusion of acetylcholine (ACh) molecules away from the receptor sites (cf. Katz & Miledi, 1973), the hydrolysis of acetylcholine, the unbinding of acetylcholine from receptor sites (Kordas, 1972*a*, *b*), or the reaction step in the membrane which possibly involves a conformational change in molecules with dipole moment (Magleby & Stevens, 1972*b*; Gage & McBurney, 1974).

We have attempted to define the site and mode of action of the alcohols because they probably have the simplest structure of all biologically active organic molecules and most of their physical properties are well known. Therefore, unlike the situation with many complex drug molecules, there is some hope of determining the physico-chemical basis of their action. More importantly, it was hoped that an investigation of the action of alcohols might give more information about the nature of the process which determines the characteristics of the post-synaptic conductance change caused by acetylcholine.

The results reported here are quantitatively consistent with the idea that the rate-limiting process during the decay of m.e.p.c.s is a chemical reaction involving dipoles occurring in a lipid environment in the post-synaptic membrane.

Some of the results have been described briefly elsewhere (McBurney & Gage, 1972; McBurney, 1973; Gage, McBurney & Schneider, 1974).

METHODS

The preparation, standard solution and recording techniques were basically as described previously (Gage & McBurney, 1972), and in the preceding paper (Gage & McBurney, 1974).

Alcohol solutions were made by adding the pure alcohols (AR) to the standard solution. It was noticed that a white precipitate (presumably calcium phosphate) formed in solutions containing ethanol concentrations greater than 0·5 M. Therefore phosphate buffer was left out of these solutions (containing more than 0·5 M ethanol) and no precipitate then formed.

Procedures used to insert four micro-electrodes in one muscle fibre, analysis of 'cable' properties, and computation of the voltage responses to a current input, have been described elsewhere (Gage & McBurney, 1972, 1973).

Acetylcholine was applied iontophoretically to end-plates by passing a constant current (Gage & Eisenberg, 1969) though a micro-electrode filled with acetylcholine. A biasing current in the opposite direction prevented unwanted leakage of acetylcholine at other times. The current was monitored with an operational amplifier between the bath and ground. The power spectra of acetylcholine 'noise' (Katz & Miledi, 1970, 1971, 1972, 1973; Anderson & Stevens, 1973), whether voltage or current fluctuations (measured in voltage-clamped fibres), were determined from

digitized records obtained with an on-line laboratory computer (Lab 8/I, Digital Equipment Corporation). The 'noise' records were analysed by a time series method (Jenkins & Watts, 1968). Care was taken to avoid records containing m.e.p.c.s. Spectral density as a function of frequency (Jenkins & Watts, 1968) was calculated from 3072 digital samples obtained at 1 kHz.

Except where otherwise stated, the temperature of preparations was 19–21° C.

RESULTS

PART I. EXPERIMENTS

Effects of ethanol

Miniature end-plate potentials

Ethanol dissolved in standard Ringer solution to give concentrations from 0·1 to 1 M caused an increase in the amplitude and duration of m.e.p.p.s. The effect became more marked, as the alcohol concentration

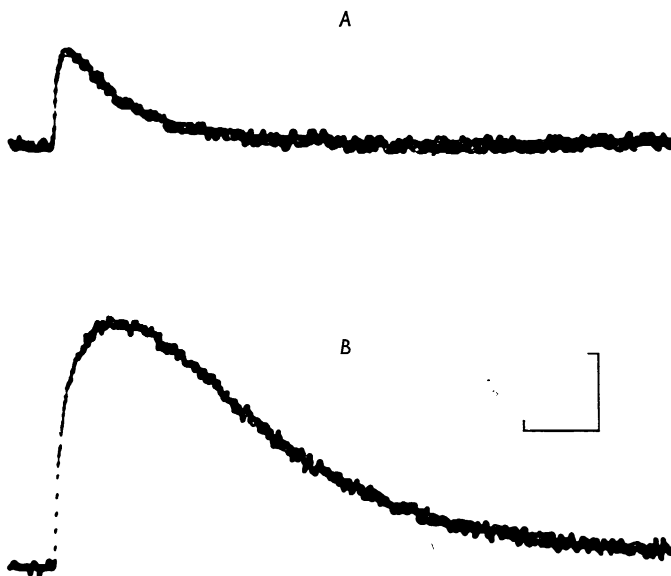

Fig. 1. The increase in amplitude and prolongation of the time course of miniature end-plate potentials caused by ethanol. The m.e.p.p. above (*A*) was recorded in standard solution. Another m.e.p.p. (*B*) was recorded from the same fibre after 10 min exposure to 0·5 M ethanol. Calibrations: vertical, 0·5 mV; horizontal, 10 msec.

was increased. An m.e.p.p. recorded in normal toad Ringer is shown in Fig. 1*A*, and another, recorded in the same fibre after 10 min in a solution containing 0·5 M ethanol, is shown in Fig. 1*B*. A prolongation and increase

in amplitude of m.e.p.p.s occurred rapidly (within minutes) after a solution change though the effect sometimes took up to 30 min to develop fully. The changes were reversible and could be reproduced many times in the same fibre.

It has been suggested that ethanol may cause an increase in m.e.p.p. amplitude and time course by increasing membrane resistance (Gage, 1965) but this seems unlikely because an increase in membrane resistance would have little effect on the amplitude of m.e.p.p.s, though it would prolong their time course (Gage & McBurney, 1973). The input resistance, space constant and time constant of three glycerol-treated fibres in which measurements were made in 0·5 M ethanol were within the range for normal 'winter' fibres (Gage & McBurney, 1973; Dulhunty & Gage, 1973). The calculated specific membrane resistance (R_m) and capacitance (C_m) for the three fibres were: $R_m = 4{\cdot}8 \pm 0{\cdot}96$ kΩ.cm^2 (mean ± S.E. of mean) and $C_m = 1{\cdot}7 \pm 0{\cdot}17$ μF.cm^{-2} (mean ± S.E. of mean). When these values are compared with those found for glycerol-treated toad sartorius fibres in standard solution (Gage & McBurney, 1973) a slightly significant increase ($0{\cdot}1 < P < 0{\cdot}15$, Student's t) is seen in R_m but there is no significant increase in C_m.

In these same three fibres, prolonged m.e.p.p.s with increased amplitudes were recorded with four micro-electrodes at four points along each fibre. From the experimentally determined passive electrical characteristics of a muscle fibre and the characteristics of the m.e.p.p.s, the amplitude and time course of the post-synaptic currents (m.e.p.c.s) which caused the m.e.p.p.s were computed (Gage & McBurney, 1973). The four m.e.p.p.s shown in Fig. 2 were recorded at the distances from the first electrode shown on the left of the Figure. An input current with the growth time and amplitude of a normal m.e.p.c. (Gage & McBurney, 1972, 1973) but with a prolonged decay time constant of 18 msec gave the computed voltage responses shown by the circles in Fig. 2. Similarly good fits were obtained in the other two fibres. As the normal decay time constant is of the order of 2–3 msec (at 20° C and a membrane potential of −70 mV) these results indicated that ethanol was prolonging m.e.p.c.s rather than affecting the passive electrical properties of muscle fibres. The prolongation of m.e.p.c.s in ethanol solutions was confirmed directly in the following experiments.

Miniature end-plate currents

M.e.p.c.s were recorded in ethanol solutions, either with extracellular electrodes or in voltage-clamped fibres. The advantage of the latter method was that a meaningful amplitude of m.e.p.c.s could be obtained. An m.e.p.c. recorded in standard solution in a voltage-clamped fibre is shown in Fig. 3*A*. Within 10 min of changing to a solution containing 0·5 M ethanol,

the time course of m.e.p.c.s was clearly prolonged (Fig. 3*B*), but further prolongation often occurred for up to 30 min. In Fig. 3*C* (standard solution) and Fig. 3*D* (0·5 M ethanol) the same effect is shown for m.e.p.c.s recorded extracellularly in another experiment. There was no significant change in the growth phase of m.e.p.c.s but there was often a slight in-

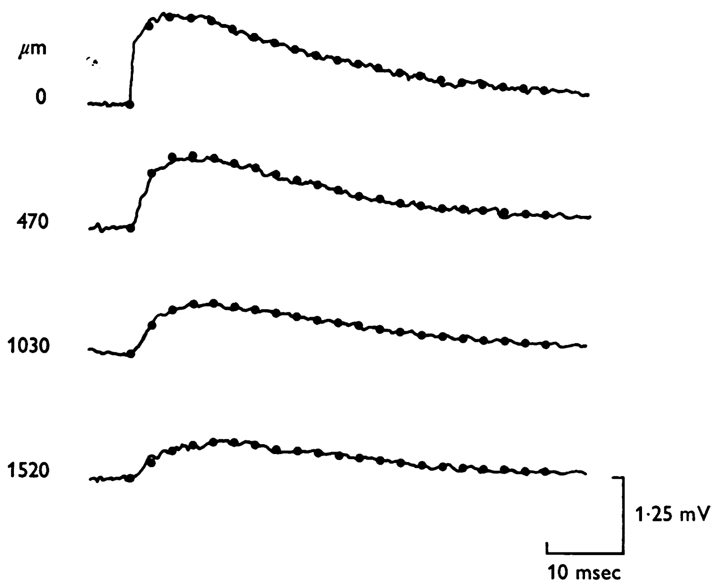

Fig. 2. A miniature end-plate potential recorded with four micro-electrodes in a muscle fibre exposed to 0·5 M ethanol in standard solution. The distances (μm) of three of the electrodes from the first (reference) electrode are shown on the left of each trace. The circles show the voltage responses (calculated for this fibre at the separations indicated) to a current input with growth time of 200 μsec and decay time constant of 18 msec. Calibrations: vertical, 1·25 mV; horizontal, 10 msec.

crease in amplitude. The decay phase, although much longer than normal, remained exponential. The decay phases of three m.e.p.c.s recorded extracellularly in one fibre at three different concentrations of ethanol (0·125 M, triangles; 0·25 M, open squares; 0·5 M, filled squares) are shown in Fig. 4 and can be seen to conform well to exponential functions with single time constants.

The effect of ethanol concentration

It can be seen in Fig. 4 that the prolongation of the time constant of decay of m.e.p.c.s was greater the higher the ethanol concentration. The influence of a range of ethanol concentrations on the decay phase of m.e.p.c.s was therefore investigated further in four preparations in which

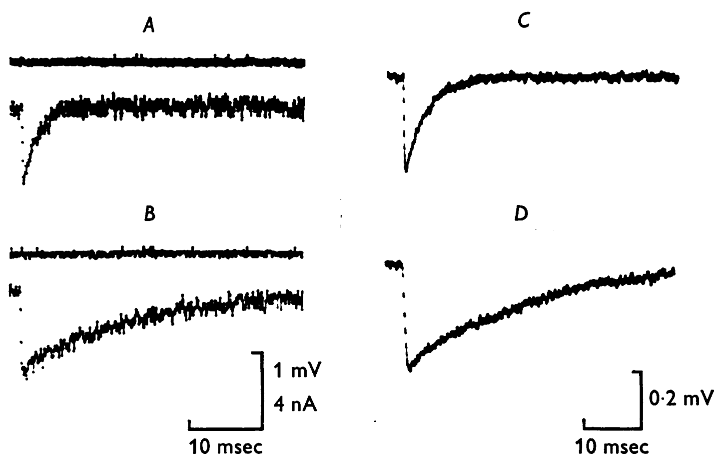

Fig. 3. Prolongation of m.e.p.c.s caused by ethanol. Records *A* (standard solution) and *B* (0·5 M ethanol solution) were obtained in a voltage-clamped fibre. Traces *C* (standard solution) and *D* (0·5 M ethanol solution) were recorded extracellularly. Calibrations, for *A* and *B*: vertical, 1 mV, 4 nA, horizontal, 10 msec; for *C* and *D*: vertical, 0·2 mV, horizontal, 10 msec.

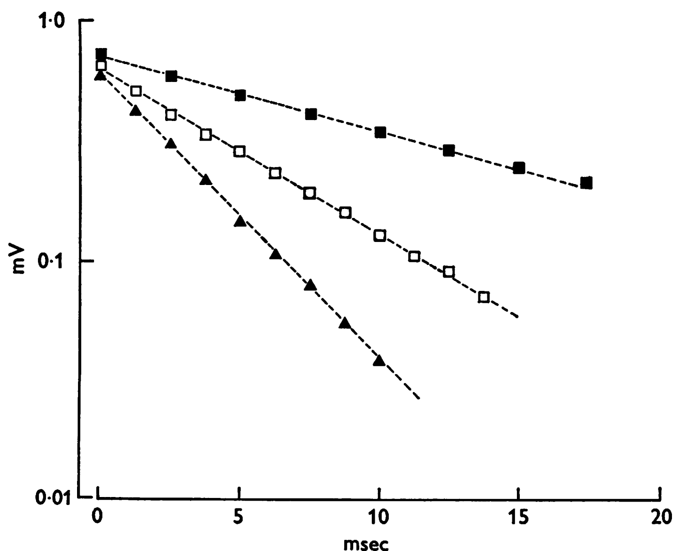

Fig. 4. The exponential decay of m.e.p.c.s recorded extracellularly in one fibre for three different concentrations of ethanol: 0·125 M (triangles), 0·25 M (open squares) and 0·5 M (filled squares). Note the logarithmic ordinate.

m.e.p.c.s were recorded extracellularly. Preparations were exposed to ethanol for at least 30 min before m.e.p.c.s were photographed. A graph of the time constant of decay of m.e.p.c.s against ethanol concentration is shown in Fig. 5. The points were obtained by averaging the time constants

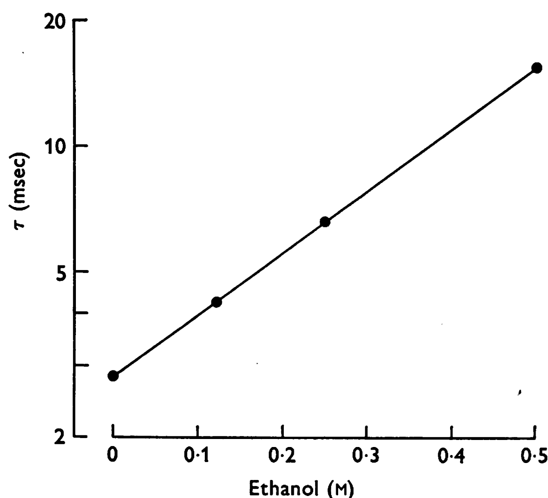

Fig. 5. The exponential relationship between ethanol concentration and the time constant of decay (τ) of m.e.p.c.s recorded extracellularly. Note the logarithmic ordinate.

measured from twenty m.e.p.c.s at each concentration in this preparation. The mean time constant of decay plus standard errors, for the experiment illustrated, are given in Table 1. The relationship between decay time constant, τ, and the ethanol concentration, C_2, could be described, in each of four experiments, by the equation

$$\tau = \tau_S . \exp (B.C_2), \tag{1}$$

where τ_S is the decay time constant in standard solution (without alcohol) and B is a constant.

Ethanol does not affect the voltage-sensitivity of m.e.p.c.s

It has recently been shown that a cholinesterase inhibitor, diisopropylfluorophosphate (DFP) can reduce the voltage sensitivity of m.e.p.c.s (Kuba, Albuquerque & Barnard, 1973). If ethanol lengthened m.e.p.c.s by inhibiting acetylcholinesterase it might have a similar effect. However, ethanol does not affect the voltage sensitivity of τ. M.e.p.c.s were recorded in voltage-clamped fibres exposed to 0·5 M ethanol at various membrane potentials. Although the decay phases of m.e.p.c.s were markedly pro-

longed, with the time constant of decay lengthened up to sevenfold, there was still further prolongation at hyperpolarized membrane potentials, and shortening at depolarized potentials. Results obtained in four such experiments gave a $\tau(0)$ of $11{\cdot}4 \pm 2{\cdot}5$ msec (mean ± S.E. of mean) and an H of $-104{\cdot}6 \pm 12{\cdot}7$ mV (mean ± S.E. of mean). When these values are compared with those found for m.e.p.c.s recorded in standard Ringer ($\tau(0) = 1{\cdot}38 \pm 0{\cdot}09$ msec; $H = -102{\cdot}5 \pm 3{\cdot}1$ mV; Gage & McBurney, 1974) it can be seen that the parameter changed by the alcohol is $\tau(0)$ but that H is not altered. Although ethanol does not affect the voltage sensitivity of m.e.p.c.s it could be inhibiting acetylcholinesterase and prolonging m.e.p.c.s like neostigmine, which does not affect voltage-sensitivity (Magleby & Stevens, 1972*a*; Gage & McBurney, 1974).

TABLE 1. The effect of ethanol on the time constant of decay of miniature end-plate currents

Concentration (M)	0	0·125	0·25	0·5
Mean τ (msec)	2·8	4·1	6·6	15·5
S.E. of mean	0·038	0·081	0·103	0·166

Ethanol increases the mean duration of 'elementary' events

Conceivably, ethanol could be influencing the time course of m.e.p.c.s by inhibiting hydrolysis of acetylcholine or by causing changes in the geometry of the synaptic cleft. Under such circumstances, it has been shown by Katz & Miledi (1973) that m.e.p.c.s can be prolonged, but the duration of elementary events underlying acetylcholine 'noise' (Katz & Miledi, 1970, 1971, 1972, 1973; Anderson & Stevens, 1973) is unchanged. To test the possibility that ethanol might have these effects, we examined the effects of ethanol on the membrane depolarization, and on the voltage and current 'noise', caused by acetylcholine in end-plate regions.

Ethanol (0·5 M) caused a two- to threefold increase in the steady level of depolarization produced by application of acetylcholine to an end-plate region. There were also considerable changes in the power spectra (Jenkins & Watts, 1968) of voltage noise and current noise caused by acetylcholine. In two experiments in which the voltage noise produced by application of acetylcholine was examined in standard solution and then in a solution containing 0·5 M ethanol the half-power frequency of the noise was reduced from 26 and 30 Hz (standard solution) to 8·5 and 11 Hz (0·5 M ethanol) respectively. In one experiment in which the current noise was recorded under voltage-clamp conditions, the half-power frequency of the noise was reduced from 70 Hz (standard solution) to 19 Hz (10 min in a solution containing 0·5 M ethanol). At the same time, the time constant of decay of m.e.p.c.s had increased from 2·5 msec to 14 msec. From these time

constants, the predicted half-power frequencies would be 64 Hz and 11·4 Hz respectively (Anderson & Stevens, 1973). A slight decrease (approximately 20 %) in the amplitude of the elementary conductance change was observed in the presence of ethanol. Whether or not this change proves to be significant will depend on future experiments. We feel that there is sufficient agreement between the observed and predicted half-power frequencies obtained in this experiment to indicate that ethanol prolongs miniature end-plate currents by prolonging the duration of the elementary molecular events. The prolongation of m.e.p.c.s does not appear to be due to an inhibitory action of ethanol on cholinesterase activity.

Similar effects of propanol, butanol and pentanol

The effects of some other straight-chain alcohols were tested in a similar way because it has been shown that methanol and propanol also increase the amplitude of m.e.p.p.s (Gage, 1965). Propanol, butanol and pentanol all prolonged m.e.p.c.s and the decay of these prolonged currents also remained exponential.

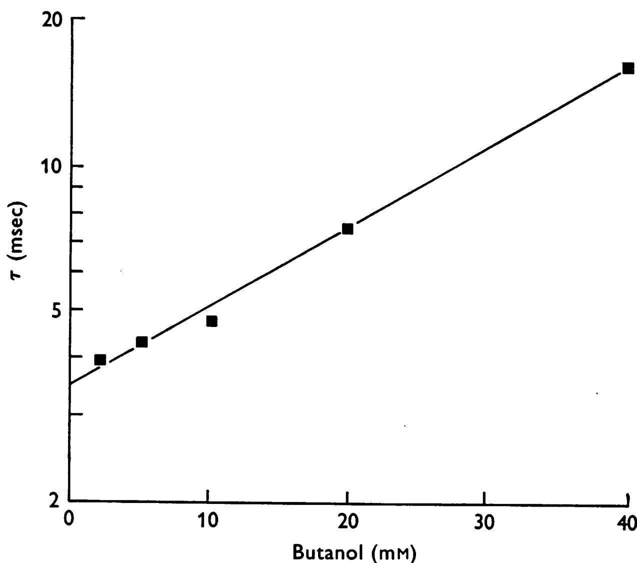

Fig. 6. The exponential relationship between butanol concentration and the time constant of decay (τ) of m.e.p.c.s recorded extracellularly. Note the logarithmic ordinate.

The relationship between the concentration of an alcohol and the decay time constant of m.e.p.c.s was exponential for each alcohol. This exponential relationship is illustrated for butanol in Fig. 6. The graph shows pooled results from two to five preparations for each alcohol concentration; the

S.E. of the means are smaller than the symbols. The lengthening of the decay time constant of m.e.p.c.s caused by each of the three alcohols occurred rapidly as with ethanol, within minutes of solution changes, but records were not taken for the first 30 min in an attempt to obtain equilibrium conditions.

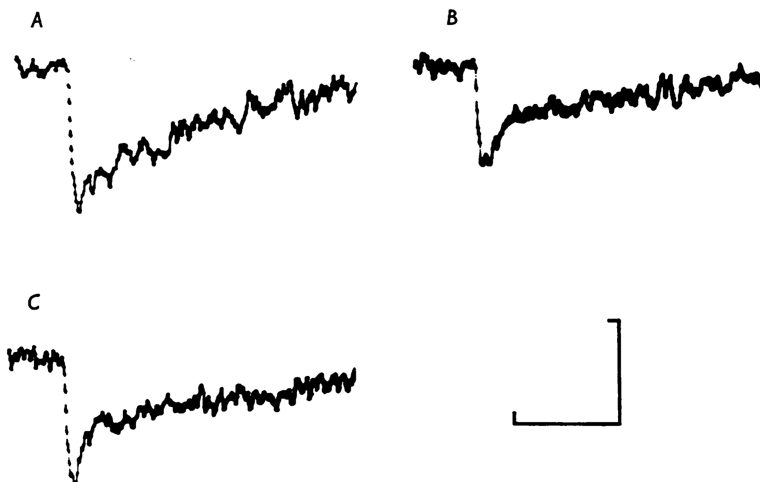

Fig. 7. Biphasic m.e.p.c.s (*B* and *C*) recorded in 1 mM hexanol. A control m.e.p.c. (*A*) recorded in standard solution in the same experiment is shown for comparison. Calibrations: vertical, 0·2 mV; horizontal, 2·5 msec.

Effect of hexanol

Hexanol produced a prolongation of m.e.p.c.s at concentrations below 0·5 mM. Above this concentration, the decay phase of m.e.p.c.s appeared to have two rates; a brief, initial, rapid phase followed by a slower phase. A normal m.e.p.c. recorded in a fibre is shown in Fig. 7*A* and 'biphasic' m.e.p.c.s recorded in 1 mM hexanol in the same fibre are shown in Fig. 7, *B* and *C*.

The relationship between alcohol chain length and potency

The decay of m.e.p.c.s seen with all the alcohols from C_2 to C_5 conformed to the relationship

$$\tau = \tau_s . \exp (B_N . C_N), \quad (2)$$

where τ_s is the decay time constant in standard solution, C_N is the concentration of a straight chain alcohol with N carbon atoms and B_N is a constant which changed with each alcohol. The slope (B_N) of the relationship between the logarithm of the decay time constant of an m.e.p.c. and alcohol concentration (C_N) increased as the chain length increased. For

example, the relationship was much steeper with hexanol than with propanol and this is illustrated in Fig. 8. M.e.p.c.s were recorded at different concentrations of hexanol and propanol in seven preparations. Each point in Fig. 8 represents the average decay time constant recorded from 10 to 25 m.e.p.c.s at a given alcohol concentration. The decay time

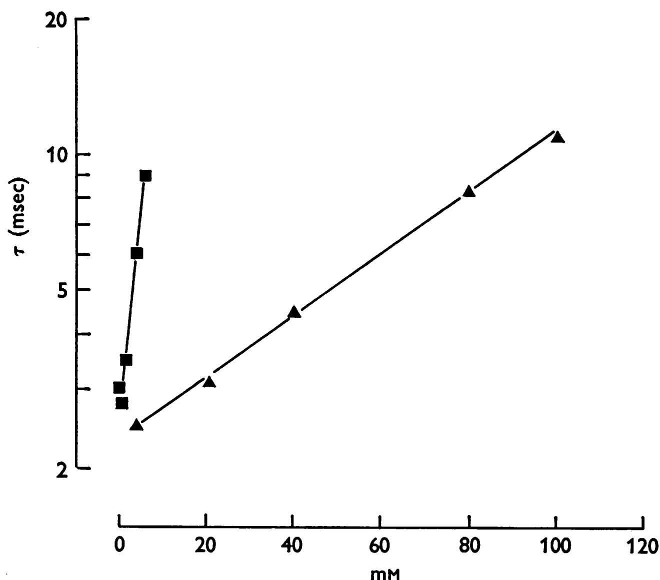

Fig. 8. A graph of the time constant of decay (τ) of m.e.p.c.s (ordinate) against alcohol concentration, for propanol (triangles) and hexanol (slow phase, squares). The much greater potency of hexanol than propanol is evident from the difference in slopes.

constant for hexanol was measured from the slow part of the decay. It can be seen that equivalent m.e.p.c. prolongations were produced by much lower concentrations of hexanol then propanol. These results indicate a relationship between B_N (eqn. (2)) and N, the number of carbon atoms in an alcohol. In fact, it was found that this relationship was also exponential and could be described by the equation

$$B_N = D.\exp(F.N), \tag{3}$$

where D and F are constants. This is illustrated in Fig. 9 which shows a graph of log B_N against N. Because of reservations about the validity of using only the slow phase of m.e.p.c.s in hexanol as a measure of its effect, the line in Fig. 9 was fitted to the lower four points and extrapolated (interrupted line) to $N = 6$. The triangle gives the averaged data obtained from the slow phase of the decay of m.e.p.c.s in hexanol and is clearly close to the predicted value.

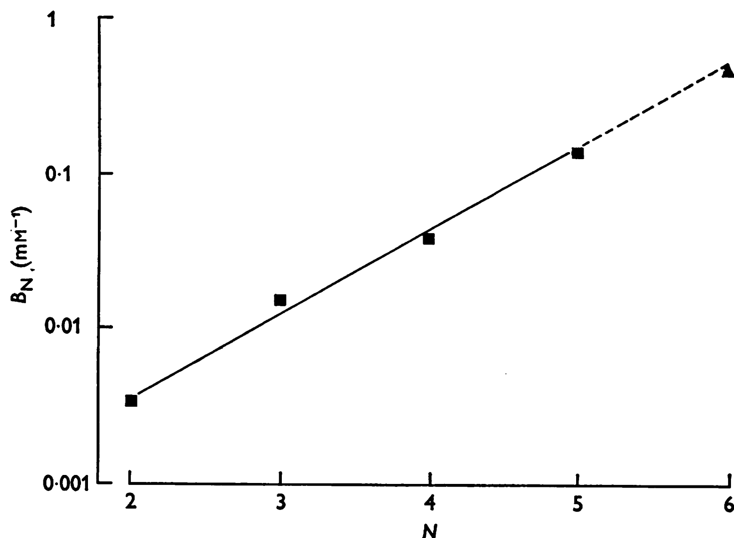

Fig. 9. A graph of the slope B_N (obtained from graphs as shown in Figs. 5, 6 and 8 of τ against alcohol concentration) against carbon chain length N (see eqn. (3)). Note the logarithmic ordinate. The line was fitted by eye to $N = 2$ to 5 and extrapolated to $N = 6$ to show the fit to the slope measured from the slow phase of m.e.p.c.s in hexanol (triangle).

PART II. ANALYSIS

The results indicate that the aliphatic alcohols affect directly the first-order, rate-limiting reaction which determines the time course of the decay of end-plate conductance changes. It seemed possible, therefore, that some information about the nature or location of the reaction might be obtained by looking for a physico-chemical basis for this effect.

Because the decay of end-plate currents is affected by membrane potential, it has been proposed that the decay of the post-synaptic conductance change in response to acetylcholine is governed by the rate of a reaction involving molecules with dipole moments so that the reaction would be influenced by the membrane field (Magleby & Stevens, 1972*a*, *b*; Anderson & Stevens, 1973; Gage & McBurney, 1974). Of course, changes in membrane potential could be affecting the reaction in some other way. It has been suggested, for example, that the membrane field may affect the rate-limiting reaction by changing the 'order' of the lipid phase (Gage, McBurney & Van Helden, 1974). This would be an 'environmental' change. It seems possible that the alcohols are influencing the rate-limiting reaction step by an action on an important feature of the environment of the reacting molecules also.

It was shown in Fig. 9 that the effectiveness of the alcohols (as measured from B_N which relates ln τ to alcohol concentration) increased exponentially with alcohol chain length (N). The oil/water partition coefficients of these alcohols also increase exponentially with alcohol chain length. This is illustrated in Fig. 10 in which 'membrane-buffer' partition coefficients

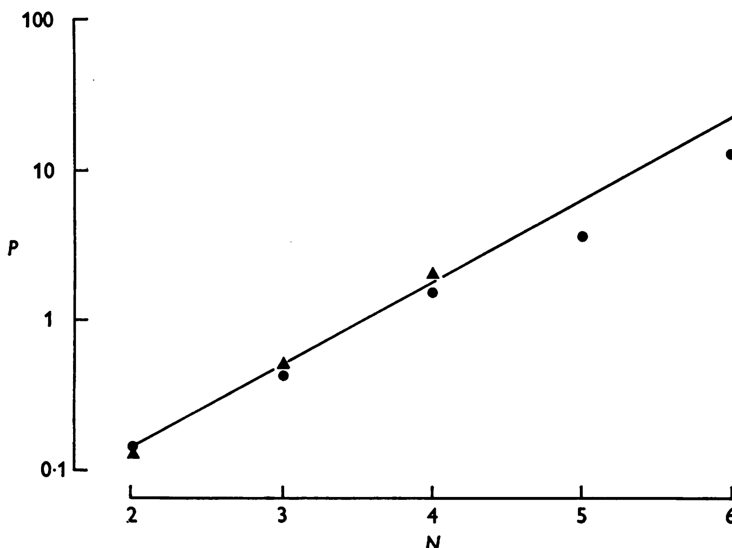

Fig. 10. A graph showing the relationship between partition coefficients (membrane-buffer, circles; oil/water, triangles) and carbon chain length, N. (Oil/water partition coefficients are multiplied by 4 to approximate the membrane-buffer partition coefficients; see Roth & Seeman, 1972.) The superimposed straight line has the slope of the line shown in Fig. 9 (B_N against N). Note the logarithmic ordinate.

(circles; Roth & Seeman, 1972) are plotted against N, the number of carbon atoms in an alcohol. Also included are oil/water partition coefficients (triangles; Wright & Diamond, 1969) which have been multiplied by 4 (see Roth & Seeman, 1972) for comparative purposes. Superimposed is a line obtained from the results shown in Fig. 9 showing the rate of change of B_N (the slope of log τ against alcohol concentration) with N. The very good agreement between the effectiveness of the alcohols and their partition coefficients strongly suggests that they exert their effects within the lipid phase of the post-synaptic membrane. That is, the alcohols may be changing the environment of a rate-limiting reaction within the membrane.

The question may now be raised as to how the alcohols might affect this environment. The answer we offer is based on the established influence of the dielectric constant of a medium on the rate of reactions within it, if

the reacting molecules have dipole moments (Glasstone, Laidler & Eyring, 1941). Considering a reaction $G^* \rightarrow G^\ddagger$ in which G^* represents a molecule in the 'open' configuration (Gage & McBurney, 1974) and $G^\ddagger$ the transition state between the 'open' and 'closed' configurations, the relationship between reaction rate, α, and dielectric constant, D, is given by the equation (Glasstone *et al.* 1941)

$$\ln \alpha = \ln \alpha_0 - \frac{1}{kT} \cdot \frac{D-1}{2D+1} \cdot \left(\frac{u_*^2}{r_*^3} - \frac{u_\ddagger^2}{r_\ddagger^3}\right) + \Sigma \frac{\phi}{kT}, \tag{4}$$

where α is the rate of disappearance of reactant, α_0 is the rate of reaction in a medium with a dielectric constant of 1 (specific reaction rate), D is the dielectric constant of the environment, k is Boltzman's constant, T is the absolute temperature (°K), u_* and $u_\ddagger$ are the dipole moments, and r_* and $r_\ddagger$ are the effective molecular radii, of G^* and $G^\ddagger$ respectively, and ϕ is a term describing the deviation from ideal behaviour due to non-electrostatic forces.

Because the effect of the alcohols on the first-order reaction responsible for the decay of m.e.p.c.s appears to be related to their concentration in the post-synaptic membrane, we assume that the reaction responsible for the decay of m.e.p.c.s occurs within the membrane. We also assume that the reacting molecules have dipole moments and that non-electrostatic forces are relatively insignificant. If these assumptions are valid, the decay of m.e.p.c.s should obey the relationship

$$\ln \tau = \ln \tau_0 + \frac{1}{kT} \cdot \frac{D_m - 1}{2D_m + 1} \cdot \left(\frac{u_*^2}{r_*^3} - \frac{u_\ddagger^2}{r_\ddagger^3}\right), \tag{5}$$

where τ (= $1/\alpha$) is the time constant of decay of m.e.p.c.s, τ_0 is the specific time constant of decay, and D_m is the dielectric constant of the membrane material. If $(u_*^2/r_*^3 - u_\ddagger^2/r_\ddagger^3)$ is constant, the equation may be written

$$\tau = \tau_0 . \exp [G . (D_m - 1)/(2D_m + 1)], \tag{6}$$

where $$G = \frac{1}{kT} \cdot \left(\frac{u_*^2}{r_*^3} - \frac{u_\ddagger^2}{r_\ddagger^3}\right).$$

This equation predicts an exponential relationship between τ and $(D_m - 1)/(2D_m + 1)$. Now if $(D_m - 1)/(2D_m + 1)$ were to change linearly with alcohol concentration, this might explain the exponential relationship between τ and alcohol concentration (Figs. 5, 6 and 8; eqn. (2)).

If there is homogeneous mixing of membrane lipid and alcohol, the dielectric constant, D_m, of the resulting membrane mixture should be a linear function of the weight fraction of the alcohol, provided the weight fraction is small (Smyth, 1955).

That is,

$$D_m = D_l + x.D_a, \tag{7}$$

where D_m is the dielectric constant of the resulting membrane mixture, D_l is the dielectric constant of the membrane lipid, D_a is the dielectric constant of the alcohol, and x is the weight fraction of the alcohol in the membrane.

It can then be shown, to a first-order approximation, that

$$\frac{D_m - 1}{2D_m + 1} = \frac{D_l - 1}{2D_l + 1} + \frac{3.x.D_a}{(2D_l + 1)^2}. \tag{8}$$

Substituting in eqn. (6)

$$\ln \tau = \ln \tau_0 + G\left[\frac{D_l - 1}{2D_l + 1} + \frac{3.x.D_a}{(2D_l + 1)^2}\right]. \tag{9}$$

If τ_s is the time constant of decay of m.e.p.c.s recorded when no alcohol is present in the membrane, then

$$\frac{\tau}{\tau_s} = \exp\left[G.\frac{3.x.D_a}{(2D_l + 1)^2}\right]. \tag{10}$$

The weight fraction, x, of alcohol in the membrane is related to the aqueous concentration, C, of the alcohol, and to the alcohol partition coefficient, P_a, where

$$P_a = \frac{\text{number of moles of alcohol/kg of membrane}}{\text{number of moles of alcohol/kg of saline}}.$$

As the number of moles of alcohol per kilogram of saline is the aqueous concentration of alcohol, C, the weight fraction of alcohol can be represented by

$$x = \text{weight of alcohol (kg) per kilogram of membrane} = P_a.M_a.C, \tag{11}$$

where M_a is the molecular weight of the alcohol (kg.mole^{-1}).

Now substituting for x in eqn. (10)

$$\frac{\tau}{\tau_s} = \exp\left\{\left[G.\frac{3.P_a.M_a.D_a}{(2D_l + 1)^2}\right].C\right\}. \tag{12}$$

This equation describes an exponential relationship between the time constant of decay of m.e.p.c.s and the aqueous concentration of the alcohol which is, in fact, the observed relationship (Figs. 5, 6 and 8; eqn. (2)).

The slope of the relationship depends on the product of the molecular mass, the dielectric constant, and the partition coefficient of the alcohol.

It is interesting to note that, for the alcohols that were used in these experiments, the product $M_a.D_a$ is approximately constant (values from Weast, 1972). Hence the slope of the relationship between τ and C for different alcohols should be approximately proportional to the variation in

their partition coefficients. Once again this is the observed relationship (see Fig. 10).

To examine these predictions, we calculated directly (from eqns. (7) and (11)) the terms $(D_m - 1)/(2D_m + 1)$ for different aqueous concentrations of these alcohols: ethanol, propanol, and butanol. The dielectric constant of the membrane lipid was assumed to be 3, membrane-buffer partition coefficients were taken from Roth & Seeman (1972) and values for the dielectric constants of the alcohols were obtained from *The Handbook of Chemistry and Physics* (Weast, 1972). The results obtained are illustrated

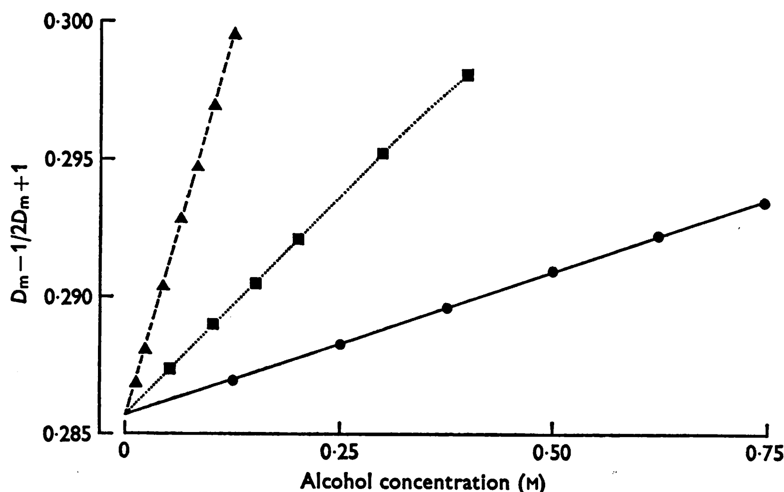

Fig. 11. A graph of $(D_m - 1)/(2D_m + 1)$ against alcohol concentration, for ethanol (circles), propanol (squares) and butanol (triangles). D_m, the dielectric constant of a mixture of membrane lipid and alcohol, was calculated as described in the text.

in Fig. 11. It can be seen that the relationship between the aqueous concentration of an alcohol and $(D_m - 1)/(2D_m + 1)$ was indeed reasonably linear over the range of alcohol concentrations used experimentally.

DISCUSSION

In this paper we have described the effects of some aliphatic alcohols on the post-synaptic conductance change generated by a quantum of acetylcholine. In the presence of the alcohols ethanol to pentanol, the decay phase of miniature end-plate currents is prolonged (Fig. 3) and this causes an increase in the amplitude and duration of m.e.p.p.s (Figs. 1 and 2).

It seems most unlikely that the effect of alcohols is due to an inhibition of cholinesterase activity. The results obtained for acetylcholine-induced depolarization and noise indicate that ethanol, unlike anticholinesterases

(Katz & Miledi, 1973), prolongs the duration of the elementary events themselves. Furthermore, the prolonged decay of m.e.p.c.s in ethanol remains exponential and is sensitive to changes in membrane potential in the normal way.

From these results it can be concluded that, in the presence of ethanol, the normal, first-order, voltage-sensitive reaction still controls the slowed rate of decay of the conductance change and that ethanol slows the reaction rate. It seems reasonable to assume that the other alcohols, from C_3 to C_5, act in a similar way.

As the chain length of the alcohols increased, lower aqueous concentrations were necessary to obtain a given prolongation of m.e.p.c.s. It was shown that this could be explained in terms of the membrane-buffer partition coefficients of the alcohols. That is, the evidence strongly suggests that the alcohols exert their effects in the post-synaptic membrane rather than in the aqueous phase.

This observation, together with the voltage sensitivity of the decay of m.e.p.c.s which suggests that molecules with a dipole moment are involved in the rate-limiting reaction, led us to examine whether the alcohols might be changing the dielectric constant in the region of the rate-limiting reaction. Our results are quantitatively consistent with this idea. We know of no other plausible mechanism which would give an exponential relationship between the time constant of decay of m.e.p.c.s and alcohol concentration. The change in dielectric constant, even in 0·5 M ethanol, although too small to cause a detectable change in membrane capacitance, would be large enough to cause a large change in reaction rate.

A dielectric constant of 3 was chosen arbitrarily for the membrane. The value seems reasonable from considerations of the capacitance and thickness of membranes, and the dielectric constants of lipids (see, for example, Katz, 1966). Whatever the actual value of D_l, an exponential relationship between the time constant of decay of m.e.p.c.s and aqueous alcohol concentration would still be predicted from eqns. (6) and (7).

A value of $1\cdot5\times10^{-11}$ e.s.u.^{2} cm^{-1} was obtained for $u_*^2/r_*^3 - u_\ddagger^2/r_\ddagger^3$ from the relationship between $\log\tau$ and ethanol concentration. $(D_m-1)/(2D_m+1)$ was calculated for each ethanol concentration using eqns. (7) and (11), and

$$\frac{kT\,.\,\mathrm{d}(\ln\tau)}{\mathrm{d}[(D_m-1)/(2D_m+1)]}$$

gave the value for $u_*^2/r_*^3 - u_\ddagger^2/r_\ddagger^3$. The fact that

$$\frac{\mathrm{d}(\ln\tau)}{\mathrm{d}[(D_m-1)/(2D_m+1)]}$$

was positive indicated that u_*^2/r_*^3 was greater than $u_\ddagger^2/r_\ddagger^3$. If the effective radius of the molecule in open configuration is the same as in the transi-

tion configuration, there must be a decrease in dipole moment in the molecule determining the rate of closing of conductance 'channels'. To carry this argument further, if the change in dipole moment is about 40–50 debye (Magleby & Stevens, 1972*b*) in a protein molecule with dipole moment of 200–500 debye (McClellan, 1963) the effective radius of the molecules would be of the order of 10–15 Å.

Although we have proposed a possible way in which aliphatic alcohols might prolong m.e.p.c.s, some results obtained with hexanol cannot be explained by this mechanism alone. With higher concentrations of hexanol, and sometimes after prolonged (more than 1 hr) exposure to the other alcohols, the decay of m.e.p.c.s was initially rapid, but then became slower with a clear point of inflexion (Fig. 7). It seems very likely that two different actions of the alcohols are responsible for the two different rates of decay seen under these circumstances. We have suggested that the slowing of the decay of m.e.p.c.s by alcohols is due to a change in the dielectric constant of the membrane in the region of the reaction determining the rate of decay of m.e.p.c.s. We have also suggested elsewhere (McBurney & Gage, 1972; McBurney, 1973; Gage, McBurney & Van Helden, 1974) that longer chain alcohols which make the decay of m.e.p.c.s faster, are changing the fluidity or viscosity of the region of membrane in which a conformational change in a protein responsible for the decay phase of m.e.p.c.s occurs. A biphasic decay of m.e.p.c.s is also seen with procaine (Gage & Armstrong, 1968; see also, Furukawa, 1957; Maeno, 1966; Steinbach, 1968; Deguchi & Narahashi, 1971). It is interesting to note that the membrane-buffer partition coefficient for procaine (4·5; Roth & Seeman, 1972) is between the partition coefficients for pentanol (3·6) and hexanol (13·0). It may be that an effect of procaine on the dielectric constant or the fluidity of membranes underlies its anaesthetic action.

In the previous paper (Gage & McBurney, 1974) it was shown that temperature and membrane potential had little effect on the growth phase of m.e.p.c.s. Again, although the decay of m.e.p.c.s was greatly prolonged by alcohols, there was no significant change in the growth phase. If the time course of the growth phase were related to the 'forward' rate of the same reaction that determines the time course of the decay phase, it might have been expected that the alcohols would have produced a significant change in both growth and decay phases. That they did not, supports the conclusion (Gage & McBurney, 1974) that some other process, such as diffusion across the synaptic cleft, governs the growth phase of m.e.p.c.s.

Finally, two interesting points emerge from these results with the alcohols. First, because the environment of a molecular reaction occurring

in a biological membrane may be an important determinant of the rate of that reaction, the rates of biologically important reactions occurring *in situ* may be quite different from the rates measured in a 'test-tube environment', despite the fact that the molecules themselves are unchanged. Secondly, because the rate of the decay phase of a synaptic current can be an important determinant of the input–output function of a synapse (Gage & McBurney, 1973) and because it is possible that the environment of the important reacting molecules may play a large part in determining the duration of this phase of the current flow, it is possible that it is in fact the 'environmental factors' which are modified in certain adaptive and pathological processes at synapses.

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