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Hydrostatic pressure modifies the action of octanol and atropine on frog endplate conductance

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1 The effects of octanol, ethanol and atropine were examined on the time course of decay (τ_D) of miniature endplate currents (m.e.p.cs) in the frog neuromuscular junction at normal and high pressure.

2 Octanol (25–100 μM) decreased reversibly the τ_D of m.e.p.cs in a dose-dependent manner, 100 μM reducing τ_D to 0.39 of the control value. Higher concentrations (200–500 μM) additionally depressed the amplitude of m.e.p.cs.

3 Hydrostatic pressure (3.19 and 5.25 MPa) reduced the τ_D of octanol (25–100 μM)-shortened m.e.p.cs. Thus 3.19 MPa and 5.25 MPa reduced the τ_D in the presence of 100 μM octanol to 0.75 and 0.78 of the octanol treated values. This effect was not completely reversed on decompression. The m.e.p.c. amplitude is reversibly decreased by pressure in the presence of octanol.

4 Hydrostatic pressure (3.19–15.55 MPa) did not modify the effect of ethanol on τ_D. At 10.40 and 15.55 MPa the τ_D was increased equally in the absence or presence of ethanol.

5 Atropine (60 μM) reduced the τ_D and amplitude of m.e.p.cs to 0.33 and 0.63 of the control values. These effects were compeletely reversible. Hydrostatic pressure (3.19 and 5.25 MPa) reduced the τ_D of atropine-shortened m.e.p.cs to 0.82 and 0.77 of the atropine-treated values respectively. This effect was not completely reversed on decompression. Hydrostatic pressure also reversibly depressed the amplitude of atropine-treated m.e.p.cs.

6 The implications of these drug-hydrostatic pressure interactions are discussed.

Introduction

Many structurally different anaesthetics depress neuromuscular transmission by reducing the amplitude and increasing the rate of decay of endplate currents (for review see Gage & Hamill, 1981). It has been suggested that such acceleration of the decay is brought about either by speeding up the rate of the reaction normally controlling the lifetime of endplate channels (α), or by blockage of the activated channels. For example, Gage *et al.* (1974, 1978) put forward the first possibility as an explanation for the action of octanol on the time constant of decay of miniature endplate currents (m.e.p.cs). Adams (1976) suggested, as an alternative, the channel-blocking scheme to explain the effect of barbiturates.

Studies with the androstanol spin label or spin-labelled fatty acids show that octanol increases the fluidity of natural membranes (Grisham & Barnett, 1973) and lipid bilayers (Miller & Pang, 1976; Finch & Kiesow, 1979). It is possible therefore that, as suggested by Gage *et al.* (1974, 1978), octanol increases α by increasing the fluidity of the lipids surrounding the acetylcholine receptor. In contrast high pressure (> 10.4 MPa) decreases the fluidity of artificial bilayers (Trudell *et al.*, 1973; Boggs *et al.*, 1976), and counters the disordering effect of octanol in erythrocyte membranes (Finch & Kiesow, 1979). The numerous membrane actions of high pressure in excitable cells have been reviewed by Wann & Macdonald (1980) and Macdonald (1984).

In this study we have examined the action of high hydrostatic pressure on the time course and amplitude of m.e.p.cs in the presence of octanol. In this way we hoped to assess whether a change in membrane fluidity was a likely mechanism for the action of octanol. Additionally, the pressure sensitivity of the action of ethanol, an alcohol with the opposite postsynaptic endplate action to octanol, and of atropine (a putative channel blocker) was tested for comparison. A brief account of some of these results has been given previously (Wann *et al.*, 1980).

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Methods

All experiments were performed on sartorius muscles isolated from *Rana temporaria* and *Rana pipiens*, of either sex. In order to minimize problems associated with movement (e.g. during transfer of the preparation to the pressure vessel whilst recording from endplate regions) the muscles were wound onto a rod, stretched to approximately *in situ* length, and placed in a Perspex bath of 1.0 ml capacity (see Stefani & Schmidt, 1972). The Ringer solution contained (mM): NaCl 115, KCl 2.5, $CaCl_2$ 1.0 and, Tris buffer 2.0, pH = 7.4. The sodium ion concentration was raised to 165 mM in some experiments, thus increasing the frequency of miniature endplate currents (m.e.p.cs) in order to offset the potent depression of frequency produced by hydrostatic pressure (see Ashford *et al.*, 1982). The drugs, octan-l-ol and atropine (both Sigma) were dissolved directly in the normal Ringer solution.

Spontaneous m.e.p.cs were recorded extracellularly using glass micro-electrodes filled with 1 M NaCl in agar. These electrodes had tip resistances of 0.2–0.8 MΩ, and diameters of 5–15 μm. The m.e.p.cs were 'captured' manually on an oscilloscope screen or by use of a transient recorder. The time course of m.e.p.cs was measured either from film of oscilloscope traces magnified with a film projector and projected onto calibrated graph paper, or directly from the 'hard copy'. The decay phase of m.e.p.cs was analysed by a log-linear (least squares) regression analysis from the current peak to 10–20% of the final value. M.e.p.cs with a correlation coefficient of 0.98 or better were used. The m.e.p.c. growth time was measured as the time for a m.e.p.c. to increase from 20% to 80% of its maximum amplitude.

The high pressure equipment and techniques have been described previously (Harper *et al.*, 1975; Wann *et al.*, 1979). Hydrostatic pressure was used in all the experiments described here, the compression medium being liquid paraffin. Pressure was applied in steps of 1.03 MPa at one minute intervals (101,325 Pa = 1 atm.) until the desired pressure was reached for data collection (usually 3.19 or 5.25 MPa). This compression rate resulted in a temperature increase in the Ringer solution close to the recording electrode of 0.5°C/10.4 MPa which dissipated within 10 min. No correction for any temperature change was made when analysing the m.e.p.c. decay rate. All the pressure data are given in absolute units i.e. 0.1 MPa = normal ambient pressure, and all the experiments were carried out at 20°C.

Table 1 The effects of octanol and atropine on miniature endplate currents

Drug (μM)	*Amplitude (% of control)*	*Time constant of decay (% of control)*
Octanol 25	N.S.	74.3 ± 5.6
Octanol 50	N.S.	59.5 ± 5.1
Octanol 100	N.S.	39.0 ± 2.6
Atropine 60	62.8 ± 2.2	33.4 ± 2.5

The data were obtained from 3–6 individual experiments for each concentration of drug. Between 20 and 40 m.e.p.cs were analysed in each experiment. Data shown as the mean ± s.e.mean.

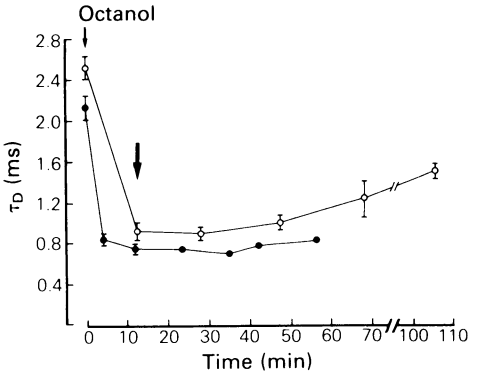

Figure 1 The time course of the reduction in the time constant of decay (τD) of miniature endplate currents by 0.1 mM octanol in normal Ringer solution outside the pressure vessel (●) and in the pressure vessel where there is a Ringer -liquid paraffin interface (○). The data were obtained from two different end-plates. The second arrow indicates the time at which the Ringer solution comes into contact with the liquid paraffin in the pressure vessel. Each point is the average of 10–15 m.e.p.cs (the vertical lines denote ± 1 s.e. where larger than the symbols).

Results

Effects of Octanol on m.e.p.cs

Previous work has shown that octanol (0.1 to 1.0 mM) reversibly depressed the amplitude and the duration of the m.e.p.cs recorded at the amphibian neuromuscular junction (Gage *et al.*, 1974; 1978). We find that lower concentrations of octanol (25–100 μM) also reduce the duration of the decay of m.e.p.cs (see e.g. Table 1 and Figure 1), and have no significant effect on amplitude (Table 1). The onset of octanol's action is rapid, a steady state is reached within 3–4 min and the effect is maintained over at least an hour (Figure 1). Higher concentrations of octanol (0.2–0.5 mM) markedly depress the amplitude and increase the rate of decay further. Octanol at 1.0 mM completely abolishes the m.e.p.cs. The

Table 2 (A) The effect of hydrostatic pressure on the miniature endplate current decay rate in the presence of octanol or atropine and (B) the results of a typical experiment showing the irreversibility of the pressure effect and the full reversal of the action of octanol

A	*Time constant of decay (%)*[a]		
Drug (μM)	3.19 MPa	5.25 MPa	0.10 MPa[b]
Octanol 25	89.3 ± 3.3	86.6 ± 2.8	90.4 ± 1.4
Octanol 50	83.7 ± 2.1	78.1 ± 2.0	86.1 ± 2.5
Octanol 100	74.9 ± 2.1	77.7 ± 1.9	80.8 ± 2.9
Atropine 60	82.1 ± 1.5	77.0 ± 2.5	90.2 ± 1.6

B	*Pressure*	*Time constant of decay*[a]
Solution	(MPa)	(ms)
Normal Ringer	0.10	1.45 ± 0.06
25 μM Octanol	0.10	1.22 ± 0.06
	5.25	1.09 ± 0.04*
	0.10	1.08 ± 0.04*
Normal Ringer	0.10	1.42 ± 0.06

(A) The data were obtained from 2–6 individual experiments for each concentration of drug. Between 15–30 m.e.p.cs were analysed for each pressure in individual experiments. The data show the means ± s.e.mean.

[a] Percentage of control (i.e. pre-pressure (0.10 MPa) data in presence of drug).

[b] Post-pressurization.

(B),[a] Mean ± s.e. mean and * $P < 0.05$ when compared to data of octanol at 0.10 MPa.

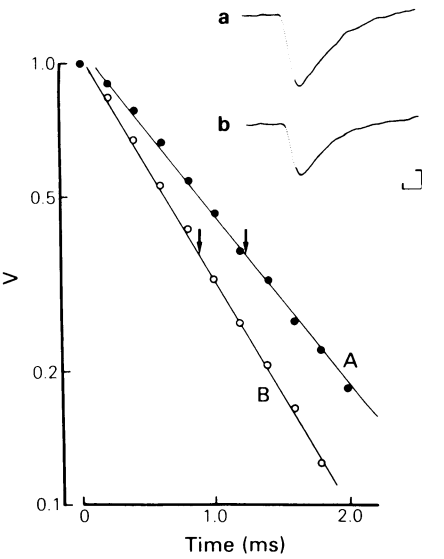

Figure 2 The effect of hydrostatic pressure on rate of decay of miniature endplate currents and the amplitude in the presence of octanol (50 μM). (Representative m.e.p.cs in octanol at 0.10 MPa (a) and 5.25 MPa (b) are shown. Calibrations: vertical 100 μV horizontal, 0.5 ms.) The decays of these currents are plotted semilogarithmically as a function of time (after the peak). The straight lines (fitted by regression analysis) illustrate that the decays are exponential with single time constants of 1.24 ms and 0.86 ms at 0.10 MPa (A) and 5.25 MPa (B) respectively. Ordinates: normalized amplitude, V; abscissae: time (ms).

effects of octanol at all concentrations are completely reversed on returning to normal Ringer solution. Octanol (25 μM–1 mM) has no significant effect on the time course of the m.e.p.c. growth phase.

In the pressure vessel the octanol containing Ringer solution is in contact with the liquid paraffin compression medium for the duration of the experiment. It is possible that during this time the aqueous concentration of octanol drops, due to the partitioning of the apolar alcohol into the liquid paraffin (apolar) phase. This effect may explain the apparent partial reversal of octanol's action observed after about one hour (0 ——— 0 in Figure 1). The presence of liquid paraffin did not influence the results in the first 40 min, therefore all data were collected at pressure within 40 min of the preparation being placed in the pressure vessel. It is unlikely that hydrostatic pressures in the range used here alter the partitioning of octanol (see Trudell *et al.*, 1973; Miller & Yu, 1977).

The pressure sensitivity of the action of octanol

It has been found previously that a hydrostatic pressure of 10.40 MPa has no significant effect on the decay rate of m.e.p.cs in the presence of 0.1 mM octanol (Wann *et al.*, 1980). As hydrostatic pressure (in the range 10.40–15.55 MPa) produces a small but significant increase in the time constant of decay of m.e.p.cs in the absence of the alcohol (Ashford *et al.*, 1979; 1982), it was argued that octanol 'protected' against the effects of hydrostatic pressure. However, high pressure reduces reversibly the amplitude of m.e.p.cs in the presence of 0.1 mM octanol whereas only a small reduction was noted in its absence. Indeed, at pressures of 10.40 MPa and up to 15.55 MPa the majority of m.e.p.cs were difficult to discern from the base line noise and thus few were large enough to enable an accurate determination of the m.e.p.c. decay time constant. We have now investigated further the effects of hydrostatic pressure on octanol-shortened m.e.p.cs with particular attention to the action of pressures up to 5.25 MPa.

In the presence of octanol (25 to 100 μM) hydrostatic pressures (3.19 and 5.25 MPa), surprisingly, actually increase the m.e.p.c. decay rate (Figure 2 and Table 2A and B) and also significantly reduce the m.e.p.c. amplitude (see Figure 2 and Table 3). The

Table 3 The effect of hydrostatic pressure on the miniature endplate current amplitude in the presence of octanol or atropine

Drug (μM)	*M.e.p.c. amplitude (%)* 3.19 MPa	5.25 MPa	0.10 MPa (post pressurization)
Octanol 25	97.0 ± 2.5	76.1 ± 2.9	95.7 ± 2.3
Octanol 50	79.6 ± 4.3	68.8 ± 3.5	95.0 ± 3.1
Octanol 100	71.2 ± 2.3	60.5 ± 2.9	95.1 ± 3.2
Atropine 60	82.5 ± 1.8	59.5 ± 2.5	98.2 ± 2.9

The data were obtained from 2–6 individual experiments for each concentration of drug. Between 15–30 m.e.p.cs were analysed for each pressure in individual experiments. Values are means ± s.e. mean and are expressed as a percentage of control values (i.e. pre-pressure (0.10 MPa) data in presence of drug).

decay of m.e.p.cs although faster at a pressure of 5.15 MPa, remains exponential with a single time constant. This is illustrated in Figure 2 where the decays of typical m.e.p.cs at 0.10 MPa (A) and 5.25 MPa (B) in the presence of 50 μM octanol are plotted semilogarithmically against time. The time constants of decay are 1.24 ms for the current at atmospheric pressure, and 0.86 ms for the current at 5.25 MPa. Table 2A shows the pooled data (expressed as percentage changes from octanol-treated currents at 0.10 MPa) from several experiments examining the accelerating effect of hydrostatic pressure (3.19 and 5.25 MPa) on the decay rate of m.e.p.cs. Interestingly, this effect is not completely reversible on decompression to 0.10 MPa, the decay phase of the m.e.p.cs remaining significantly shorter. However, subsequent washing of the preparation with normal Ringer solution causes the time constant of decay to revert to pretreated values. An example of this effect in one experiment, is given in Table 2B. In contrast, the reduction of the m.e.p.c. amplitude by hydrostatic pressure (3.19 and 5.25 MPa) in the presence of octanol (25 to 100 μM) was totally reversible on decompression to atmospheric pressure (Table 3). These pressures had no significant effect on the time course of the growth phase in the presence of octanol.

Table 4 Effects of hydrostatic pressure on miniature endplate currents in the presence of ethanol

Solution	*Pressure* (MPa)	*Time constant of decay*[a] (ms)
Normal Ringer	0.10	1.70 ± 0.06
Ethanol 0.4 M	0.10	7.45 ± 0.24
	3.19	8.21 ± 0.37
	5.25	7.64 ± 0.27
	7.31	8.19 ± 0.28
	10.40	9.05 ± 0.43*
	15.55	9.69 ± 0.49*

Pooled data from five experiments. Between 15–20 m.e.p.cs were analysed for each pressure in individual experiments.
[a] Mean ± s.e.mean.
* $P < 0.001$, when compared with ethanol data at 0.10 MPa

Pressure sensitivity of ethanol – lengthened m.e.p.cs

It was of interest to determine whether m.e.p.cs treated with a short chain rather than a long chain aliphatic alcohol showed a similar pressure sensitivity. Unlike octanol the shorter chain alcohols (e.g. ethanol) prolong considerably the decay phase of m.e.p.cs at amphibian (Gage *et al.*, 1975) and mammalian (Quastel & Linder, 1975) neuromuscular junctions. In the present study, ethanol (0.4 M) reversibly produced about a four fold increase in the time constant of decay of m.e.p.cs in agreement with that observed by Gage *et al.* (1975) and Quastel & Linder (1975). There was no significant change in the amplitude of the m.e.p.cs or the time course of the growth phase. Hydrostatic pressure below 10.40 MPa had no significant effect on the decay phase time course or the amplitude of m.e.p.cs in the presence of ethanol. However, although there was a large variability in the current decays, pressures of 10.40 MPa and 15.55 MPa produced a significant prolongation of the m.e.p.c. decay (see Table 4). In four out of five experiments this effect of hydrostatic pressure was completely reversible. Hydrostatic pressure had no effect on the time course of the growth phase in the presence of ethanol.

Pressure modifies the effects of atropine on me.p.cs

Atropine is a non-competitive antagonist at the neuromuscular junction. It increases the decay rate of endplate currents and m.e.p.cs (Alder & Albuquerque, 1976; Feltz & Large, 1976), and shortens the mean channel lifetime (Katz & Miledi, 1973). Since the effect of atropine is thus similar to that of octanol, the action of hydrostatic pressure was tested on m.e.p.cs in the presence of atropine.

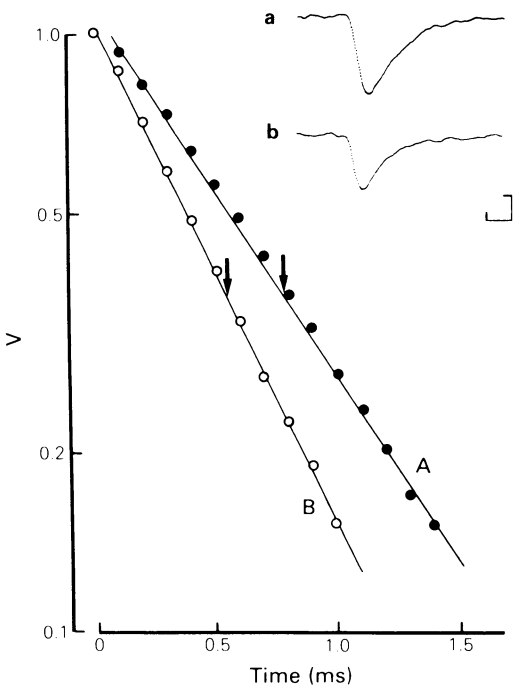

Figure 3 The effect of hydrostatic pressure on the decay rate and amplitude of miniature endplate currents in the presence of atropine (60 μM). Representative m.e.p.cs are shown in atropine at 0.10 MPa (a) and 5.25 MPa (b). Calibrations: vertical 100 μV; horizontal, 0.5 ms. Semilogarithmic plots of the decay phases of these currents are also shown. The straight lines (fitted by regression analysis) illustrate the exponential nature of the current decay. The time constants of decay at 0.10 MPa (A) and 5.25 MPa (B) are 0.76 and 0.54 ms, respectively. Ordinates: normalized amplitude, V; abscissae: time (ms).

Application of atropine at a concentration of 60 μM, produces a marked increase in the decay rate, and a reduction in the amplitude of m.e.p.cs (see Table 1) which was completely reversible on washing with normal Ringer solution. The reduction in decay time constant produced by atropine followed a similar time course to that of octanol, and was not altered by the presence of liquid paraffin over a period of 45 min. On applying hydrostatic pressure to atropine-treated preparations, the most noticeable effect was an immediate and marked reduction in the m.e.p.c. amplitude at relatively low pressures (i.e. less than 5.25 MPa). Indeed at pressures greater than 5.25 MPa the m.e.p.cs were extremely small and were difficult to distinguish from the baseline noise. This effect of pressure on the m.e.p.c. amplitude in the presence of atropine was reversible on decompression (Table 3).

Additionally, the m.e.p.c. decay phase time course in the presence of 60 μM atropine was reduced by hydrostatic pressure (Table 2A). This is illustrated in Figure 3 where the decays of typical m.e.p.c.s. at 0.10 MPa (A) and 5.25 MPa (B), in the presence of atropine, are plotted semilogarithmically against time. The time constants of decay are 0.76 ms for (A) and 0.54 ms for (B). As in the experiments with octanol this effect was not completely reversible on decompression (Table 2A). Atropine at all pressures had no significant effect on the time course of the m.e.p.c. growth phase.

Discussion

Octanol shortens the lifetime of acetylcholine-activated ionic channels. There are two ways in which this could be achieved. Octanol could either block the activated ionic channel or increase the normal rate constant of decay. The relevant reactions are usually represented by the following simplified kinetic scheme:

$$A + C \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} O \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} B$$

Where A is acetylcholine; C, O and B represent the closed, open and blocked states of the acetylcholine receptor-ionophore complex respectively; β and α are the rate constants for opening and closing of channels and k_1 and k_{-1} are the rate constants for blocking and unblocking of open channels. In this scheme there are two reactions leading away from the open state, α and k_1, i.e. the normal closing reaction and the blocking reaction. Octanol, by reducing channel lifetime, increases the rate at which 'O' disappears. It can do so only by increasing α or by converting the conducting channel into the blocked state 'B' given by the rate k_1.

Gage *et al.* (1978) favoured the former idea, that octanol decreases channel lifetime by allowing the original reaction controlling the closing of channel to proceed at a faster rate ($n\alpha$). Their evidence is that the temperature and voltage sensitivity of the m.e.p.c. decay rate and mean channel lifetime were, in most cases, unaltered in the presence of octanol. Thus for a blocking model to hold, the reaction k_1 would have to have a similar voltage and temperature sensitivity to α. One possible mechanism offered by these authors is that octanol increased α by increasing membrane fluidity.

The results obtained in this study show that octanol at low concentrations (25–100 μM) reversibly increases the rate of decay of m.e.p.cs. This concentration range is lower than that required in previous

investigations on the amphibian neuromuscular junction (Gage *et al.*, 1974; 1978), and it seems unlikely that such low concentrations of octanol fluidize the lipid environment of the receptor. Additionally in the presence of octanol, at these concentrations, moderate hydrostatic pressures (3.19–5.25 MPa) reduce significantly the time constant of decay of m.e.p.cs. Hydrostatic pressure is known to reduce fluidity in lipid bilayer and biological membranes (Wann & Macdonald, 1980). On fluidity considerations alone we would expect, therefore, that hydrostatic pressure would oppose the effect of octanol. From our data we conclude that octanol does not increase α by increasing the fluidity of lipids surrounding the acetylcholine receptor, which is consistent with the known temperature independent nature of octanol's action (Gage *et al.*, 1978).

In a previous publication octanol (0.1 mM) was considered to 'protect' against the lengthening of the m.e.p.c. decay induced by hydrostatic pressures of 10.40 MPa or greater (Wann *et al.*, 1980). Protection can now be attributed to the dual effect of hydrostatic pressure. At 10.4 MPa and above the potentiating effect of hydrostatic pressure on octanol's action offsets the normal lengthening effect of pressure on the m.e.p.c. decay.

Hydrostatic pressures up to 5.25 MPa have no effect on the normal rate of decay of m.e.p.c. (Ashford *et al.*, 1982). If the reaction controlling the channel closure is the same in the presence of octanol, then similarly we might expect that pressures up to 5.25 MPa will have no effect. The data provided here show that such pressures do modify the decay rate in the presence of octanol, thus raising the possibility that another reaction governs the rate of decay in the presence of octanol. Alternatively, it is possible that pressure somehow enhances the binding of octanol to the postsynaptic membrane, so increasing its effectiveness.

It has been proposed that atropine increases the rate of decay of m.e.p.cs and decreases channel lifetime by blocking open ion channels (Feltz *et al.*, 1977; Adler *et al.*, 1978) i.e. reaction k_1 also determines the rate of decay. Hydrostatic pressure (3.19 and 5.25 MPa) potentiates the effect of atropine, like octanol, on the m.e.p.c. decay rate. Therefore, it is plausible that the increased rate of m.e.p.c. decay produced by atropine and octanol is due to a similar mechanism, which is block of the open ionic channel, and that this block is pressure-dependent. If we accept this as a possibility then, following Adams (1976), in the presence of octanol the open channel can either close normally (α) or become blocked (k_1) so that the rate constant for m.e.p.c. decay will be governed by $\alpha + Ck_1$ where C is the octanol concentration. Thus if the difference between the rate constant of decay in control and octanol-containing

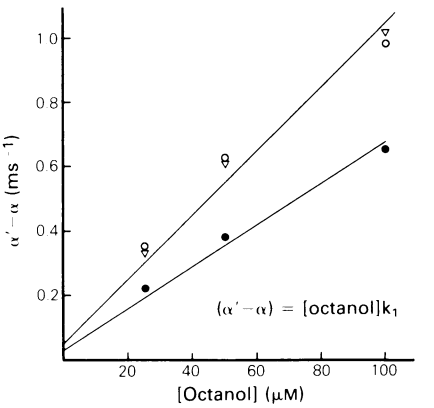

Figure 4 The relationship between the difference in the rates of decay of miniature endplate currents in octanol and control Ringer solution and the aqueous octanol concentration. Data are shown for 0.1 MPa (●), 3.19 MPa (▽) and 5.25 MPa (○). Each point is the mean of data from 2–6 experiments. The lines are fitted by regression analysis, the slopes being $6.5 \times 10^6 M^{-1} s^{-1}$ at 0.1 MPa and $9.6 \times 10^6 M^{-1} s^{-1}$ at 3.19 MPa. The fit of the data at 5.25 MPa is not shown but was almost superimposable with a slope of $1.01 \times 10^7 M^{-1} s^{-1}$. In each case $r = 0.99$.

Ringer solution is plotted against the octanol concentration an approximate value for k_1 can be obtained from the slope of the line. This analysis is shown in Figure 4 for 0.10 MPa, 3.19 MPa and 5.25 MPa. At atmospheric pressure k_1 is $6.5 \times 10^6 M^{-1} s^{-1}$ and there was little difference between the data for 3.19 MPa and 5.25 MPa, the values of k_1 being $1.01 \times 10^7 M^{-1} s^{-1}$ and $9.6 \times 10^6 M^{-1} s^{-1}$, respectively. Although only one concentration of atropine was tested, a similar calculation gives values for k_1 of $1.6 \times 10^7 M^{-1} s^{-1}$, $2.1 \times 10^7 M^{-1} s^{-1}$ and $2.0 \times 10^7 M^{-1} s^{-1}$ for pressures of 0.10 MPa, 3.19 MPa and 5.25 MPa, respectively. Therefore, in kinetic terms, pressure acts (according to this model) by increasing the rate constant k_1 although this effect is maximal at 3.19 MPa in the case of both octanol and atropine.

Of further interest is the observation that the pressure-induced increase in the rate of decay of m.e.p.cs is not completely reversible for either octanol or atropine, i.e. the currents remain faster on decompression. However, when octanol is washed out after decompression, the decay rate reverts to normal pretreatment values. This may indicate that pressure induced an irreversible (or at least very slowly reversed) conformational change in the receptor-channel complex thus resulting in the exposure of more binding sites for octanol. It is interesting to note in this context that the pressures employed in these experiments are within the human diving range.

Another feature of the interaction between octanol or atropine and high pressure was revealed in the studies of the m.e.p.c. amplitude. Although absolute values cannot be estimated reliably with the extracellular electrode technique, it was clear that hydrostatic pressure reduced the m.e.p.c. amplitude more markedly in the presence of either octanol or atropine. This finding should be substantiated in voltage-clamp experiments. Such a reduction in the m.e.p.c. amplitude means that in the presence of either drug pressure reduces either the number of channels opened or the unitary conductance of these channels.

Ethanol lengthens the m.e.p.c. decay and increases the lifetime of individual channels (Gage *et al.*, 1975; Quastel & Linder, 1975). Pressure (10.40–15.55 MPa) increased significantly the time constant of decay in the presence of ethanol. Indeed the increase (20–30%) was of similar magnitude to that observed (20–40%) in the absence of ethanol. It has been shown previously that the temperature dependence of ethanol-lengthened m.e.p.cs is similar to that of control m.e.p.cs in mouse diaphragm (Robertson & Wann, 1984). These pressure and temperature data strongly support the idea that the reaction controlling the decay rate in ethanol solutions is the same as that in normal Ringer solution. The pressure experiments with ethanol also show that the modifying action of high pressure does not apply to all the alcohols.

The molecular mechanisms leading to the different effects of octanol and ethanol described above are at present unknown. In part such differences probably arise from the different binding sites of the two alcohols. In the absence of the appropriate information on the mechanism of action of the alcohols it is difficult to interpret our alcohol-pressure data. What is clear, however, is that our findings are quite incompatible with the critical volume hypothesis (Miller *et al.*, 1973).

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