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THE EFFECTS OF HYDROSTATIC PRESSURE ON THE SPONTANEOUS RELEASE OF TRANSMITTER AT THE FROG NEUROMUSCULAR JUNCTION

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SUMMARY

1. The effects of hydrostatic pressure (0·1–15·55 MPa) on the spontaneous release of transmitter at the frog neuromuscular junction were investigated.

2. The major effect of high pressure is on the release mechanism, pressure (0·1–10·40 MPa) producing an exponential decrease in frequency of the miniature end-plate currents in normal Ringer solution. The frequency decreases to 0·52 and 0·24 of the control value at 5·25 and 10·40 MPa respectively. This effect is reversible on decompression.

3. The sensitivity of the release process to high pressure is unaltered in 10 mM-K^+, 6 mM- and 10 mM-Ca^{2+} and hypertonic (165 mM-NaCl) Ringer solution, although the high Ca^{2+} media shift the threshold for the pressure effect to higher pressures.

4. Higher pressure (10·40–15·55 MPa) produces a small increase in the time constant of decay (τ_D) of m.e.p.c.s with no effect on the growth phase. A pressure of 15·55 MPa increases τ_D to 1·35 of the control value.

5. The possible actions of high pressure on both the pre- and post-synaptic processes are briefly discussed.

INTRODUCTION

It has long been known that high pressure produces marked effects on isolated nerve and muscle (see Wann & Macdonald, 1980). The effects of high pressure on the motor activity of a variety of animal species including man have now also been reported (Brauer, 1975; Bennett, 1975; Harris, 1979). In animals high pressure induces irregular motor activity comprising firstly tremors, then at higher pressures clonic or tonic–clonic convulsions (see Brauer, 1975). These motor disturbances along with other symptoms constitute the high pressure nervous syndrome or h.p.n.s. (Brauer, 1975). At higher pressures (for mammals, amphibians and crustaceans this is around

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10·40–15·55 MPa) inhibition of motor activity is produced (Macdonald & Miller, 1976; Wilcock, Wann & Macdonald, 1978). (The units in MPa used in the paper correspond to the pressure guage readings of 150 (10 atm), 300, 450, 600, 750 (50 atm) p.s.i. etc. At present gauges are not calibrated in MPa units enabling rounded numbers to be used conveniently. The odd numbers used in our plots are a consequence of this.)

It has been shown that high hydrostatic pressure (5·25–20·80 MPa) reduces the amplitude of the excitatory junction potential (e.j.p.) at the lobster neuromuscular junction (Campenot, 1975), so that a decrease in neuromuscular transmission could contribute to the depression of motor activity in crustaceans. Further, it has been argued that the reduction in e.j.p. is due to a decreased release of transmitter rather than post-synaptic factors (Campenot, 1975). In the vesicular model of transmitter release, fusion of vesicles with the preterminal membrane may be initiated by a phase change in the membrane lipids (e.g. Papahadjopoulos, Vail, Newton, Nir, Jacobson, Poste & Lazo, 1977). Since high pressure has well-defined effects on the phase transition behaviour and fluidity of model membrane bilayers (Wann & Macdonald, 1980), it may prove to be a useful probe of neuromuscular transmission.

We have therefore investigated the pre- and post-synaptic effects of high pressure using the frog neuromuscular junction. Our data show that high pressure affects both the pre- and post-synaptic events. The spontaneous quantal release of transmitter (miniature end-plate current and miniature end-plate potential frequency) was very sensitive to pressure and was depressed; the post-synaptic conductance change was less sensitive and its duration was increased. Some of these results have been presented in a preliminary form (Ashford, Macdonald & Wann, 1979).

METHODS

All experiments were carried out on sartorius muscles dissected from both male and female *Rana temporaria* or *Rana pipiens*. The sartorius muscles were wound on to a rod which fitted into a Perspex bath of 1·0 ml capacity (see Stefani & Schmidt, 1972). This method proved useful in minimizing movement problems associated with transferring the preparation to the pressure vessel whilst recording intra- or extracellularly from end-plate regions. The Ringer solution had the following composition (mM): NaCl, 115; KCl, 2·5; $CaCl_2$, 1·0; Tris buffer, 2·0. The pH was 7·4. Miniature end-plate currents (m.e.p.c.s) were recorded extracellularly with a focal glass electrode (tip resistance 0·2–0·8 MΩ) of tip diameter 5–15 μm filled with a 1% agar/1 M-NaCl solution. Miniature end-plate potentials (m.e.p.p.s) were recorded with glass micro-electrodes (tip resistance 5–10 MΩ) filled with 3 M-KCl. Frequency was measured continuously using a Siemens oscillomink ink recorder.

Spontaneous m.e.p.c.s were 'captured' manually on an oscilloscope screen or by use of a transient recorder (Datalab 901) which stored and subsequently displayed the m.e.p.c.s in a stationary position on the oscilloscope screen. These m.e.p.c.s were photographed and 'hard copies' could be obtained if required. The growth time was measured from the digital form of the m.e.p.c. as the time for a m.e.p.c. to increase from 20% to 80% of its maximum amplitude. Alternatively, the time course of the m.e.p.c.s was measured from film of oscilloscope traces magnified with a film projector on to calibrated graph paper. The decay phase of the m.e.p.c.s was analysed by a log-linear (least squares) regression analysis from the current peak to 10–20% of the final value. Plots from m.e.p.c.s showing a correlation coefficient of 0·98 or better were used. All experiments were performed at 20 °C.

Pressure apparatus

The equipment and our techniques have been described previously (Harper, Macdonald & Wann, 1975; Wann, Macdonald, Harper & Wilcock, 1979). Hydrostatic pressure was always used, the compression medium being liquid paraffin.

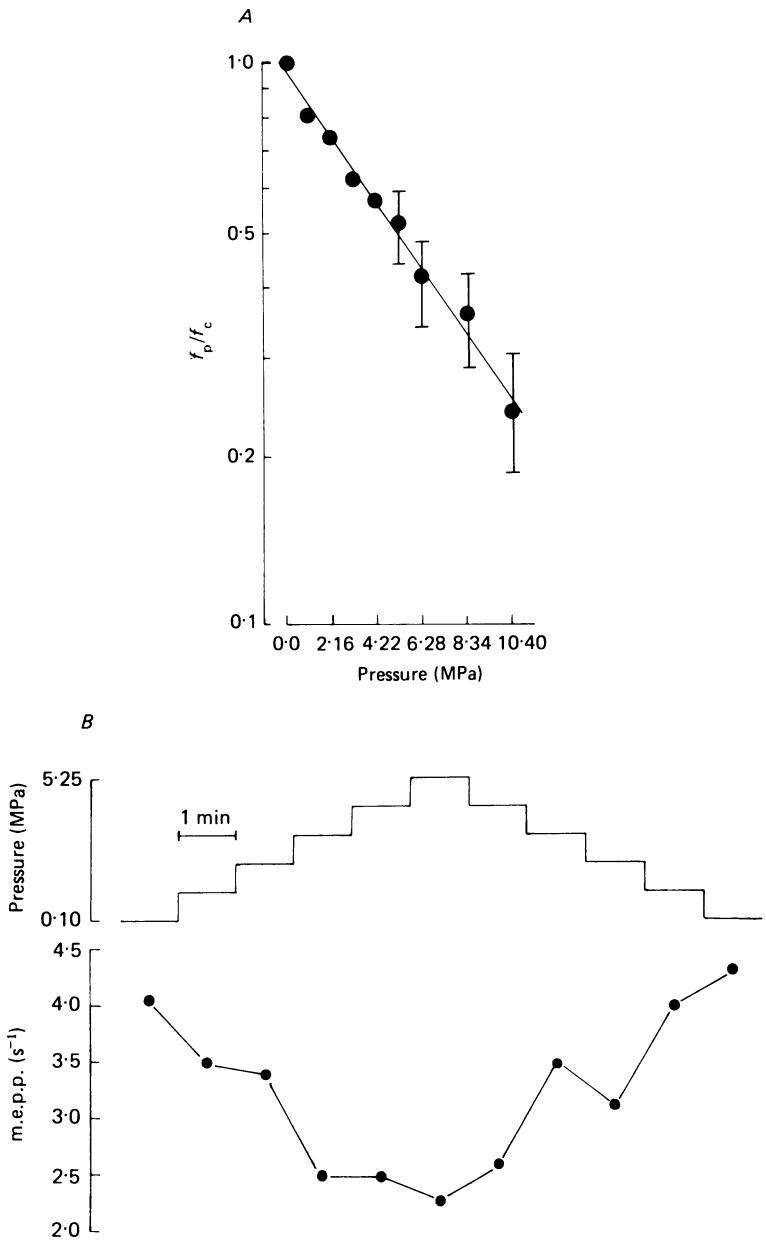

Fig. 1. The effect of hydrostatic pressure on the spontaneous quantal release of transmitter. *A*, a semi-logarithmic plot of the effect of hydrostatic pressure on m.e.p.c. frequency. Ordinate: m.e.p.c. frequency (f_p) as a ratio of control frequency at 0·10 MPa (f_c). Abcissa: pressure (MPa). The data were obtained from five separate experiments. The line has been fitted by regression analysis ($r = 0{\cdot}99$); the bar heights represent 1 × S.E. (where these are not shown the S.E. values are smaller than the symbol size). *B*, the effect of compression (up to 5·25 MPa) followed by decompression in 1·03 MPa steps on m.e.p.p. frequency. The data were obtained from a single junction. Ordinates: m.e.p.p. frequency (s^{-1}) and hydrostatic pressure (MPa).

Pressure was applied in steps of 1·03 MPa at 1 min intervals (101325 Pa = 1 atm) in the experiments examining the release of transmitter. Compression at this rate resulted in a maximum temperature increase in the Ringer solution close to the recording electrode of 0·5 deg C/10·40 MPa which equilibrated within 10 min. The m.e.p.c. or m.e.p.p. frequency was measured during the middle 30 s of each compression step (i.e. 15–45 s) and was not corrected for any temperature change. In the experiments where the effects of pressure on m.e.p.c. parameters were studied, the pressure steps were either 2·58 or 5·15 MPa applied at between 10 and 15 min intervals to enable data collection to be made (the m.e.p.c. frequency was low at pressure), and characteristics of the m.e.p.c. were measured at a time when the temperature had equilibrated.

All data are plotted in absolute units, i.e. 0·1 MPa = normal ambient pressure.

RESULTS

Presynaptic effects

Normal release

In our first experiments the effects of hydrostatic pressure on m.e.p.c. frequency were examined. The effect of hydrostatic pressure (0·1–10·40 MPa) on such quantal release is shown in Fig. 1*A* (note that 0·1 MPa = atmospheric pressure). The frequency was measured during compression, the frequency at any one pressure (f_p) and the frequency at 0·1 MPa (f_c) being expressed as a ratio. Pressure produces an exponential decrease in frequency which is significant ($P < 0·05$) at 1·13 MPa and above. The frequency decreases to $0·52 \pm 0·07$ and $0·24 \pm 0·08$ ($\bar{x} \pm 1 \times$ S.E., $n = 5$) of the control value at 5·25 and 10·40 MPa respectively. This effect is reversible on decompression.

Application of pressure could produce a slight movement of the electrode tip resulting in an apparent reduction in the frequency of transmitter release. It was therefore important to confirm these findings with intracellular recordings. Fig. 1*B* shows the results of a single experiment. High pressure clearly produces a reversible reduction in m.e.p.p. frequency. In eight experiments 5·25 MPa reduced the frequency to a mean of $0·54 \pm 0·03$ ($\bar{x} \pm 1 \times$ S.E., $n = 8$) of the control value. There was therefore good quantitative agreement between experiments in which either m.e.p.c.s or m.e.p.p.s were recorded over this pressure range. In three other experiments on compression the pressure was held for 5 min at 2·16 MPa and 10 min at 5·25 MPa before decompression at the normal rate. The m.e.p.p. frequency remained constant during such short stops at pressure and returned to normal only on decompression. In some other experiments a longer stay at pressure (5·25 MPa) resulted in some recovery of m.e.p.p. frequency. Thus in two experiments the frequency decreased to 0·44 and 0·47 of the control value on compression to 5·25 MPa. In the absence of decompression the frequency was 0·49 and 0·54 15 min later and 0·61 and 0·72 of the control value after 40 min.

The spontaneous release of transmitter in normal Ringer solution is thus very sensitive to pressure. It was consequently of interest to test whether the spontaneous release of transmitter under modified ionic conditions showed the same sensitivity. The effects of high pressure were tested on preparations exposed to (*a*) a high K^+_o (10 mM) solution, (*b*) high Ca^{2+}_o solutions, and (*c*) a hypertonic Ringer solution (165 mM-NaCl).

Release in 10 mM-K^+_o

Hydrostatic pressure (5·25–10·40 MPa) produces a small depolarization (< 5 mV) of skeletal muscle fibres. This depolarization added to that induced by the 10 mM-K^+_o bathing medium reducing the amplitude of the m.e.p.p.s and making it difficult to analyse the effects of high pressure on m.e.p.p. frequency. Results from one of two successful experiments where m.e.p.p.s could easily be distinguished from the base

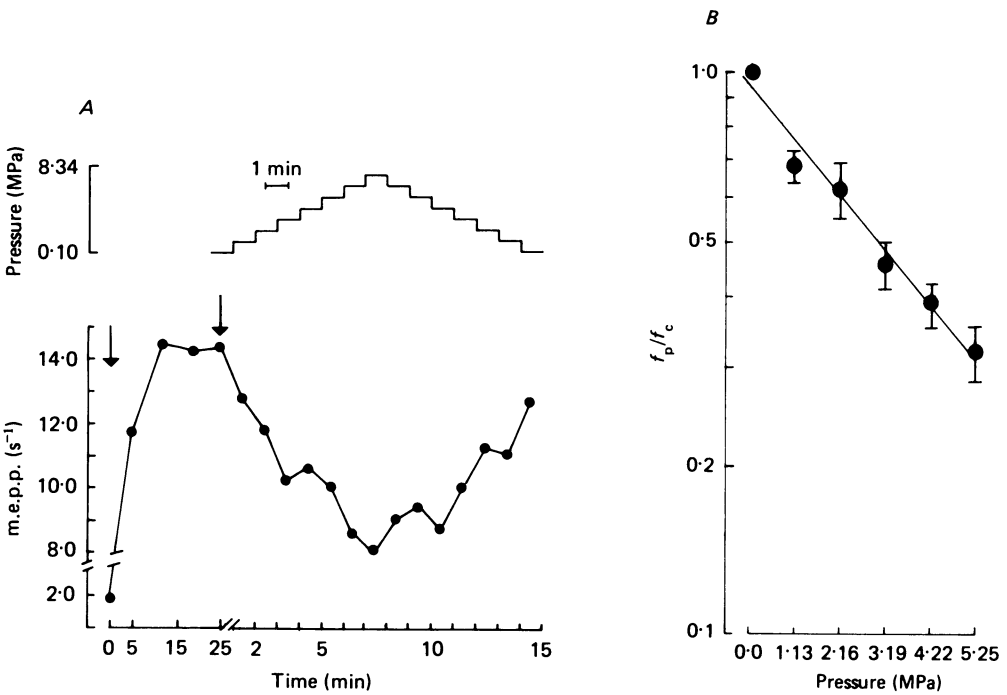

Fig. 2. The depression of the spontaneous quantal release of transmitter by hydrostatic pressure in 10 mM-K^+ Ringer solution. *A*, the effect of hydrostatic pressure on m.e.p.p. frequency in the presence of 10 mM-K^+, added at zero time (denoted by the first arrow). The m.e.p.p. frequency was followed over a period of 25 min before application of pressure. Ordinates: m.e.p.p. frequency (s^{-1}) and hydrostatic pressure (MPa). Note the different time scale commencing at the second arrow. *B*, a semi-logarithmic plot of the effect of hydrostatic pressure on m.e.p.c. frequency in the presence of 10 mM-K^+ Ringer solution. Note the exponential decline in frequency with increasing pressure. Ordinate: m.e.p.c. frequency (f_p) as a ratio of the control frequency (f_c) after 30 min in 10 mM-K^+ Ringer. Abscissa: pressure (MPa). The points show the means of three separate experiments. The line has been fitted by regression analysis ($r = 0·99$); the bar heights represent 1 × S.E.

line noise are shown in Fig. 2*A*. It was found that 10 mM-K^+_o increased the m.e.p.p. frequency over 7-fold in approximately 10 min and high pressure reversibly reduced the m.e.p.p. frequency in the high K^+_o solution (Fig. 2*A*).

It was more convenient, because of the better signal-to-noise ratio, to record continuously the effect of high pressure on m.e.p.c. frequency in 10 mM-K^+_o. Pooled data from three experiments are shown in Fig. 2*B*. High pressure ($\geqslant$ 1·13 MPa)

reduces the m.e.p.c. frequency significantly in 10 mM-K^+_o. Indeed the m.e.p.c. frequency decrease is more marked than in control Ringer solution (cf. Figs. 1*A* and 2*B*) although the difference is not significant ($P > 0.05$). A pressure of 5·25 MPa reduced the m.e.p.c. frequency in 10 mM-K^+_o to 0.32 ± 0.04 ($\bar{x} \pm 1 \times$ S.E., $n = 3$) of the control value.

Release in 6 mM-Ca^{2+}_o and 10 mM-Ca^{2+}_o

The effects of up to 5·25 MPa on m.e.p.p. frequency were tested on six muscles bathed in 6 mM-Ca^{2+}_o and three muscles bathed in 10 mM-Ca^{2+}_o. The control Ringer solution was osmotically adjusted with sucrose to match the high Ca^{2+} solutions. In

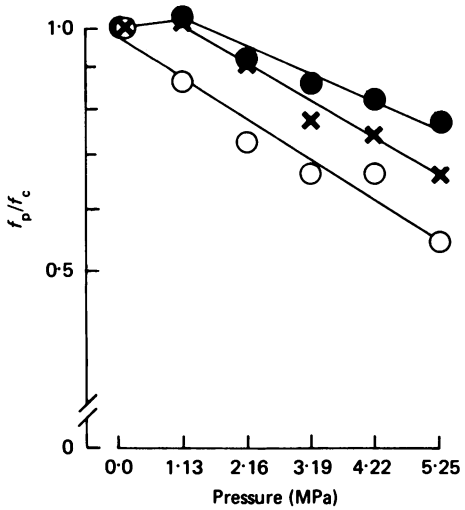

Fig. 3. The reduction of m.e.p.p. frequency by hydrostatic pressure in the presence of raised external Ca^{2+} concentration. Ordinate: m.e.p.p. frequency (f_p) as a ratio of the control frequency (f_c) once the frequency had reached a steady state in the high Ca^{2+} Ringer solution. Abscissa: pressure (MPa). Open circles, control data in 1 mM-Ca^{2+}_o (eight experiments); crosses, data obtained with 6 mM-Ca^{2+}_o (six experiments); filled circles, data obtained with 10 mM-Ca^{2+}_o (three experiments). The lines were fitted by regression analysis ($r \geqslant 0.97$).

some experiments, particularly in 6 mM-Ca^{2+}_o, very little change in frequency was observed on addition of the high Ca^{2+} solution. However, in other experiments in 6 mM-Ca^{2+}_o the frequency increased up to 3-fold. The maximum increase in 10 mM-Ca^{2+}_o was 3·5-fold. High pressure was applied approximately 30 min after addition of the high Ca^{2+} medium when a new steady state had been reached. The effect of pressure did not depend on the base line frequency in the high Ca^{2+} medium. Pooled data from these experiments are shown, along with results obtained in control Ringer solution, in Fig. 3.

Two interesting points emerge. First, in both the 6 mM-and 10 mM-Ca^{2+}_o solutions at 1·13 MPa the mean frequency increases slightly relative to the frequency in high Ca^{2+} at 0·1 MPa. At 1·13 MPa in 10 mM-Ca^{2+}_o this increase was statistically significant ($P < 0.05$). Secondly, the mean m.e.p.p. frequency in 10 mM-Ca^{2+}_o is significantly

higher ($P < 0.05$) than in 1 mM-Ca^{2+}_o for all pressures tested, and the mean m.e.p.p. frequency in 6 mM-Ca^{2+}_o is significantly higher than in 1 mM-Ca^{2+}_o for all pressures except 4·22 MPa. The f_p/f_c ratio at 1·13 MPa is 0.86 ± 0.03 ($\bar{x} \pm 1 \times$ S.E., $n = 8$) in normal Ringer solution, 1.03 ± 0.09 ($\bar{x} \pm 1 \times$ S.E., $n = 6$) in 6 mM-Ca^{2+}_o and 1.07 ± 0.03 ($\bar{x} \pm 1 \times$ S.E., $n = 3$) in 10 mM-Ca^{2+}_o Ringer. Corresponding f_p/f_c ratios at 5·25 MPa are 0.54 ± 0.03 ($\bar{x} \pm 1 \times$ S.E., $n = 8$), 0.66 ± 0.01 ($\bar{x} \pm 1 \times$ S.E., $n = 6$) and 0.77 ± 0.03 ($\bar{x} \pm 1 \times$ S.E., $n = 3$). Clearly, high Ca^{2+} solutions increase the initial 'threshold' pressure required to reduce the spontaneous release of transmitter, with little effect on the sensitivity of the release mechanism to high pressure (compare the differences between the data at 1·13 MPa and 5·25 MPa).

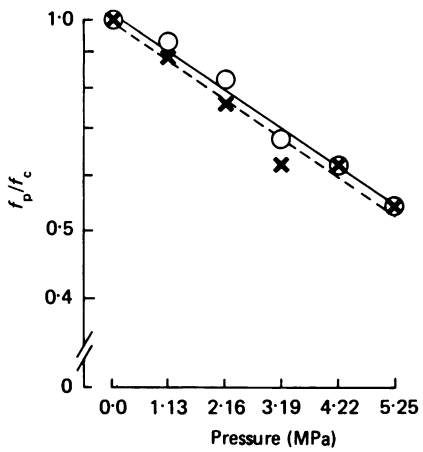

Fig. 4. The effect of hydrostatic pressure on m.e.p.p. and m.e.p.c. frequency in hypertonic Ringer solution (165 mM-NaCl). Ordinate: m.e.p.p./m.e.p.c. frequency (f_p) as a ratio of the control frequency (f_c). Abscissa: pressure (MPa). Note the exponential relationship. Open circles, m.e.p.p. frequency data (three experiments), $r = 0.99$; crosses, m.e.p.c. frequency data (six experiments), $r = 0.98$.

Release in a hypertonic medium

Hypertonic Ringer solution (165 mM-NaCl) is known to increase the spontaneous release of transmitter markedly by a mechanism which does not require Ca^{2+}_o (see Shimoni, Alnaes & Rahamimoff, 1977). The effect of a range of hydrostatic pressures (1·13–5·25 MPa) was thus tested on preparations exposed to such a bathing solution for 30 min. At this time the frequency of release had reached a new steady state. Results from such experiments are shown in Fig. 4. The depression of both m.e.p.p. and m.e.p.c. frequency produced by hydrostatic pressure in the presence of hypertonic Ringer solution is identical to that observed in normal Ringer solution. The m.e.p.p. frequency and m.e.p.c. frequency decrease to 0.54 ± 0.04 ($\bar{x} \pm 1 \times$ S.E., $n = 3$) and 0.54 ± 0.03 ($\bar{x} \pm 1 \times$ S.E., $n = 6$) respectively at 5·25 MPa.

Post-synaptic effects

Effect on extracellular m.e.p.c. parameters

High hydrostatic pressure (10·40–15·55 MPa) produced a small significant increase in the time constant of decay, τ_D, of m.e.p.c.s, the decay phase remaining exponential (Fig. 5). A typical result is shown in Fig. 6*A*. Below 7·82 MPa pressure had no effect; thereafter it produced a linear increase in τ_D. A lengthening of m.e.p.c. decay at pressures greater than 10·40 MPa was observed in fourteen experiments, the mean

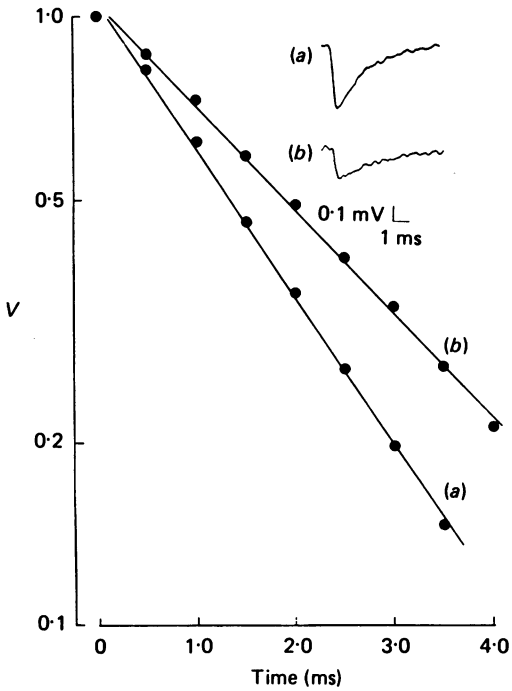

Fig. 5. Semi-logarithmic plot of the decay phase of m.e.p.c.s recorded extracellularly from one end-plate at 0·10 MPa (*a*) and 15·55 MPa (*b*). The straight lines (fitted by regression analysis) illustrate the exponential nature of the current decay. Ordinate: normalized amplitude, *V*. Abscissa: time (ms). The time constants of decay at 0·10 MPa and 15·55 MPa are 1·81 and 2·54 ms respectively. Insets show the currents from which the data are plotted.

increase in τ_D being 35 % at 15·55 MPa. The reversibility of this effect of pressure was not systematically investigated although in initial experiments reversibility was obtained on decompression. Reliable data at pressures higher than 15·55 MPa proved difficult to collect.

Previously it has been reported that the activities of several acetylcholinesterases are inhibited by high pressure (Hochachka, 1974). Inhibition of acetylcholinesterase activity at the neuromuscular junction increases τ_D of m.e.p.c.s. To test whether the increase in τ_D produced by high pressure was due to partial inhibition of acetylcholinesterase activity, several experiments were conducted in the presence of

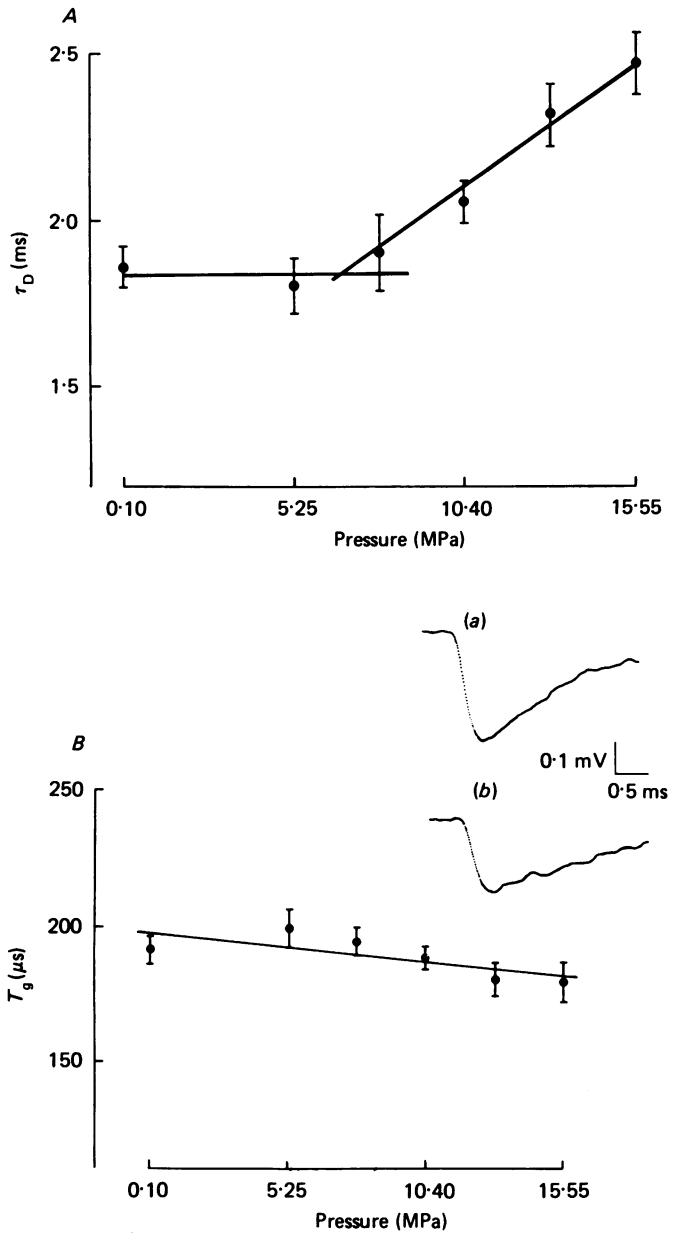

Fig. 6. The effect of hydrostatic pressure on the time constant of decay (τ_D) and the growth time (T_g) of m.e.p.c.s recorded extracellularly.

A, τ_D plotted as a function of pressure; points show the means ± S.E. of fifteen to twenty m.e.p.c.s recorded from a single end-plate. Ordinate: τ_D (ms). Abscissa: pressure (MPa). The data obtained at 7·82 MPa and above are fitted by a regression line, $r = 0·99$.

B, Growth times (T_g) of m.e.p.c.s measured over a range of pressures. Data points show the means ± S.E. of at least forty m.e.p.c.s at each pressure. The continuous line was fitted by linear regression ($r = 0·75$). Ordinate T_g (μs). Abscissa: pressure (MPa). Insets show representative m.e.p.c.s, (*a*) recorded at 0·10 MPa and (*b*) at 15·55 MPa.

tubocurarine ($7{\cdot}5 \times 10^{-7}$ M). Tubocurarine reduces τ_D of m.e.p.c.s lengthened by an anticholinesterase action (see Katz & Miledi, 1973) and it has been suggested that at pressure the action of tubocurarine is enhanced (Gountis-Bonikos, Kendig & Cohen, 1977). The m.e.p.c. decay was, however, lengthened by a similar amount by high pressure in experiments using tubocurarine, suggesting that high pressure was not inhibiting the acetylcholinesterase.

In five experiments pressure (0·1–15·55 MPa) had no effect on the growth phase of extracellularly recorded m.e.p.c.s. These pooled results are shown in Fig. 6*B*, and again suggest that there is no effect of high pressure on acetylcholinesterase activity. The amplitude of extracellular m.e.p.c.s was depressed consistently at high pressure although this was not systematically investigated since there is the possibility that a slight displacement of the electrode tip during pressurization contributes to the reduction. Voltage clamp measurements are currently underway to confirm that a real amplitude depression occurs.

Amplitude histograms of m.e.p.p.s collected at 0·1, 5·25 and 10·40 MPa showed that no shift in the distribution of m.e.p.p. amplitudes occurred at pressure. This was expected in view of the small effect of high pressure on τ_D and the amplitude of m.e.p.c.s.

DISCUSSION

The experiments reported here show clearly that the dominant effect of high pressure on the spontaneous release of transmitter at the amphibian neuromuscular junction occurs presynaptically. In experiments on the pressurized crustacean neuromuscular junction Campenot (1975) concluded from data on the coefficient of variation of the amplitude of the excitatory junction potential that high pressure was acting on the release of transmitter.

In this study the spontaneous release of transmitter is markedly depressed by pressure under a variety of conditions. The finding that high pressure reduces similarly the quantal release of transmitter in experiments where release has been previously increased by a Ca^{2+}-dependent mechanism (high K^+_o Ringer solution) or Ca^{2+}-independent means (hypertonic Ringer solution) might suggest that the target was a basic event or structure essential to the release process occurring at the preterminal membrane.

If we adopt the vesicular hypothesis then current models of vesicular release divide the process into (*a*) assembly of the active zone at the nerve terminal membrane and (*b*) the exocytotic-fusion event (e.g. Kelly, Deutsch, Carlson & Wagner, 1979). The exocytotic-fusion event is the Ca^{2+}-dependent process. Morphological specializations in the presynaptic membrane have been identified and it is suggested that these represent the release sites or active zones (see Heuser & Reese, 1979). Perhaps the assembly of these sites is sensitive to high pressure. Previous work indicates that Ca^{2+} ions isothermally induce a bilayer change creating phase boundaries which are a prerequisite for the fusion event (Marsh, Radda & Ritchie, 1976; Papahadjopoulos *et al.* 1977). The effect of pressure on the endothermic phase transition of bilayers conforms to the thermodynamic relationship $dT/dp = T.\Delta V/\Delta H$ with $dT/dp < 0{\cdot}03$ deg C/0·1 MPa in pure phospholipid liposomes and simple cell mem-

brane bilayers (Wann & Macdonald, 1980). There are no data on the effect of pressure on the more complex Ca^{2+}-induced isothermal transitions, so that it is difficult to predict whether pressure would favour or oppose such transitions. It is interesting that in the experiments reported here, raising the $Ca^{2+}{}_o$ concentration shifted the 'threshold' for the onset of the effect of high pressure on the spontaneous release process to a higher pressure (Fig. 3). This may mean that such phase changes are opposed by pressure. Alternatively it is possible that high pressure somehow increases the energy barrier to apposition of the vesicle and preterminal membrane thus keeping them apart.

The effect of temperature on m.e.p.p. frequency is not linear (Duncan & Statham, 1977), but over the temperature range where the effect is greatest a reduction of 4 °C (i.e. from 25 to 21 °C) is equivalent to 10·40 MPa (i.e. 0·04 °C/0·1 MPa). Over the temperature range where the effect on m.e.p.p. frequency is less (< 20 °C) the dT/dp ratio is even higher. Such a temperature–pressure equivalence is higher than that reported for phase transition behaviour of neutral lipid bilayers at pressure (Wann & Macdonald, 1980). Unfortunately the appropriate dT/dp data do not exist for neuronal membranes, making it difficult to take this argument any further. It would be interesting to compare the effects of high hydrostatic pressure on the evoked release of transmitter with the effects observed on spontaneous release. Due to the high safety factor for transmission it seems unlikely that the evoked release of transmitter could be depressed by high hydrostatic pressure in the range 0·1–10·40 MPa to result in complete failure of neuromuscular transmission. Indeed we have observed that neuromuscular transmission in the frog does not fail at 20·80 MPa (unpublished observations). The precise effects of high hydrostatic pressure on the evoked release of transmitter at the frog neuromuscular junction remain to be investigated. Previous studies show that hydrostatic pressure (7·520 MPa) reduces neuromuscular-transmission in crustaceans (Campenot, 1975) and helium pressure (10·40 MPa) depresses neuromuscular transmission in the rat (Kendig & Cohen, 1976). We suggest that depressed release of transmitter is the major factor causing the depression of transmission. It is still a matter of speculation as to the contribution which such depression of neuromuscular transmission makes to the motor paralysis which is reported in whole animals at somewhat lower pressures. At present the susceptibility of the presynaptic release mechanism of central synapses is unknown. It is not therefore possible to draw any conclusions concerning the relevance of the observed presynaptic effect of high pressure to the disorders of central nervous function reported at high pressure (Brauer, 1975). This too must await further investigation.

The effects of high hydrostatic pressure on post-synaptic events were less marked. The decay phase of the m.e.p.c. was most sensitive, this being lengthened by pressures above 10·40 MPa. One possibility is that the reaction controlling the decay rate is influenced by the microviscosity of the membrane lipid. This proposal is of interest for two reasons. First, it has been suggested previously that in the case of general anaesthetics which produce the opposite effect (i.e. they reduce the decay time) this is achieved by a reduction of the microviscosity of membrane lipids (for a review see Gage & Hamill, 1981). Secondly, it is known that pressure and general anaesthetics can act antagonistically in whole animals, isolated excitable cells and model bilayers

(Miller, 1975; Kendig, 1980). Our results suggest that channel lifetime is increased slightly by high pressure; however, a direct test of this requires measurements of single-channel parameters at pressure. The growth phase of the m.e.p.c. was unaffected over the pressure range studied. This is in agreement with previous experiments with ethanol or variations of temperature which showed that the growth phase and decay phase of the m.e.p.c. are controlled by rather different processes (Gage & McBurney, 1975; Gage, McBurney & Schneider, 1975).

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