Nitrogen narcosis and pressure reversal of anesthetic effects in node of Ranvier

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KENDIG, JOAN J. *Nitrogen narcosis and pressure reversal of anesthetic effects in node of Ranvier.* Am. J. Physiol. 246 (Cell Physiol. 15): C91–C95, 1984.—To compare sodium channel block by hyperbaric nitrogen with that induced by other anesthetics and to examine the basis for pressure antagonism to anesthetic condition block, voltage clamped nodes of Ranvier were exposed to nitrogen at pressures at 1–14 atm alone and in combination with helium to a total pressure of up to 100 atm. At 7 and 14 atm nitrogen, sodium currents were reversibly depressed without accompanying changes in the current-voltage relation. The curve relating steady-state inactivation (h_∞) to voltage was shifted in the hyperpolarizing direction, as is the case with other general anesthetic agents. The time constant of inactivation (τ_h) was slightly decreased at depolarized potentials. The preceding companion paper demonstrated an opposite effect of hyperbaric helium on the properties of sodium inactivation. Addition of helium pressure in the presence of nitrogen at 14 atm did not increase peak sodium current with inactivation maximally removed, but it did shift the h_∞ curve back toward control levels, thus increasing sodium current at points on the slope of the curve. It is proposed that these opposing shifts in steady-state inactivation levels are the basis for pressure antagonism to anesthetic conduction block. In the case of inert gases and volatile anesthetic agents, the antagonism may be direct but has not been shown to be so. In the case of the local anesthetic benzocaine, differences in the voltage dependence of anesthetic and pressure-induced changes in τ_h indicate the antagonism is indirect.

sodium channels; anesthetic agents; conduction block; hyperbaric pressure; pressure-anesthetic antagonism

AT PRESSURES above 1 atm inert gases such as xenon and nitrogen exert anesthetic effects; the pressure required to induce anesthesia varies inversely with lipid solubility (6, 7, 10, 25, 28). It is often assumed that anesthesia by the inert gases is fundamentally similar to anesthesia by volatile general anesthetic agents (6, 7). Like other general anesthetic agents (8, 13, 15, 33), inert gases induce an anesthetic state that is reversed by exposure to high hydrostatic or helium pressures (16, 25, 28); they also antagonize some of the effects of pressure in intact animals and humans (3, 10) and in isolated nerve preparations (23). A second assumption, which has motivated many studies on pressure, is that the phenomenon of pressure reversal of anesthesia represents direct antagonism between the physical actions of pressure and those of anesthetics on important molecular structures in excitable membrane (26–28). In lipid bilayer model membranes, such a direct antagonism has been shown (16, 31, 32). However, more recent studies suggest that the interaction between pressure and anesthetics is more complex than can be represented by direct antagonism (9). The present experiments were designed to test the first of these assumptions by examining the effects of hyperbaric nitrogen on sodium channels in the node of Ranvier and comparing them with the effects of local and volatile general anesthetics reported in our previous studies on the same preparation (5, 17, 22). The preceding companion paper (19) examined the effects of hyperbaric helium alone. In the present paper, the assumption of direct antagonism was tested by examining the combined effects of hyperbaric nitrogen and high pressures of helium in the same preparation compared with the effect of either gas alone.

METHODS

Sciatic nerve axons from *Xenopus laevis* were prepared for voltage clamp of the node of Ranvier under hyperbaric conditions as described in the preceding companion paper (19). Potassium channels were blocked by tetraethylammonium chloride. After stabilization in the pressure chamber, control measurements were made of the sodium current-voltage relation, the inactivation-voltage curve, and the time constant of inactivation (τ_h) as measured both by the time course of sodium current decay and by a three-pulse protocol, as described in the preceding companion paper. Nitrogen was admitted from a commercial pressurized cylinder to pressures of up to 14 atm nitrogen in the pressure chamber. After temperatures at the node and in the gas phase above it had returned to control levels, the measurements were repeated. The chamber was vented and measurements again made following decompression and return of temperatures to control levels. Four nodes were successfully carried through this protocol, and an additional two were exposed to two different nitrogen pressures. In experiments in which the combined effects of nitrogen and helium pressure were examined, measurements were made at 1 atm and at a single nitrogen pressure of 14 atm as described above. Helium was then admitted to bring the total pressure to 68 atm, and the measurements were repeated after temperature stabilization and again after 15 min. The second measurement was found necessary, because initial poor mixing of helium with the nitrogen already in the chamber led to a transient increase in effective nitrogen pressure near the node, as evidenced by an exacerbation of the nitrogen effects

immediately on compression by helium. This had resolved by the second measurement, which represents the steady-state effect of the nitrogen-helium combination and constitutes the results reported below. No decompression stage was attempted in the combined nitrogen-helium experiments. The results are from successful experiments on four nodes.

RESULTS

Nitrogen. At 7 atm, nitrogen produced a slight, and at 14 atm a significant, decrease in peak sodium current, without a clearcut shift in the current-voltage relationship or the sodium current reversal potential (Fig. 1). The effects of nitrogen on peak current amplitude were reversible on decompression (Fig. 1). At 14 atm, there was a shift in the inactivation (h_∞) curve to more hyperpolarized potentials by 5–15 mV (Figs. 1 and 2). This is plotted in Fig. 2 in two ways: with all sodium currents expressed as a percent of the maximum control, to show the absolute depression of peak sodium current by nitrogen, and with each point expressed as a percent of the maximum achieved at that nitrogen pressure, to reveal any changes in the slope of the curve in hyperbaric nitrogen. There were no significant or consistent slope changes. The shift in the h_∞ curve induced by nitrogen was reversed on decompression (Fig. 2).

Nitrogen and helium. On compression of the nitrogen-treated node up to 100 atm with helium, there was no increase in the nitrogen-depressed peak sodium current measured at potentials at which h_∞ was 1. At points on the slope of the inactivation-voltage curve, however, inward currents at any given membrane voltage were increased. Compression of the nitrogen-treated node to 70 atm with helium restored the h_∞ curve toward its control (1 atm) position on the voltage axis (Fig. 3). In this respect, hyperbaric helium pressure behaved in the presence of nitrogen in the same way as helium pressure alone (19), producing a shift in the inactivation curve along the voltage axis in the direction opposite to that induced by nitrogen.

Is the antagonism direct? The opposing effects of pressure and nitrogen on steady-state inactivation thus constitutes pressure antagonism to the effect of an anes-

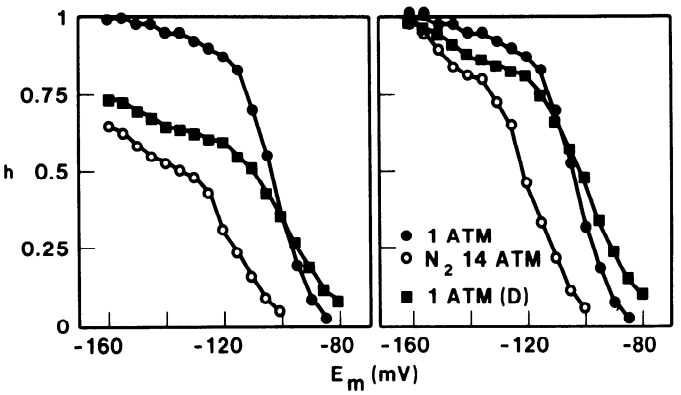

FIG. 2. Hyperpolarizing shift in inactivation-voltage curve on compression with 14 atm nitrogen and restoration toward control levels following decompression. Membrane voltage (E_m) was set by 50-ms prepulses before test pulse. *Left*: curves plotted with all values relative to control maximum. Note that there was some irreversible loss in maximum sodium current over time required for compression-decompression cycle. *Right*: same data plotted with each condition relative to its own maximum. There was no consistent change in slope of curve at its midpoint. Node 1.26.82.

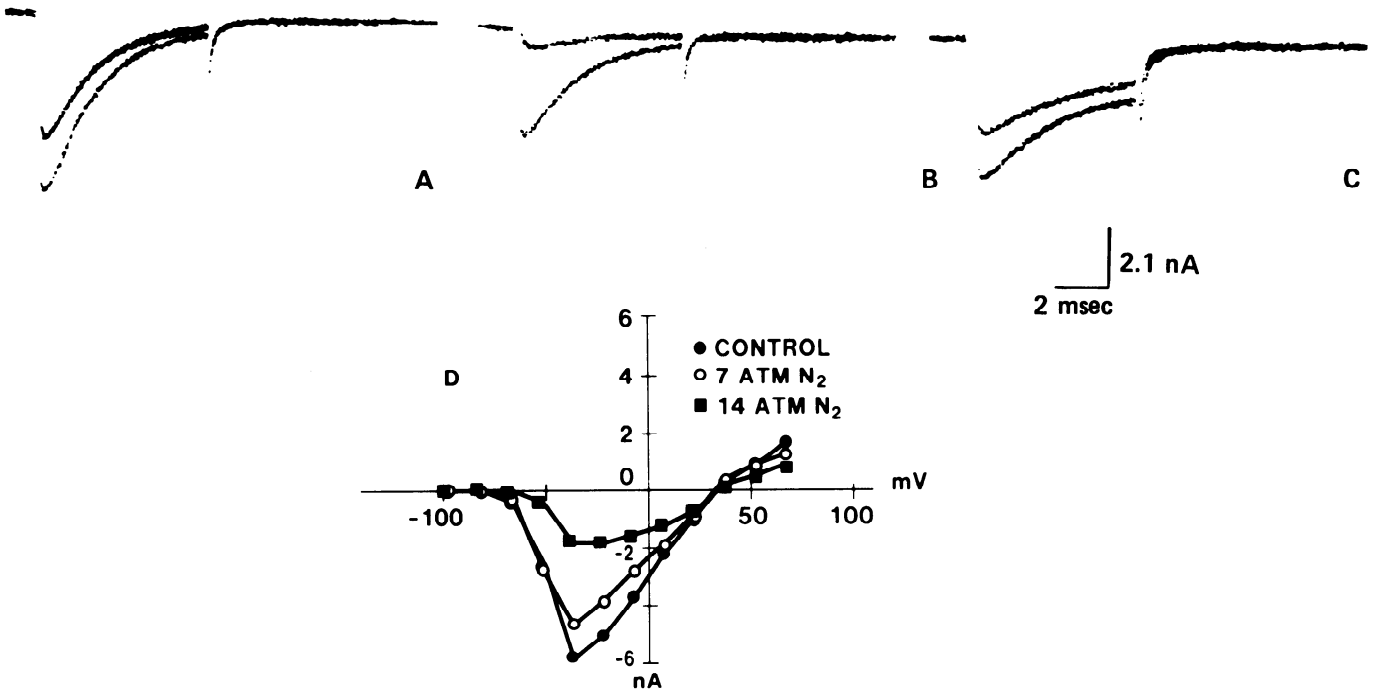

FIG. 1. Effects of nitrogen on sodium currents in node of Ranvier; potassium currents blocked by tetraethylammonium. *A*: control responses to a depolarizing test pulse 60 mV positive to holding potential alone and preceded by a 50-mV, 50-ms hyperpolarizing prepulse to maximize steady-state inactivation. *B*: same node after exposure to 14 atm nitrogen. *C*: reversibility on decompression to 1 atm. Node 1.26.82 no. 1. *D*: current-voltage plot of sodium currents in another node at 7 and 14 atm nitrogen; no consistent shift along voltage axis accompanied depression of current amplitude. Node 1.26.82 no. 2.

thetic agent. Is it a direct antagonism or are pressure and nitrogen acting on different processes? Inactivation in Hodgkin-Huxley terms is governed by voltage-dependent rate constants, α_h and β_h. As reported in the preceding companion paper (19), helium pressure effects on the time constant of inactivation are voltage dependent, being pronounced at very depolarized potentials and minimal at hyperpolarized potentials. The voltage dependence is consistent with a selective pressure effect on the processes that underly development of the inactive state, with relatively little effect on the processes underlying recovery from the inactive to the resting state. Measurements of τ_h were made on nodes exposed to 14 atm nitrogen alone and to 14 atm nitrogen plus 54 atm helium to bring the total pressure to 68 atm. The effect of nitrogen on τ_h was very slight (Fig. 4); there may have been some decrease at depolarized potentials. As described in the preceding companion paper, helium pressure increased τ_h selectively at depolarized, but not at hyperpolarized, potentials.

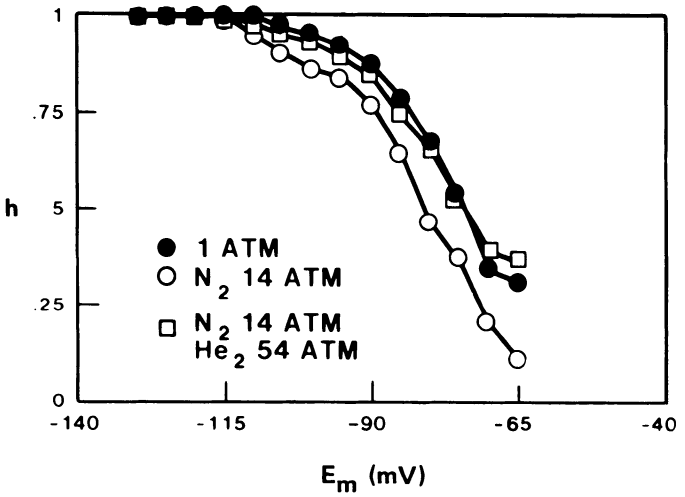

FIG. 3. Hyperpolarizing shift in steady-state activation-voltage curve in 14 atm nitrogen and its reversal on further compression with helium to a total pressure of 68 atm; all points are relative to control (1 atm) maximum current. Methods as described in Fig. 2. Node 2.2.82.

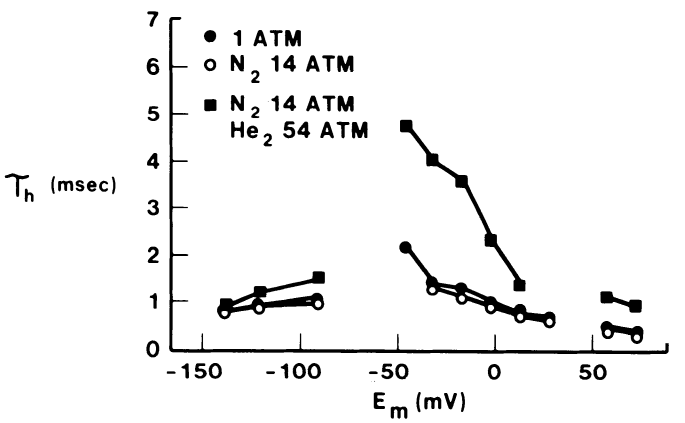

FIG. 4. Time constant of inactivation (τ_h) over range −150 to +70 mV. At potentials positive to −50 mV, τ_h was measured as decay of sodium current. At more negative potentials, a 3-pulse protocol was used. Although decay of the sodium current at depolarized potentials is consistently faster in 14 atm nitrogen than in 1 atm, the effect is not great. On compression with helium to a total pressure of 68 atm, there is a large voltage-dependent increase in τ_h. E_m, membrane voltage. Node 2.2.82.

DISCUSSION

The pronounced effect of rather modest nitrogen pressures on sodium currents was somewhat surprising, because 14 atm is below the anesthetic pressure of nitrogen (6). Other anesthetic agents significantly depress sodium currents in this axon only at levels at or above their anesthetic partial pressures. However, nitrogen at 14 atm does appear to behave like all other anesthetics so far studied, in that a portion of the decrease in sodium current at the normal resting potential is due to an increase in the percentage of channels in the inactive state, as evidenced by the hyperpolarizing shift in the inactivation curve. This property has been documented for the volatile general anesthetics (2, 22), barbiturates (18, 22), and local anesthetics (12, 22).

The present results establish a possible basis for pressure reversal of conduction block. Hyperbaric pressure antagonizes conduction block by volatile agents and some local anesthetics (17, 20, 21, 24, 30). The antagonism is limited; if the amplitude of the compound action potential is depressed by more than approximately 10%, hyperbaric pressure does not completely restore it (17). The increase in the amplitude of the partly blocked compound action potential is limited to about 10% of the control value (21, 24). In myelinated peripheral nerve, the only antagonistic interaction we have observed between pressure and anesthetics consists of opposing shifts in the voltage dependence of the inactivation curve. It therefore seems reasonable to propose that the limited pressure reversal of anesthetic conduction block in vertebrate axons is due to the upward shift in the number of resting (noninactivated) channels at the normal resting potential. Such an upward shift would be sufficient to restore conduction only in axons just past the point of conduction failure but insufficient to restore conduction in more deeply blocked axons. For this proposed mechanism to work, it is not necessary that the particular agent block conduction in part by depressing the level of the inactivation constant, as an increase in sodium channel availability will alleviate partial sodium channel block, voltage dependent or not. This proposed mechanism can thus also account for pressure reversal of ethanol-induced conduction block (30), although ethanol does not change steady-state inactivation (1). However, it is probable that pressure antagonism to conduction block will be more extensive in the case of agents which in fact exert a block that is partially voltage dependent. The higher probability of pressure reversal of conduction failure with agents that shift h_∞ downward may account in part for our observation that pressure successfully antagonizes conduction block by benzocaine and lidocaine, which exert significant tonic effects on inactivation, but not by procaine or QX572 (20), which may be expected to exert relatively small effects on inactivation at low stimulus frequencies (5, 12).

The molecular conclusions that can be drawn from the present results are limited. Although there is a slight tendency for the shift in h_∞ with nitrogen to be associated

with a decrease in τ_h at very depolarized potentials, it is not significant in this preparation compared with the τ_h decrease reported for ether and halothane in another preparation (2). Nor, in spite of several attempts, have we been able to show that a voltage-dependent τ_h decrease in this preparation is responsible for the apparent h_∞ shifts produced by either ether or phenobarbital (22). Therefore, although it is tempting to speculate that at least some of the lipid-soluble conduction blocking agents may share a common action in this axon membrane by facilitating sodium channel inactivation, the evidence does not permit this conclusion. Furthermore, even if this conclusion could be drawn, inactivation almost certainly corresponds to a sequence of several channel state changes, the rate constants governing any one of which affect the others.

A more distinct, however negative, conclusion can be drawn about an assumption that has motivated much of the research into hyperbaric pressure-anesthetic interactions. The fact that a wide variety of anesthetic agents, of many different structures, is subject to pressure antagonism has been thought to support the hypothesis that all the agents share a common site of action. The assumption on which this line of reasoning was based is that pressure "reversal" of anesthesia represents a direct opposing effect of pressure and anesthetic agents on the same fundamental processes in nerve membrane. If our hypothesis concerning pressure reversal of anesthetic conduction block is correct, then the case of the neutral local anesthetic benzocaine presents a clear counter example that falsifies the assumption for this agent. In the node of Ranvier, this agent, like other anesthetics so far examined, shifts the inactivation curve in the hyperpolarizing direction (12). As we have shown, however, in the case of benzocaine, the shift is due to an effect of benzocaine on recovery from inactivation, rather than on its development (22). We have also previously shown that benzocaine conduction block is antagonized by pressure (20). If the antagonism is due, as we postulate, to opposing effects of pressure and anesthetics on the level of steady-state sodium inactivation, then pressure antagonism to benzocaine conduction block cannot be direct. The distinctly different voltage dependencies of benzocaine and pressure effects on τ_h show that they are acting on different processes. The possibility remains, however, that pressure antagonism to other agents such as volatile or gaseous anesthetics may be direct; however, the present results do not confirm this.

These results have demonstrated a possible site of pressure-anesthetic antagonism in the inactivation process of the voltage-dependent sodium channel. Their relevance to pressure antagonism to anesthesia in intact animals remains to be clarified.

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