J. Physiol. (1981), **311**, *pp.* 325–339
With 7 text-figures
Printed in Great Britain

THE ACTION OF HIGH HYDROSTATIC PRESSURE ON THE MEMBRANE CURRENTS OF *HELIX* NEURONES

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(*Received 20 November 1979*)

SUMMARY

1. The actions of high hydrostatic pressure (10·4, 20·8 MPa) on the membrane currents of *Helix* neurones were examined under voltage clamp.

2. High hydrostatic pressure (20·8 MPa) reduced the maximum inward current to 0·78 and the delayed outward current, measured at the inward current reversal potential, to 0·75 of their value at atmospheric pressure.

3. High hydrostatic pressure shifted the curve relating the inward current conductance to membrane potential to more positive values but the maximum conductance was unaltered.

4. The rates of activation of the inward and delayed outward currents were slowed by pressure.

5. The steady-state level and time course of inactivation of the inward current was unaffected by high pressure over the investigated range.

6. The effects of high hydrostatic pressure on the fast outward current identified in gastropod neurones by Connor & Stevens (1971) were also examined. 20·8 MPa reduced the current measured at −30 mV to 0·71 of its control value.

7. The rate of activation of the fast outward current was slowed by high pressure but the time constant of inactivation was unchanged.

8. The majority of the effects of high hydrostatic pressure were completely reversible upon decompression.

9. These results are discussed with reference to the known effects of high hydrostatic pressure on the action potential and discharge frequency of gastropod neurones. Possible sites and mechanisms of pressure action on the excitable cell are briefly discussed.

INTRODUCTION

Over the last 5 years there have been numerous reports of the effects of either high hydrostatic pressure or helium pressure on the electrical activity of various excitable cell systems. In particular the effects of both hydrostatic and helium pressure have been tested on the action potential of nerve axons and *Helix* ganglion cells (Roth, 1975; Wann, Macdonald & Harper, 1979*a*; Kendig & Cohen, 1977) and the effects of helium pressure have been tested on the action potential of skeletal muscle (Henderson, Morin & Lanphier, 1975). Additionally, the effects of both high

0022-3751/81/3520-0609 $07.50

hydrostatic and helium pressure have been investigated on various aspects of synaptic transmission (Campenot, 1975; Kendig, Trudell & Cohen, 1975; Kendig and Cohen, 1976; Ashford, Macdonald and Wann, 1979; Wann *et al.* 1979*a*).

Many of these studies have no doubt been prompted by the observation that high hydrostatic pressure produces marked motor disturbances in a wide range of animal species (see Brauer, 1975; Macdonald, 1975) and by the expectation that certain marine life must be adapted to its high pressure environment. Also high pressure has often been used to test various hypotheses of anaesthetic action since high pressure can offset the effects of some anaesthetics in whole animals (Lever, Miller, Paton & Smith, 1971; Miller, 1975). At the cellular level high pressure has been demonstrated to offset partially the conduction block produced by some anaesthetics in peripheral nerves (e.g. Spyropoulos, 1957; Kendig & Cohen, 1977), but interpretation of such pressure-anaesthetic interactions will remain obscure until more detailed investigations of the effects of pressure are carried out.

Preliminary voltage-clamp studies on the squid axon have shown that high helium pressure slows the kinetics of the ion currents flowing during the action potential (Henderson & Gilbert, 1975). Comparable data using hydrostatic pressure are lacking.

Previously we have demonstrated that high hydrostatic pressure produces marked slowing of the depolarizing and repolarizing phases of the somatic action potential of *Helix* neurones (Wann *et al.* 1979*a*). We now describe a voltage-clamp analysis of the effects of high hydrostatic pressure on the action potential currents. A preliminary account of some of these results has been reported in Harper, Macdonald & Wann (1977).

METHODS

Experiments were performed with ganglion cells from *Helix pomatia*. Giant cells located on the dorsal aspect of the left and right parietal ganglia of the suboesophageal mass were used. Where possible neurones were identified according to Parmentier (1973). The ganglia were bathed in normal Ringer solution containing (mM) NaCl, 80; KCl 4; $CaCl_2$, 8; $MgCl_2$, 5; Tris-HCl, 5; pH 7·8. The micro-electrodes for intracellular recording and current injection were filled with 3 M-KCl and 2 M-K citrate respectively and had resistances of 2–5 MΩ.

Voltage-clamp circuit. A conventional two intracellular micro-electrode clamp system was employed. Two micro-electrodes with their tips fixed at a distance of 20–80 μm were inserted simultaneously into a neurone. The current passing electrode was wrapped in a grounded platinum foil shield to within 0·5 mm of its tip to minimize capacity coupling. Membrane potential was measured differentially between the 3 M-KCl filled micro-electrode and a Ringer-agar macro-electrode in the tissue bath. The second intracellular electrode was connected to the output of the potential control amplifier (Teledyne-Philbrick 1022). The operating closed loop gain of this amplifier was 3×10^3. With a rectangular command pulse the membrane potential change was 90% complete within 200–500 μsec.

Membrane currents were measured with a virtual ground connected to the bath through a second independent macro-electrode.

In the analysis of the delayed outward current a series resistance (R_s) compensation circuit was included in the clamp system. The input resistance of *Helix pomatia* neurones is 1–20 MΩ which is in parallel with the membrane capacity. There is also a smaller resistance R_s which is in series with the capacity. R_s may be calculated from records of the capacity current (Hodgkin, Huxley & Katz, 1952), and was found to be 10–15 kΩ. This figure consists of contributions from the restricted diffusive space surrounding the cell membrane, the bulk Ringer solution filling the bath and the macro-electrode. The membrane potential error without compensation is $I_m . R_s$ where I_m is the membrane current. The maximum inward current is approximately 3×10^{-7} A, thus an error of 5 mV may result. However the delayed outward current may be as high as 2×10^{-6} A in the

voltage range under study so that an error of 30 mV is possible in this case. It was only feasible to compensate for approximately 80% of the series resistance (R_s) without inducing oscillations in the system. The value of the series resistance was not measurably altered by pressure. However a modest change of this component would be necessary to permit detection (i.e. 10–20%). Voltage and current signals were filtered at 2·5 kHz and recorded on photographic film for subsequent analysis. Current records were corrected for leakage by extrapolation from suitably scaled hyperpolarising pulses.

Pressure apparatus. All experiments were carried out using a horizontally mounted cylindrical pressure vessel of 10·8 cm internal diameter. The design of the vessel and its associated electrophysiological apparatus have been previously discussed (Wann, Macdonald, Harper & Wilcock, 1979*b*). Here it is necessary to emphasize how difficult it was to avoid significant displacement of the micro-electrodes during an experiment. The design of the equipment required the selected neurone to be impaled whilst the recording apparatus was mounted on the laboratory bench. The apparatus was then gently manoeuvred into the pressure vessel. The second vulnerable stage came when the vessel was filled with oil, and finally difficulties were occasionally encountered during pressurization. Displacement of one or both of the recording electrodes during the transfer and filling procedures occurred in approximately seventy per cent of experiments making it impracticable to confine the experiments to precisely identified neurones.

The data reported here are believed to be free from movement artifact for three reasons. First, each individual recording showed no sign of mechanical disturbance (e.g. abrupt changes in behaviour) and secondly, many effects were completely reversible on decompression. Finally many of the measurements of the action potential characteristics and leakage conductance of *Helix* neurones under pressure are identical to those obtained with a smaller pressure vessel of different geometry (Harper, Macdonald & Wann, 1975), in which the mechanical disturbance accompanying compression would be quite different to that occurring in the vessel used in the present investigation.

Hydrostatic pressure (i.e. no gas phase present, the compression medium being light mineral oil, liquid paraffin, B.P.) was applied in steps of 1·03 MPa min^{-1} (101325 Pa = 1 atmosphere or 10 m seawater). The temperature of the pressure vessel was kept constant $\pm 0{\cdot}2$ °C at values between 18 and 22 °C. However the compression rate resulted in a temperature increment of 1·5 °C in the Ringer solution in the tissue bath with pressurisation to 20·8 MPa. This temperature increase was not dissipated until 1·5 hr and the data are therefore corrected where necessary using the appropriate Q_{10} values measured at atmospheric pressure (our own unpublished data). All measurements were taken ten minutes after attainment of pressure. However, data recorded at this time and after thirty minutes at pressure were not significantly different.

The tip potentials of 3 M-KCl filled microelectrodes were measured at pressure. An 80 mM-K isethionate solution of pH 7·5 was chosen to approximate the intracellular medium. The tip potential (normally $\leqslant -5$ mV) increased by $-0{\cdot}38 \pm 0{\cdot}22$ mV ($\bar{x} \pm$ S.E., $n = 4$) at 20·8 MPa. This tip potential change was not corrected for.

For all data means $\pm$S.E. of the mean values are quoted.

RESULTS

Electrical parameters

Leakage currents. These changed linearly with membrane potential over the range -60 to -100 mV. In many instances more negative potentials produced inward going, anomalous rectification. High pressure increases the leakage current in the linear voltage range. Thus 20·8 MPa increases the leakage conductance to $1{\cdot}50 \pm 0{\cdot}04$ ($n = 11$) of its control value. This effect was fully reversible on decompression. This result confirms the observations of Wann *et al.* (1979*a*) in which a comparable decrease of the input resistance of *Helix* neurones was reported for similar pressures.

Input capacitance. The time constant (τ_m) of the neurone was measured under current clamp conditions, the relationship $C_m = \tau_m \cdot G_m$ was used to calculate the input capacitance (C_m) where G_m is the input conductance. The input capacitance

was not significantly altered by pressure being $1{\cdot}03 \pm 0{\cdot}08$ and $1{\cdot}06 \pm 0{\cdot}05$ ($n = 5$) of its control value at 10·4 and 20·8 MPa respectively. In two cells the input capacitance was determined under voltage-clamp conditions by measuring the membrane current during injection of a 20 mV, 10 Hz ramp waveform superimposed on the holding potential. Again no measurable change of capacitance was detected on pressurisation to 20·8 MPa.

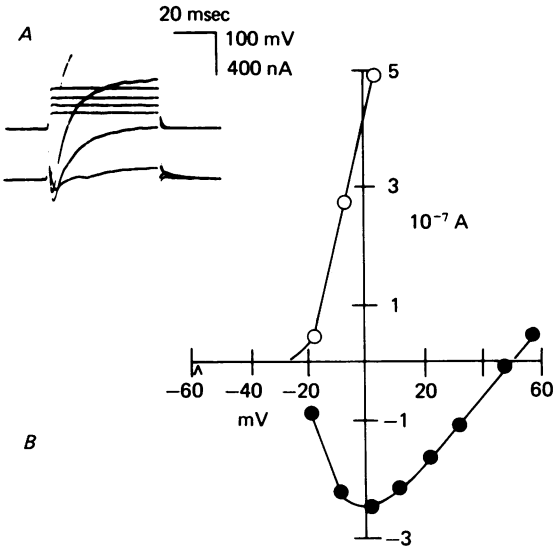

Fig. 1. *A*, membrane currents (lower traces) associated with membrane voltage steps (upper traces) recorded in the oil filled pressure vessel at atmospheric pressure. The initial positive portion of the current record is the tail of the capacitative currents which obscures the rising phase of the early current at positive test potentials. The inward current notch recorded approximately 20 msec after the start of the test pulse to −18 mV is due to the discharge of an action potential in the axon which is electrically remote from the soma and is not adequately clamped (see Standen, 1975). In this and all subsequent records downward deflections represent inward currents. Holding potential −58 mV (arrow in *B*), test potentials ranging from −18 to +47 mV, temperature 20 °C. *B*, full current–voltage relation for the inward (●) and delayed outward current (○) for the same experiment.

Inward and delayed outward currents

Inward currents (I_I) and delayed outward currents (I_O) were examined by holding the membrane potential at its initial resting value. Depolarizing clamp pulses result in the rapid development of a transient inward current followed by a delayed outward current. The time course and magnitude of these currents are a function of both the holding and test potentials. A family of membrane currents associated with increasing voltage steps recorded in the oil filled pressure vessel at atmospheric pressure is shown in Fig. 1*A* together with the full current-voltage relation for the same experiment (Fig. 1*B*). The general characteristics of the currents are similar to those described for squid axon but they have a much slower time course and the outward current dominates the inward current at test potentials positive to zero.

Effect of high pressure on the inward current–voltage relation

High hydrostatic pressure decreased the amplitude of the inward current (see Fig. 2*A–D*, the full current–voltage relationship for the same experiment is shown in Fig. 2*E*). Compression to 20·8 MPa resulted in a significant depression of the maximum inward current to 0·78 ± 0·05 ($n = 11$) of its control value. The apparent equilibrium potential (normally in the range +40 to +50 mV) was not significantly altered by pressure, although in several instances it became less positive by < 5 mV. In four cells in which the ratio inward/leakage conductance was high (> 10) the equilibrium potential was +45·4 ± 2·2 mV and +44·9 ± 2·4 mV at 0·1 and 20·8 MPa respectively.

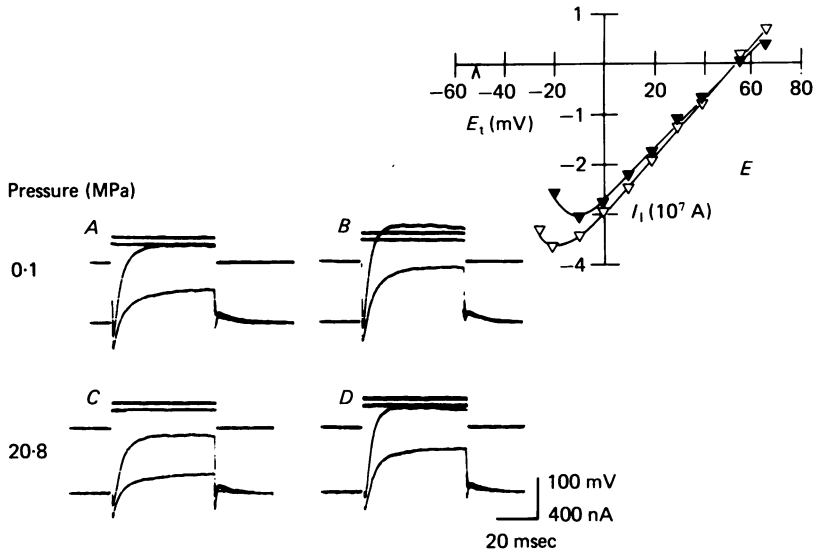

Fig. 2. Membrane currents recorded at 0·1 MPa (*A* and *B*) and 20·8 MPa (*C* and *D*). The test potentials are −1 and +19 mV (*A* and *C*) or +9 and +29 mV (*B* and *D*). RPr −1 neurone (Parmentier, 1973), holding potential −51 mV (arrow in *E*), temperature 22 °C. *E*, peak inward current plotted against test potential (E_t) recorded at 0·1 MPa (▽) and after compression to 20·8 MPa (▼).

The method used for leakage current correction assumes that there is no rectification of this component over the range of potentials studied. A marked increased in leakage conductance at positive potentials has been found by Tillotson (1979). Consequently the 'real' equilibrium potential is probably positive to the value cited here. If the leakage current is increased proportionately at all potentials by pressure the 'real' equilibrium potential will be shifted further in a positive direction. Values for this parameter should be reassessed using the instantaneous current–voltage relationship for the inward current. It was not possible to determine whether pressure caused a shift in the onset of the inward current due to contamination of the current record at low test potentials by axon spikes.

The membrane conductance for the inward current was calculated from

$$G_I = \frac{I_I}{(E_t - E_r)} \quad (1)$$

Hodgkin & Huxley (1952*a*). Where G_I is the membrane conductance at a given test

potential E_t, I_I was the peak inward current, and E_r is the apparent membrane potential at which the inward current reverses its polarity. A typical result is plotted in Fig. 3*A*. In the majority of experiments the maximum conductance of the inward current was not altered by pressures up to 20·8 MPa. However, the conductance–voltage relation was shifted to more positive test potentials.

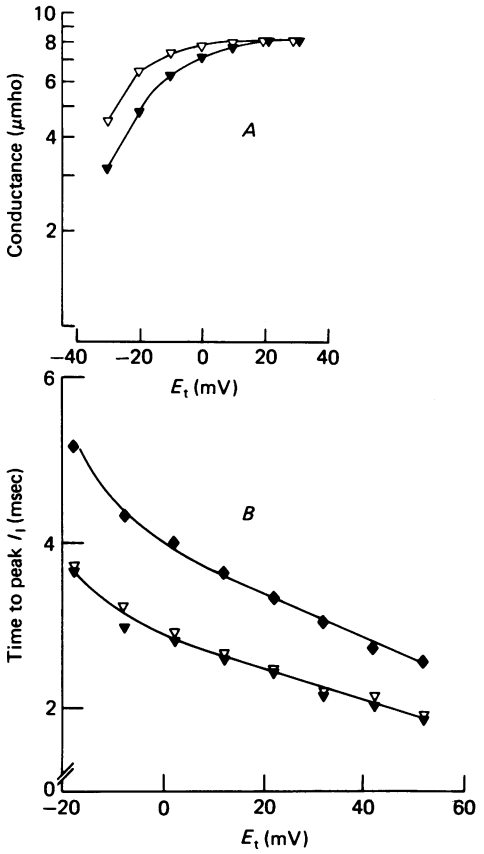

Fig. 3. Action of high hydrostatic pressure 20·8 MPa on the conductance (*A*) and time to peak of the inward current (*B*) for two different cells RPr−1 and LPr−7 respectively. *A*, peak inward current conductance plotted against test potential at 0·1 (▽) and 20·8 MPa (▼), holding potential −45 mV, temperature 18 °C. *B*, time to peak I_I, (▼) and (♦) represent data recorded at 0·1 and 20·8 MPa, (▽) post decompression data. Holding potential −48 mV, temperature 20 °C.

High hydrostatic pressure slows the activation of the inward current (Fig. 3*B*). The time to peak inward current (at zero mV) was increased to $1{\cdot}46 \pm 0{\cdot}06$ ($n = 5$) of its precompression value by 20·8 MPa. These data were corrected for the temperature increment of compression using a Q_{10} of 3 for the reciprocal of the time to peak (unpublished data). This effect of pressure was completely reversible on decompression (see Fig. 3*B*).

Inactivation is unaffected by high pressure

The steady-state inactivation level (h_∞) was examined by pulsing from the holding potential to a varying conditioning pre-potential, which was either positive or negative to the holding potential, for a duration exceeding 5 τ_m (500–1000 msec) before stepping to a fixed test potential which was usually around zero mV. The peak inward current during the test pulse, after conditioning, was expressed as a fraction of the current recorded in the absence of the conditioning prepulse giving the ratio

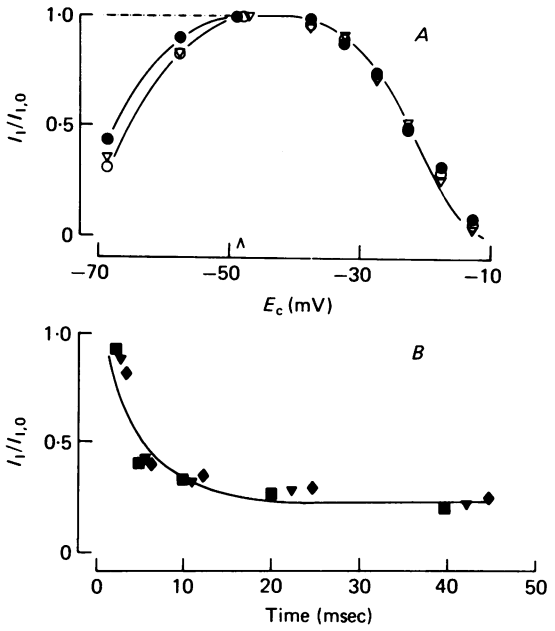

Fig. 4. *A*, the steady-state inactivation curve for the inward current at 0·1 (○) and 20·8 MPa (●), (▽) represent post-decompression data. Ordinate, the ratio of the peak inward current with and without a conditioning prepulse (I_I and $I_{I,O}$ respectively). Abscissa; the potential of the conditioning prepulse (E_c). The duration of E_c was 500 msec, holding potential −48 mV, test potential +2 mV. The continuous curve was drawn to eqn. (2) with E_h −25 mV, k_h 4 mV, temperature 20 °C. LPr-7 cell. *B*, the time course of inactivation of the inward current at 0·1 (■), 10·4 (▼) and 20·8 MPa (◆). E_c −20 mV, holding potential −45 mV, temperature 20 °C. The data are corrected for the temperature increment of compression using a Q_{10} of 3. The continuous line was drawn to

$$I_I/I_{I,O} = (I_I/I_{I,O})_\infty - [(I_I/I_{I,O})_\infty - 1] \exp(-t/\tau_h),$$

Hodgkin & Huxley (1952*b*), where $(I_I/I_{I,O})_\infty$ is the ordinate at time $t = \infty$ and τ_h is the time constant (5 msec).

$I_I/I_{I,O}$. The plot of this ratio against the conditioning potential (E_c) gives the steady state inactivation curve. The inactivation level was not altered by high pressure (Fig. 4*A*). The continuous line was drawn to

$$I_I/I_{I,O} = \left[1 + \exp\frac{E_c + E_h}{k_h}\right]^{-1} \quad (2)$$

Hodgkin & Huxley (1952*b*).

E_h is the conditioning potential at which $I_I/I_{I,O} = 0{\cdot}5$, k_h is a shape factor. A best fit was obtained using a value of 4 mV for k_h. E_h was measured as $-27{\cdot}4 \pm 1{\cdot}1$ and $27{\cdot}6 \pm 1{\cdot}5$ mV at 0·1 and 20·8 MPa respectively in five cells. As reported by Standen (1975) *Helix* neurones exhibit maximal removal of inactivation at normal resting potentials.

In these experiments a complication arises when hyperpolarizing prepulses are applied due to the activation of a fast outward current, described by Connor & Stevens (1971) and Neher (1971) for Doridiae and *Helix* neurones respectively. This current will sum algebraically with the inward current causing a reduction in the ratio $I_I/I_{I,O}$. It is of interest that this depression is decreased by pressure (Fig. 4*A*). This effect was observed in all four cells in which the steady state inactivation was examined at conditioning potentials negative to holding. This result implies that the amplitude of the fast outward current at the time of peak I_I is reduced by high pressure. The action of high pressure on this current will be described more fully in a later section.

The time course of inactivation of the inward current was studied by stepping to a fixed conditioning potential of -20 mV for a varying duration before pulsing to the test potential (zero mV). The result of such an experiment is shown in Fig. 4*B*. For the two cells examined the time course of inactivation was unaltered by pressure 20·8 MPa.

In some instances the amplitude of the inward current did not fully revert to precompression values upon decompression, even after several hours. This behaviour was also observed in the somatic action potential of *Helix* neurones (Wann *et al.* 1979*a*). However the leakage conductance and membrane potential normally showed full recovery and thus it seems unlikely that this effect is due to damage of the cell membrane, and the 'ageing' of the inward current described by Neher (1971) may contribute. Similar problems of lack of full reversibility have been reported by Henderson & Gilbert (1975).

Pressure action on the delayed outward current

The development of the delayed outward current frequently showed an abrupt shoulder on the rising phase, this in conjunction with the complexity of the tail currents has been suggested to be due to two kinetically distinct components (Meech & Standen, 1975). The faster component is similar to the delayed K current in squid giant axon, the slower component is a Ca dependent potassium current. These currents have a distinct pharmacology (Meech & Standen, 1975; Thompson, 1977).

Pressure application decreases the maximum outward current over a range of test potentials (Fig. 5, see also Fig. 2). In 8 cells the maximum outward current recorded at $+40$ mV was reduced to $0{\cdot}75 \pm 0{\cdot}03$ of its precompression value by 20·8 MPa. This decrease was in most cases fully reversed on decompression. In a previous communication (Harper *et al.* 1977) we reported that the outward current was not significantly altered by pressure. This difference in behaviour is due to measurement of I_O at the inward current null potential ($+40$ mV) in the present investigation compared to zero mV in the preliminary report.

The amplitude of the after potential of the somatic action potential in *Helix* neurones is increased by pressure, increasing by $3{\cdot}1 \pm 1{\cdot}2$ mV ($n = 12$) with 20·8 MPa (our own unpublished observations). This behaviour accompanies the membrane potential depolarization occurring on compression ($8{\cdot}3 \pm 2{\cdot}5$ mV) for the same cells. It may thus be argued that the outward current equilibrium

potential is not markedly altered, implying that the outward current conductance is decreased by pressure. This behaviour contrasts with that of the K current in squid giant axon which is unaffected by high pressure (Henderson & Gilbert, 1975).

The logarithm of the difference between the maximum outward current (measured at +40 mV), corrected for leakage and its value at earlier times was plotted against time as described by Moore, Ulbricht & Takata (1964). The slope of the linear portion of this plot was used to calculate the time constant of turn on of the outward current, which 20·8 MPa increased to $1{\cdot}64 \pm 0{\cdot}02$ ($n = 3$) of its precompression value.

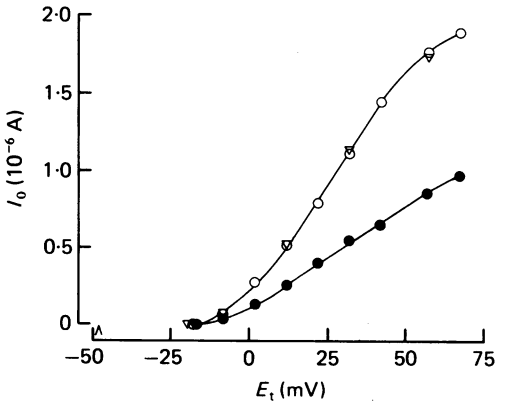

Fig. 5. The effect of pressure on the delayed outward current–voltage relation, LPr−7 cell. Abscissa, test potential; ordinate, maximum outward current measured during the 100 msec test pulse. (○), 0·1 MPa; (●), 20·8 MPa (▽) decompression values. Holding potential −48 mV (indicated by arrow), temperature 20 °C.

Fast outward current

Voltage-clamp analyses of the currents in gastropod somatic membrane have revealed that in addition to the transient, early, inward current and delayed outward current characteristic of squid giant axon there exists a fast transient outward current (Connor & Stevens, 1971; Neher, 1971; Gola & Romey, 1971). The latter current (I_A), which is carried by potassium ions is operationally distinct from the late outward current (I_O). The I_A component is inactivated at normal resting potentials but can be studied by stepping from hyperpolarized holding potentials to test potentials negative to −20 mV (Thompson, 1977). The current I_A rises rapidly to a peak amplitude which is dependent on both holding and test potentials (Fig. 6*A* and *B*) and then decays much more slowly towards zero. The decay phase is exponential and is characterized by a single time constant which was found to be approximately independent of membrane potential over the range investigated.

This result agrees with the findings of Connor & Stevens (1971) and Gola & Romey (1971). However Neher (1971) has shown that the time constant of decay of I_A is strongly voltage dependent. The discrepancies in these reports may be due to differences in the current separation procedures. In this study no measurable delayed outward current was recorded on stepping to test potentials negative to −20 mV from the normal holding potential. However a shift of inactivation level of the delayed outward current when holding at a hyperpolarized potential may result in contamination of I_A by I_O.

The action of high pressure on I_A is analogous to its effect on I_I

High hydrostatic pressure depresses the peak amplitude of I_A. Fig. 6*C* shows the effect of 20·8 MPa on the current–voltage relation of the fast outward current. In two cells 10·4 and 20·8 MPa decreased the peak amplitude of I_A to 0·87 and 0·80 and 0·72 and 0·70 of the control values respectively at a test potential of −30 mV.

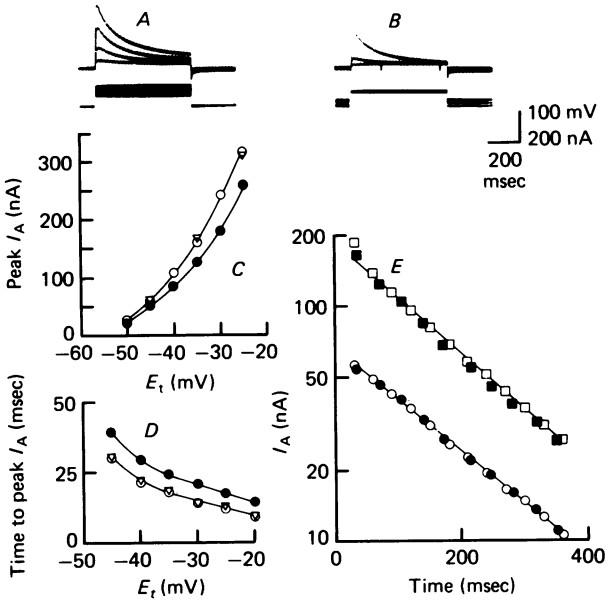

Fig. 6. *A*, fast outward currents associated with depolarizing voltage steps from −95 mV to clamping potentials from −55 to −25 mV at 10 mV intervals. *B*, voltage steps to −25 mV from varying holding potentials (−70, −60, −50 mV). *C*, current–voltage relation of I_A recorded at 0·1 (○) and 20·8 MPa (●), (▽) recovery values. *D*, effect of high pressure on the time to peak I_A. Data at 0·1 and 20·8 MPa described by (○) and (●) respectively, (▽) post-decompression data. *E*, semilogarithmic plot of the decay phase of I_A currents recorded at 0·1 and 20·8 MPa open and filled symbols respectively. Currents recorded on stepping from the holding potential to −45 mV (○, ●) and −30 mV (□, ■) are plotted. Data are corrected for the temperature increment of pressurization, assuming a Q_{10} of 2·4 for the time constant of inactivation (Neher, 1971). Holding potential for *C–E*, −95 mV, temperature 20 °C.

In order to gain a simple measurement of the kinetics of the activation of I_A during a test pulse, the time between the onset of the pulse and the peak outward current was measured and plotted as a function of membrane potential. This process was slowed by pressure (Fig. 6*D*). Increasing pressure to 20·8 MPa increased the time to peak at −30 mV to approximately 1·5 of its control value in the two cells investigated. In contrast the time constant of inactivation of I_A was not altered by pressure (see Fig. 6*E*). All effects of pressure were entirely reversible upon decompression.

A measurement of the voltage sensitivity of I_A activation (a_∞) was gained by extrapolating the exponential decay phase backward to the onset of the test pulse,

as described by Neher (1971), the calculated peak amplitude is plotted in Fig. 7. The *A* current activates at approximately −60 mV and increases sharply with depolarization. The steady-state inactivation function (b_∞) was generated by stepping to a fixed test potential (−25 mV) from more negative holding potentials. Measured peak amplitudes were expressed as a fraction of the maximum current recorded on pulsing from a very negative holding potential (Fig. 7). The continuous line was drawn

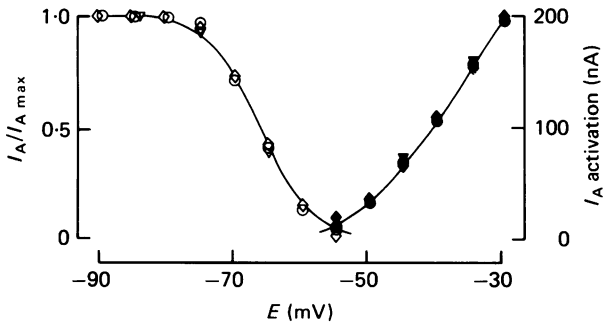

Fig. 7. Steady-state activation (a_∞) and inactivation (b_∞) of I_A, open and filled symbols respectively. (○, ●) represent data at 0·1 MPa, (◇, ◆) show data at 20·8 MPa, (▽, ▼) are post-decompression values. *E* represents the test potential and conditioning potential for a_∞ and b_∞ curves respectively. The continuous line for b_∞ was drawn to eqn. (2) with $E_h = -66$ mV, $k_h = 4$ mV. Holding potential for a_∞ −95 mV, test potential for b_∞ −25 mV.

to eqn. (2) where E_c was the holding potential. E_h was measured as $-68{\cdot}7 \pm 1{\cdot}6$ mV in four cells at atmospheric pressure. A value of 4 mV for k_h was again found to provide the best fit for the data. These data are in good agreement with the results of Neher (1971). High pressure, 10·4 and 20·8 MPa, did not alter the steady-state inactivation of I_A in the two cells studied. The potential for half inactivation (E_h) of the latter at 20·8 MPa was $-69{\cdot}0 \pm 2{\cdot}8$ mV.

DISCUSSION

The results of this study provide the most detailed voltage-clamp analysis of the effects of high hydrostatic pressure on the ionic currents of an excitable cell. In particular we are now in a position to describe more fully the effects of high pressure on the action potential. Our data have been recorded 10 and 30 min after attaining the selected pressure to ensure that the electrical behaviour of the neurone was stable and to avoid earlier temperature transients. The membrane currents recorded at these times were not different so that it seems likely that we are measuring a 'steady-state' effect of pressure. However, a study of the immediate effect of pressurization is obviously of importance.

High hydrostatic pressure slows the rate of depolarization and repolarization of the somatic action potential in *Helix* neurones. If it is assumed that the maximum inward current is primarily responsible for the peak depolarizing rate of the action potential the latter may be approximated by $dE/dt = -C_m . I_I$. Indeed the decrease

in the maximum inward current measured in this study is in good agreement with the reduction in peak depolarization rate of the action potential reported previously (Wann *et al.* 1979*a*). These parameters are reduced to 0·78 and 0·76 respectively of their control values by 20·8 MPa. A similar decrease of the inward sodium current and the depolarizing rate of the action potential has been shown in squid giant axon using comparable helium pressure (Henderson & Gilbert, 1975; Henderson *et al.* 1977). Likewise the decreased rate of activation of the inward current observed in *Helix* neurones has also been reported in squid giant axon (Henderson & Gilbert, 1975; Shrivastav, Parmentier & Bennett, 1978). Our results suggest that the time constant of inactivation and level of steady-state inactivation were unaltered by high pressure.

The somatic membrane of gastropod neurones possesses a complex inward current, comprising two additive components carried by sodium and calcium ions (Standen, 1975; Kostyuk & Krishtal, 1977). The relative contributions of these currents to the total varies between individual neurones. In addition, these currents have a distinct pharmacology and kinetics (Kostyuk & Krishtal, 1977). Thus, any similarity between the behaviour of the inward and sodium currents of *Helix* neurones and the squid giant axon respectively may be entirely fortuitous. Additionally, the presence of a poorly clamped axon process (e.g. Fig. 1*A*) complicates the measurement of the soma membrane current and it is likely that the recorded membrane current consists of significant contributions from both soma and axon. The measured membrane currents may thus be of limited value as an indication of inward current flow in the soma. A series resistance compensation was only applied in the measurement of the delayed current. In the results shown in Figs. 2 and 3*A* the maximum inward current was reduced by 60 and 70 nA respectively by 20·8 MPa. This would result in a maximum differential voltage sensing error of $\sim$ 1 mV. The voltage sensitivity of the time constant of inactivation of the inward current (τ_h) is steepest at -20 to -30 mV (Neher, 1971), and a small error in voltage sensing caused by lack of series resistance compensation may therefore be masking a small slowing effect of pressure on the kinetics of inactivation. The method used for leakage current correction assumes no rectification of this component. In addition to complicating the measurement of the early, inward, current equilibrium potential and conductance measurements this will also affect the measurement of the inactivation of this current. The above factors may contribute to the finding that high pressure is apparently differentially affecting the activation and inactivation processes. Further study is therefore required to resolve this point.

The capacitance data suggest that no changes in the membrane thickness or surface area occur at pressure. Consequently it seems unlikely that the shift of the conductance–voltage relation or the decrease in the rate of activation of the inward current is due to an increase in the transmembrane field profile produced by a change in the membrane geometry.

The current–voltage relationship of the delayed outward current was depressed by high pressure. The behaviour of the after potential indicates that the equilibrium potential was not markedly changed by pressure (our own unpublished observations). Thus in contrast to the potassium current in the squid giant axon the conductance of the delayed outward current in *Helix* neurones is decreased by pressure. Although the decrease in peak inward current may be due primarily to slowed activation, a simple reduction in the rate of activation of the outward current cannot account for the observed decrease in conductance.

The effect of pressure on the fast outward current of gastropod neurones has not previously been studied. The steady state inactivation level of I_A was apparently unaffected by high pressure (like the inward current). Similarly the rate of activation of I_A was slowed and the kinetics of inactivation unaltered. The decrease in amplitude of the fast outward current resulted from the combination of the above effects. This

together with the simultaneous increase in leakage conductance and resultant small depolarization would increase the discharge rate of *Helix* neurones in response to constant depolarizing current and also increase pacemaker activity. Indeed high hydrostatic pressure has previously been shown to increase the firing frequency of *Helix* pace-maker neurones, frequency typically trebling with compression to 20·8 MPa (Wann *et al.* 1979*a*).

A reduction of the Ca component of the inward current will decrease the Ca dependent potassium current described by Meech & Standen (1975). In addition, I_A may also be Ca dependent (Standen, 1974). A possible mechanism for pressure action on the outward currents may thus be suggested, whereby a decrease of the inward Ca conductance results in a decrease of the fast outward and delayed outward K currents. Clearly the effects of pressure on the inward Ca current require to be investigated before pursuing this argument further.

The problem of distinguishing the direct effects of high pressure on the excitable membrane channels from possible alterations in the membrane environment is at present difficult. In our experiments it was not possible to analyse the immediate effects of compression or decompression, consequently we do not know whether changes in intracellular ionic concentration are occurring during the first 10 min. The effects of high pressure on subcellular organelles, e.g. mitochondria are not known and there are few data on the metabolic effects of the range of pressures used in this study (Wann & Macdonald, 1980). Indeed the metabolic status of excitable cells at pressure remains to be investigated. If changes in ionic balance do occur these would require to be completed in 10 min since the 'steady-state' behaviour of the membrane currents between 10 and 30 min post-compression is inconsistent with slow changes in ionic balance.

Continuous recording of miniature end-plate potential frequency at the amphibian end-plate during pressurisation shows that high pressure reduces markedly the release of transmitter (Ashford *et al.* 1979). No transient increases in frequency were observed as would be expected if a rapid change in internal pH or Ca^{2+} ion concentration occurred (Baker & Crawford, 1975; Cohen & Van der Kloot, 1976). Further studies are required however to assess whether rapid changes in pH or Ca_i^{2+} occur in *Helix* nerve cells at pressure. A change in the internal surface potential caused by for example a rise in pH could explain the shift in the current–voltage relation, or the increase in time to peak, of the inward current. However, the apparent lack of shift of the inactivation curve raises the possibility that high pressure is affecting a variety of different cellular reactions.

Molar volume changes accompanying conformational and other molecular reactions determine the primary susceptibility of a system to pressure. Clearly more data are needed to specify precisely how high pressure exerts its effects.

This work was supported by S.R.C. grants to A. G. M. and K. T. W. The authors acknowledge the technical assistance of Mrs P. Geraghty and Mr A. Simpson.

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