Ionic currents in vertebrate myelinated nerve at hyperbaric pressure

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KENDIG, JOAN J. *Ionic currents in vertebrate myelinated nerve at hyperbaric pressure.* Am. J. Physiol. 246 (Cell Physiol. 15): C84–C90, 1984.—To establish a base line for a study of anesthetic-pressure antagonism in axons, voltage-clamped nodes of Ranvier from amphibian sciatic nerve were subjected to pressures of 1–100 atm. Over the time of compression, there was usually an irreversible decrease in peak inward sodium current, but there was no change in peak outward sodium current or in the current-voltage relationship. The steady-state inactivation-voltage curve was shifted 5–15 mV in the depolarizing direction at 70–100 atm. The rate of rise of the sodium current was slowed, as was the time constant of inactivation (τ_h). Increase in τ_h was markedly voltage dependent, suggesting a selective effect of pressure on β_h, the rate constant governing development of the inactive state. The rate of development of steady-state outward potassium current was also decreased, without significant change in maximum current. The effects of pressure are qualitatively similar to, but different in detail from, those reported in squid axon and different in some details from the effects of cooling in this preparation. None of the effects can presently be related to the high-pressure nervous syndrome.

node of Ranvier; sodium currents; potassium currents; hyperbaric pressure; high-pressure nervous syndrome

PRESSURE, like temperature, is a fundamental physical variable. In comparison with temperature, however, pressure has been relatively little used as a tool in the investigation of biological processes. Pressure is of particular interest in the study of excitable cells, both because of the constellation of symptoms observed in humans and other vertebrates exposed to high pressure [high-pressure nervous syndrome (HPNS)] (10, 11, 14) and because of the antagonistic interaction between hyperbaric pressure and anesthetic agents (1, 12, 15, 16). In neither case is the cellular basis of the phenomenon clear. In previous studies, we have observed that pressure to 200 atm slightly decreases the amplitude of the compound action potential of vertebrate nerves, slows conduction, and decreases the rate of change of both the rising and the falling phases of the action potential (19, 22). In crustacean peripheral nerve, a small portion of the heterogeneous axon population displays increased excitability in the form of repetitive impulse generation; otherwise, excitability is decreased (21). Studies on the squid giant axon show that pressure slows the kinetics of both sodium and potassium currents (5, 6, 13). In studies on the antagonism between pressure and anesthetic agents, we (19, 22) and others (25) have observed an antagonism between pressure and various anesthetic agents on the conducted action potential. The present studies on the effects of hyperbaric pressure on ionic currents were motivated by the desire to understand the basis for the antagonism. Some of the work has been presented in abbreviated form (17, 18).

METHODS

Preparation. The node of Ranvier of amphibian (*Rana catesbeiana* or *Xenopus laevis*) sciatic nerve axon was arranged for voltage clamping by a technique similar to that previously described (7, 20). The node with its adjacent internodes was dissected in standard frog Ringer solution (in mM): NaCl 114, KCl 2.4, $CaCl_2$ 2, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer 10, pH adjusted to 7.4 and mounted in a Vaseline gap voltage clamp chamber of the Dodge and Frankenhaeuser type. The solution in contact with the cut internodes was 120 mM KCl buffered with 20 mM HEPES, pH adjusted to 7.4. The electronics of the clamp system were substantially similar to those we have used in previous studies at normobaric pressures (7, 20). Modifications were made in the configuration of the electrodes in contact with the solution and in the dimensions and position of the voltage follower stage, to allow for positioning in the cylindrical 1.8-liter pressure chamber (Figs. 1 and 2).

Temperature control. Of special circumstances in the hyperbaric environment that might produce artifacts, temperature changes resulting from compression and decompression are probably the most important. This is of particular significance in these experiments, because the rate constants governing ion channel configuration changes have large temperature coefficients, with rate of change with 10°C increase values (Q_{10}'s) in a range between 1.8 and 3 (9). In the Vaseline gap technique, the node necessarily is at or near the surface of a relatively small volume of solution and thus is exposed to the large heat of compression of the gas phase as well as to temperature changes in the aqueous phase. Temperature of the solution around the node was controlled by a Peltier thermoelectric device beneath the Plexiglas holder (Fig. 2). The solution temperature was maintained at approximately 10°C, a standard temperature for this preparation and one that permits good resolution of ion channel time constants. Temperature was monitored by a thermistor in the pool containing the node. An addi-

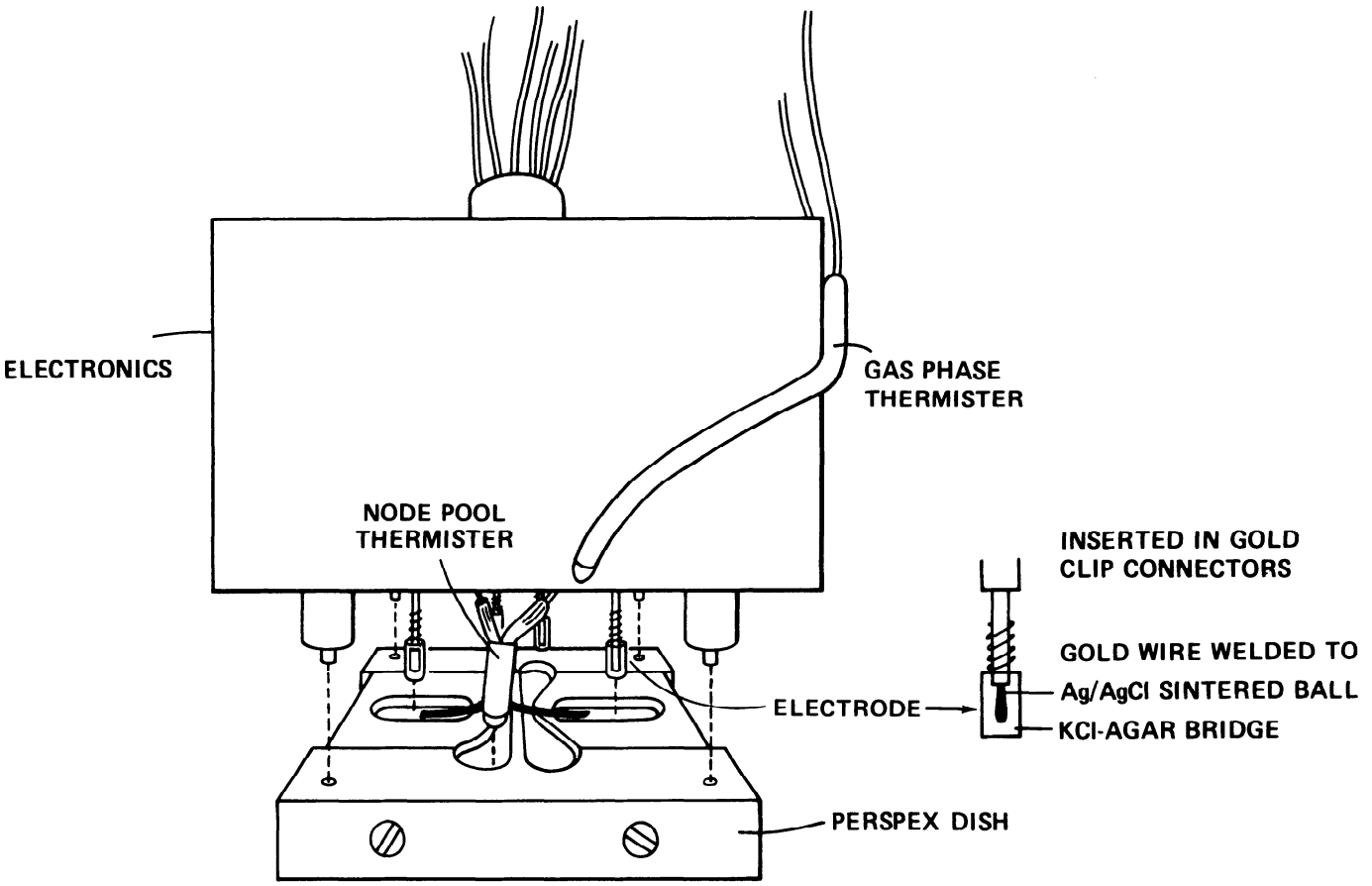

FIG. 1. Voltage follower stage and electrode interface with the Vaseline gap voltage clamp chamber. Electrodes in contact with pools were 3 M KCl-agar-filled glass sleeves fitted over sintered Ag-AgCl electrodes. The latter were welded or crimped to gold pins inserted into sockets in amplifier head. Thermistors in gas phase and in solution near node are shown; an additional thermistor is within brass casing enclosing amplifier.

tional thermistor was positioned in the gas phase above the node (Fig. 1). Measurements were made following compression when both thermistors recorded temperatures within 0.25°C of the 1-ATA control reading, to ensure that the temperature at the node itself had not changed. Temperature within the brass block enclosing the voltage follower electronics was monitored by a separate thermistor and maintained within 2°C of control temperatures. During compression and decompression, transient temperature changes were limited to ±3°C in the aqueous phase and ±5°C in the gas phase.

Pressurization. Compression was carried out by admitting helium from a commercial cylinder pressurized to 4,000 psi, at a rate slow enough to maintain temperature changes within the above limits. Compression to 100 atm, and temperature stabilization at that pressure, required approximately 45 min. Decompression was carried out by venting the chamber at a similarly controlled rate.

Voltage clamp measurements. Sodium and potassium currents were studied in isolation either by treating the preparation with tetraethylammonium chloride to block potassium channels or by substituting choline for sodium in the Ringer solution to block inward sodium currents. Leakage current was electronically subtracted from all records. Sodium and potassium current-voltage curves were established by imposing a range of depolarizing test pulses after a 50-ms prepulse at which the Hodgkin-Huxley inactivation constant (h) was set equal to 1 (sodium) or from an arbitrary −80 mV holding potential (potassium). Sodium current amplitude was measured as peak transient current and potassium as steady-state outward current. The sodium channel inactivation-voltage curve was determined by imposing 50-ms prepulses of variable voltage before a standard test pulse. The rise time of the sodium current was measured at two-thirds of the maximum amplitude ($\tau_{2/3}$), over the same potential range as the current-voltage curves. The time constant of sodium channel inactivation (τ_h) was extracted both by measuring the time required for the sodium current to decline $1/e$ the distance between peak and final values and by a three-pulse protocol in which a long conditioning pulse is followed by a pulse of variable voltage and duration and then by a test pulse. Although inactivation has been shown not to be a single exponential process (3), only a limited attempt was made to separate time constants.

Decompression. Measurements following at least partial decompression were achieved in all the nodes reported in this study. In all, data were available from eight nodes that underwent full compression-decompression cycles between 1 and 70 or 100 atm. The data were consistent with those from a further 21 nodes for which information was only available at 35 atm or which did not survive decompression.

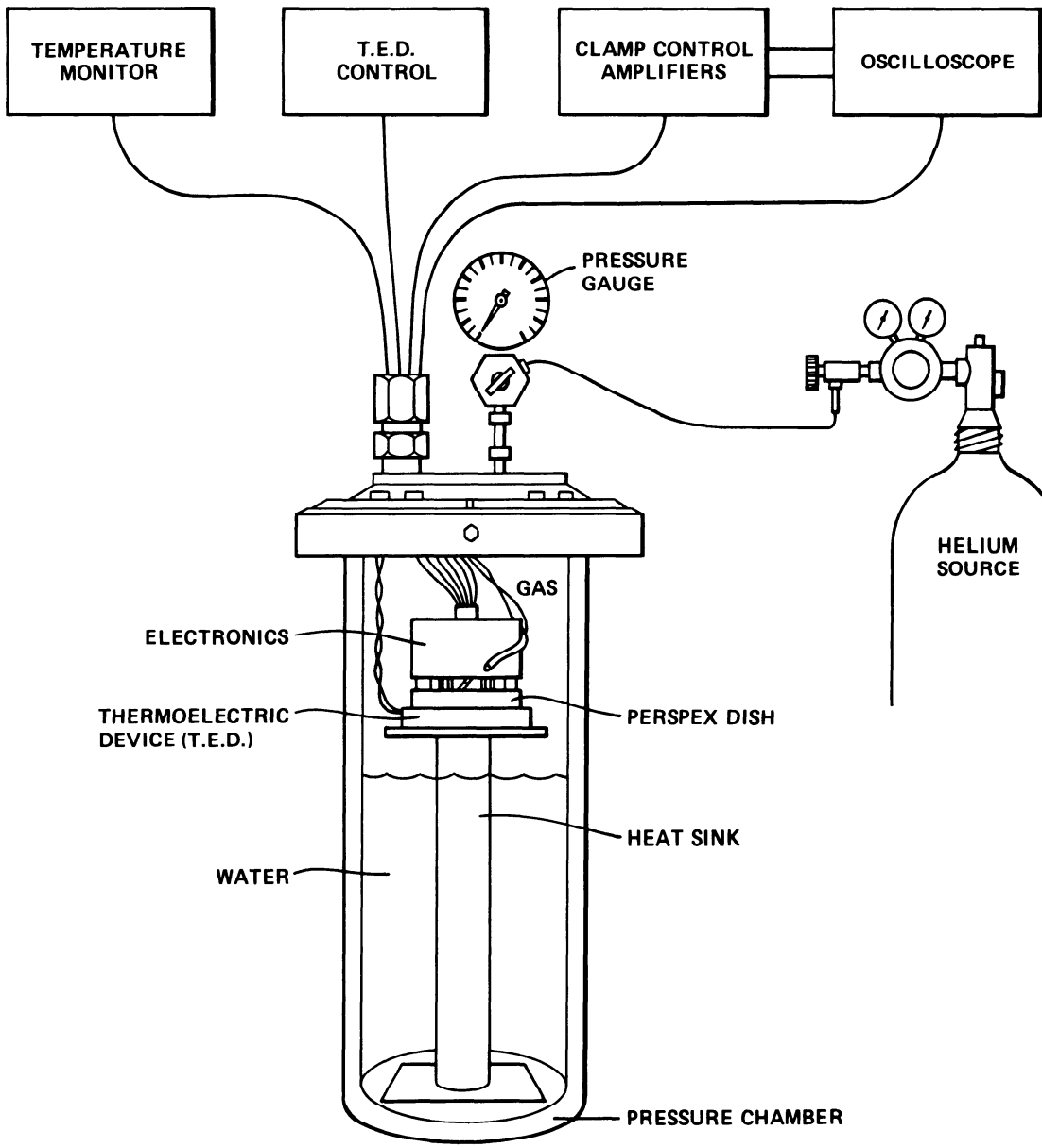

FIG. 2. Arrangement of clamp chamber within pressure vessel. Temperature is controlled by a thermoelectric device (TED) below clamp chamber, with a heat sink extending into water, which partially fills chamber. Helium is admitted from a cylinder to a predetermined pressure monitored by a gauge on chamber lid. Electrical connections are made through a Conax fitting on lid.

RESULTS

Helium pressure and sodium currents. The most striking effect of compression is a marked decrease in the rate of rise and decline of sodium currents (Fig. 3, *A* and *B*). On compression, there was usually some loss in peak inward sodium current amplitude, which was not reversible on decompression, but there was no consistent shift in the current-voltage relationship along the *X*-axis (Fig. 3*C*). Maximum outward sodium current showed no consistent change, and there was no consistent change in the sodium current reversal potential.

The curve relating steady-state inactivation (h_∞) to voltage was consistently shifted to the right, in the depolarizing direction (Fig. 4), a shift that was reversible on decompression. The magnitude of the shift varied from 5 to 15 mV at pressures of 70–100 atm. Thus for any given voltage at which h_∞ was less than 1, more sodium channels were available to conduct at hyperbaric than at normobaric pressures.

The rise time of the sodium current and τ_h were both increased at high pressures (Figs. 5 and 6). The pressure effect on the rise time was relatively independent of voltage over the range in which it was measured, i.e., potentials 30–150 mV positive to the holding potential. There was, however, a marked voltage dependence of the pressure effect on τ_h over the wider range of potentials at which this measurement was made (Fig. 6). At hyperpolarized potentials, there was little effect of pressure on τ_h, but a striking increase at more depolarized potentials. At the most positive potentials examined, around 150 mV positive to the holding potential, τ_h was two- to threefold greater at pressures of 70–100 atm than τ_h at 1 atm. There was a relatively smaller change at potentials

close to the holding potential, with no consistent change at very negative potentials. Because inactivation is a complex process, it was of interest to compare the effect of pressure on early and late phases of sodium current decay. When the decay of the sodium current was plotted on a logarithmic scale as a function of time, the effect of pressure did not appear to be consistently time dependent. It therefore does not appear that pressure selectively affects one step in inactivation more than another to any great extent.

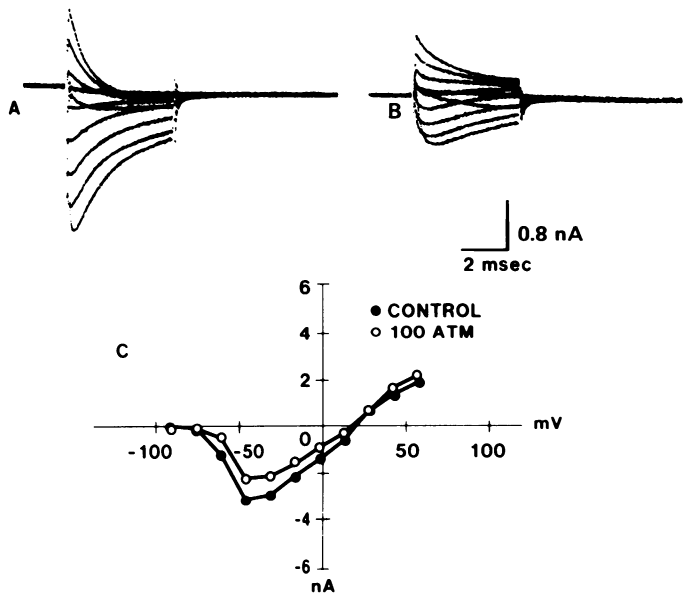

FIG. 3. Sodium currents at 1 (*A*) and 100 atm (*B*). Response to a series of depolarizing voltage steps of 7-ms duration at 15-mV increments from a holding potential of 83 mV. Potassium channels blocked with tetraethylammonium. Node temperature is 9.5°C. Currents are slowed at all potentials. Leakage current, electronically subtracted, did not change on compression. Effect of pressure on current time course was partially reversed on decompression. Node 1.11.82. Current-voltage relation shape for a different node (*C*) was not altered by compression, although currents were smaller after compression. Node 11.16.81, temperature 8.5°C.

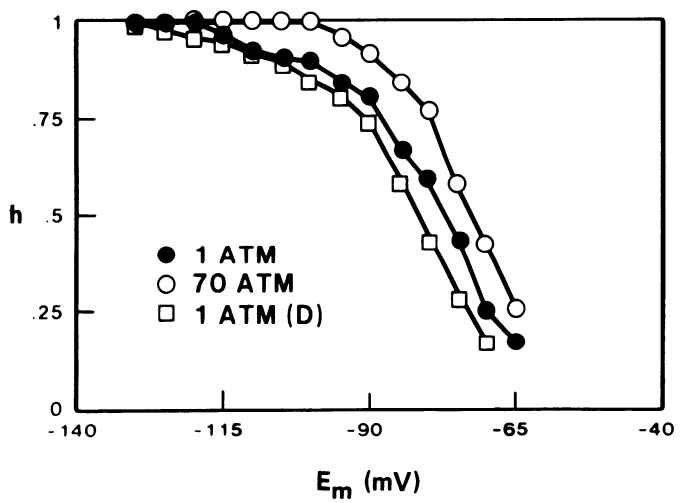

FIG. 4. Shift in voltage dependence of steady-state inactivation at 70 atm. *Vertical scale*, peak sodium current referred to maximum sodium current elicited at hyperpolarized potentials (h_∞); *horizontal scale*, amplitude of a 50-ms voltage step preceding a depolarizing test step to −23 mV. τ_m, membrane voltage. Shift in h_∞ observed at 70 atm is reversed on decompression to 1 atm. Node 12.21.81, temperature 10.5°C.

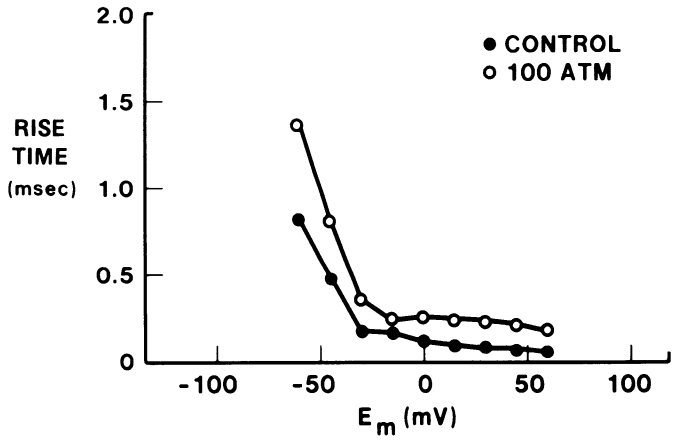

FIG. 5. Sodium current rise time vs. membrane voltage (E_m) at 1 and at 100 atm. Relative displacement of curve appears larger at more depolarized potentials, suggesting some voltage dependence of the effect of pressure on time constant of sodium channel activation; however, rise time was measured over a limited voltage range. Node 11.16.81, temperature 8.5°C.

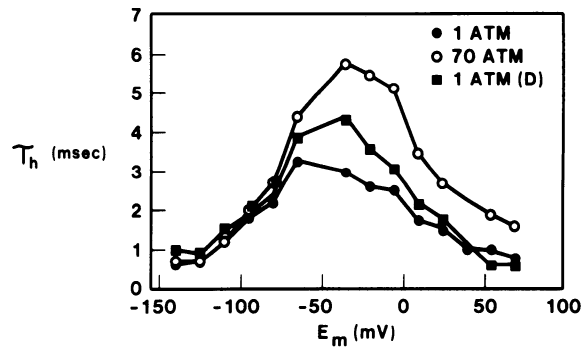

FIG. 6. Time constant of inactivation (τ_h) vs. membrane voltage (E_m) at 1 and 70 atm and on decompression to 1 atm. τ_h was measured by a 3-pulse protocol at potentials negative to −50 mV, and as sodium current decay at more positive potentials. The increase in τ_h following compression is voltage dependent, being more pronounced at the more depolarized potentials. Recovery of τ_h on decompression was incomplete. Node 12.21.81, temperature 10.5°C.

Potassium currents. A similar slowing of the activation of potassium channel currents was observed (Fig. 7). The slowing involved both a decrease in the rate of rise of the current and a small delay in turn-on. No attempt was made to quantify time constant changes; it is known that there are at least two and perhaps three populations of potassium channels in the node (8).

Leakage and holding current. There was no consistent change in leakage current. As described in preliminary reports of these studies, many nodes display an inward drift in holding current upon compression (17, 18). Although we have been able to produce similar changes in holding current by treatment with inhibitors of active sodium transport (unpublished data), we have not been able to identify this effect of pressure as an inhibition of active sodium-potassium exchange in part because the shift in holding current with either pressure or active transport inhibitors varies considerably from axon to axon. Nodes analyzed for the present report were selected because they displayed either no shift in holding current or shifts of less than 0.1 μA. This selection was facilitated

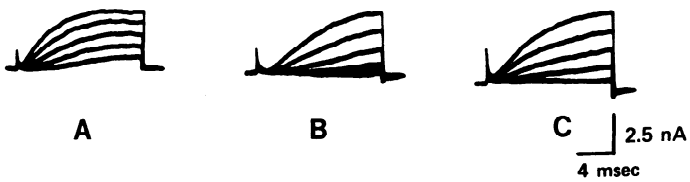

FIG. 7. Potassium currents at 1 (*A*) and 70 (*B*) atm; sodium currents blocked by choline substitution. Depolarizing test pulses in 15-mV increments from −35 to +25 mV. There is an increase in both delay to current onset and in time constant of activation, but there was no significant change in maximum potassium current. On decompression to 1 atm (*C*), pressure-induced delay in potassium activation is partially reversed. Node 12.10.79, temperature 10.5°C.

by the casual observation that nodes which displayed a large shift in holding current on cooling also displayed a large shift on compression. This is also consistent with, but not proof of, the identification of holding current shift with active transport changes.

Reversibility on decompression. As noted above, the decline in peak inward sodium current amplitude observed at pressures above 35 atm was not reversed on decompression, even in those cases where the preparation was successfully brought back to 1 atm and, in two cases, was maintained at 1 atm for up to 20 min. In view of the time required for a compression-decompression cycle, this irreversible loss of sodium current cannot be distinguished from the usual loss of current that occurs in this preparation over time. The shift in the voltage dependence of sodium inactivation and the increases in time constants for both sodium and potassium currents were all at least partly reversible on decompression.

DISCUSSION

Possible sources of error in hyperbaric measurements. Temperature was carefully monitored in these experiments, and adiabatic temperature changes can almost certainly be eliminated as a source of any of the effects ascribed to pressure. In the preparations analyzed for this study, there was no evidence that compression was physically altering the Vaseline seals on which the technique depends in any way that contributed to the observed effects. Changes in holding current were observed in some but not all nodes, the behavior of which was otherwise identical; there was no consistent change in the clamp settling time upon compression or in apparent membrane resistance or capacitance. With the HEPES buffer, changes in pH do not occur over the pressure range examined. Likewise, significant changes in electrode properties and ionic conductivity are unlikely at these modest pressures.

It is possible that some of the observed effects might be ascribed to helium rather than to pressure per se. This possibility cannot be directly ruled out for this preparation, in which a gas phase in close contact with the nerve is unavoidable. Although helium has sometimes been suggested to exert specific effects on excitable cells different from pressure effects, no specific helium effect has been documented. In addition, the present results in the node of Ranvier are qualitatively similar to effects reported for pressure in a squid axon preparation in which helium was not used (5, 6). The effects of helium pressure, however, have been suggested to be less than those of hydrostatic pressure because of helium's solubility in lipids (2).

Significance of pressure effects. An increase in membrane time constants is one of the most consistently observed effects of pressure. The slowing of all phases of the ionic currents corresponds to, and accounts for, the slowing and broadening of the action potential observed in previous studies on whole nerves (22).

It is shown here that steady-state inactivation, as measured with 50-ms prepulses, is shifted in the positive direction. This is a new finding, which has not been reported in other preparations. The voltage dependence of the pressure-induced shift in the corresponding time constant τ_h indicates that in Hodgkin-Huxley terms, pressure exerts a selective effect on the β_h rate constant. In physical terms, it appears that the conformation changes that underlie development of the inactive state of the channel are much more sensitive to pressure than those that underlie recovery from the inactive state. The steady-state inactivation level is dependent on both rate constants. The observed depolarizing shift in the h_∞ curve and selective decrease in β_h are in qualitative agreement with each other.

In normal peripheral nerve axons, the increase in the level of available channels due to the inactivation shift would have little effect on the conducted action potential or axon excitability. Only in cases where conduction is impaired or marginal might it have a significant effect. The decrease in rate of repolarization following an action potential might be expected to affect membrane processes related to accommodation; we have observed a pressure-related decrease in the ability of whole sciatic nerve to follow trains of high-frequency stimuli (Kendig and Erickson, unpublished data).

We had previously reported that some axons from this nerve are depolarized by pressure, displaying a steady inward current under voltage clamp. Depolarization may tend to increase axon excitability. The axons reported in the present study were selected from those that do not display a significant change in holding current on compression. The effects on pressure described here are therefore confined to the voltage and time-dependent inward sodium and outward potassium current. For these axons, no excitability increase was apparent on compression except for the reported change in steady-state inactivation.

Comparison with squid axon. Several groups have studied squid giant axons at hyperbaric pressure (5, 6, 13, 24). In the case of potassium currents, the results are similar to those in node with respect to an increase in time constant (τ_n) (6, 13, 24). In squid, there is an increase in the late outward current, attributed by some to a decrease in potassium accumulation in the extracellular space (24) and by others to a pressure-related enhancement of conductance and rate of opening of late potassium channels (6). This is not seen in the node of Ranvier. As for sodium currents, pressure produces a shift in the current-voltage relationship in the squid axon at small depolarizations, which is not seen in the node

(5); in the latter, the maximum inward current occurs at nearly identical voltages over the pressure range even though the current amplitude is depressed. The decrease in rate of rise of the sodium current is qualitatively similar in both preparations. In neither is there evidence for strong voltage dependence of pressure effects on the rate of rise. In two studies on squid axon, no change in h_∞ (13, 24, 31) at pressure is reported; in a third, carried out at much higher pressures, there is a small shift in the h_∞ curve in the opposite direction to that observed in the node and a decrease in slope (5). However, the same strong voltage dependence of the pressure effect on τ_h is reported as is seen in the node of Ranvier (5); this should lead, as Conti et al. point out (5), to an increase in h_∞ with pressure. Why the τ_h and h_∞ measurements are consistent in the vertebrate axon and not in the squid is an unanswered question. Quantitatively, the effects of pressure appear to be smaller in squid axon than in node of Ranvier, e.g., 15% increase in τ_h for squid giant axon at 100 atm vs. a threefold increase in the vertebrate nerves (24).

Comparison of temperature and pressure effects. In many systems, there is a rough correspondence between the effects of high pressure and those of low temperature. In the node of Ranvier, low temperatures increase the time constants of the sodium and potassium currents; the Q_{10}'s for the various rate constants are well described (9). High pressure does behave qualitatively like low temperature. However, in spite of the lack of reports of voltage-dependent effects of temperature on the rate constants governing inactivation [the Q_{10}'s of α_h and β_h are reported to be nearly identical (23)], low temperature induces a shift in a steady-state inactivation in a direction opposite to that we observe at high pressure (4). It thus appears that pressure and cooling may act differently on some processes governing sodium channel inactivation at the membrane level.

Relationship of these findings to HPNS. With the exception of the depolarizing inward current we reported previously (17, 18), which was minimal or absent in the axons described in the present study, it is difficult to relate the effects of pressure in these axons directly to HPNS. In straight axons with a high safety factor such as these, none of the effects of pressure would produce significant increases in excitability of the sort thought to accompany HPNS. It might be speculated that in damaged axons or in the case of conduction through regions of low safety factor, the increase in the level of h_∞ and the prolongation of depolarization by the increase in τ_h and τ_n might improve conduction. This, however, remains to be demonstrated.

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