Pressure (≤4 ATA) increases membrane conductance and firing rate in the rat solitary complex

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Neuronal sensitivity to pressure, barosensitivity, is illustrated by high-pressure nervous syndrome, which manifests as increased central nervous system excitability when heliox or trimix is breathed at >15 atmospheres absolute (ATA). We have tested the hypothesis that smaller levels of pressure (≤4 ATA) also increase neuronal excitability. The effect of hyperbaric helium, which mimics increased hydrostatic pressure, was determined on putative CO2/H+-chemoreceptor neurons in the solitary complex in rat brain stem slices by intracellular recording. Pressure stimulated firing rate in 31% of neurons (barosensitivity) and decreased input resistance. Barosensitivity was retained during synaptic blockade and was unaffected by antioxidants. Barosensitivity was distributed among CO2/H+-chemosensitive and -insensitive neurons; in CO2/H+-chemosensitive neurons, pressure did not significantly reduce neuronal chemosensitivity. We conclude that moderate pressure stimulates certain solitary complex neurons by a mechanism that possibly involves an increased cation conductance, but that does not involve free radicals. Neuronal barosensitivity to ≤4 ATA may represent a physiological adaptive response to increased pressure or a pathophysiological response that is the early manifestation of high-pressure nervous syndrome.

brain slice; intracellular recording; cardiorespiratory control; high-pressure nervous syndrome; hyperbaric helium; hypercapnia; hypoxia; neuron

Breathing a gas mixture of oxygen and helium (heliox) or oxygen, helium, and nitrogen (trimix) at ambient pressures in excess of 15 atmospheres absolute (ATA) can result in high-pressure nervous syndrome (HPNS). Symptoms of HPNS include muscular tremors, loss of coordination, nausea, respiratory difficulties, memory deficit, and seizures, with the latter occurring in animals but not humans (1, 13, 38). It is likely that seizures were not observed in humans because they have not been exposed to high enough pressure for health reasons and not because the potential mechanism of barosensitivity differs between humans and other animals. Presently, onset of HPNS is what limits human performance at great depths while hyperbaric gas mixtures of oxygen and inert gases are breathed (13). Although the exact cellular mechanisms underlying HPNS are unknown, neurophysiological studies indicate that hyperbaric pressure increases excitability of the central nervous system (CNS) by changes in both synaptic (16, 17, 19, 50) and intrinsic membrane properties (20, 21, 24, 25, 34, 41–43, 48, 49; also reviewed in 6, 13, 22, 23, 38). It is also clear that the effects of hyperbaric pressure on the CNS are due to the direct effects of increased hydrostatic pressure (i.e., pressure per se) and not to the effects of increased gas partial pressures (i.e., narcotic actions) (13).

By contrast, it is generally believed that pressures at <15 ATA have no effects on neurons in the mammalian CNS (23, 26) and that any changes noted in the CNS at these moderate pressures must be due to increased partial pressure of O2, CO2, or N2. Few studies, however, have tested whether moderate levels of pressure alter neuronal function in the mammalian CNS (13). Moreover, several recent studies suggest that small-to-moderate levels of pressure, well below 10 ATA, can alter function at the invertebrate neuromuscular junction (8, 9) and of neuronal cells in a variety of tissues (28, 39). Consequently, it becomes important to reevaluate the range of neuronal barosensitivity (i.e., sensitivity to pressure) expressed by neurons in the mammalian CNS.

In our preceding paper (33), we reported that hyperbaric hyperoxia (HBO2) at 2.2–3.3 ATA increased firing rate and input resistance (Rin) of putative central CO2/H+-chemoreceptors in a cardiorespiratory control region of the brain stem known as the solitary complex (SC). This excitatory effect of hyperoxia was shown to be due to increased production of oxygen free radicals. However, it is also possible that increased pressure had an additional effect on neuronal excitability that occurred independently from that due to increased tissue PO2 and increased O2 free radicals. In the present study, therefore, we test the hypothesis that SC neurons are stimulated by moderate pressure in the absence of changes in tissue PO2, PCO2, and pH.

There were three goals in this study. First, to describe the effects of pressure per se (2–4 ATA) on...
neuronal firing rate and $R_{\text{in}}$ in SC neurons. Second, to determine whether putative CO$_2$/H$^+$ chemoreceptors in the SC, which are stimulated by HBO$_2$ (33), are likewise affected by pressure alone and, if so, whether pressure decreases neuronal CO$_2$/H$^+$ chemosensitivity. CO$_2$/H$^+$-chemosensitive neurons in the SC are thought to participate in cardiorespiratory control (40). Previous studies have shown that high levels of pressure ($\geq$15 ATA) decrease respiratory drive (44) and central chemosensitivity (45). Third, we wanted to determine whether barosensitivity to moderate levels of pressure, if it occurred, was mediated by an O$_2$ free radical-dependent mechanism. Thom (47) reported that hyperbaric helium (HBHe), which is often used to mimic the effects of increased hydrostatic pressure (33, 42–45) and other inert gases, at pressures as low as 2.8 ATA, increases production of O$_2$ free radicals (i.e., superoxide). Because we have demonstrated that CO$_2$/H$^+$-chemosensitive neurons in the SC were highly sensitive to oxidative stress (and O$_2$ free radicals) (33), we wanted to determine whether the use of HBHe, similarly, in our brain stem slice preparation resulted in increased O$_2$ free radical production. Thus we planned to determine whether putative CO$_2$/H$^+$-chemosensitive neurons in the SC are thought to be healthy when it had a membrane potential more negative than −40 mV, as measured from the Axo-

METHODS

General methods regarding preparation of the in vitro rat brain slice for conducting electrophysiological measurements while changing barometric pressure (P$_n$), P$_{O_2}$, and/or P$_{CO_2}$ have been described previously (12, 33).

Test Conditions

Compression medium. As in previous studies (9, 16, 17, 19, 42–45), helium was chosen as the compression medium to mimic hydrostatic pressure. All inert gases exert narcotic actions that are directly related to their lipid solubility (2, 6, 13). Helium is of very low lipid solubility (2), and, for this reason, the narcotic effects of helium are reported to be negligible, except at very high pressures, $\geq$100 ATA (13, 36, 46), which are much higher than the levels of P$_n$ used in this study. Thus any changes in neuronal excitability, as determined by a change in firing rate during HBHe (i.e., barosen-
sitivity), were attributed to the effects of pressure per se rather than the narcotic effects of HBHe (13).

Before compression, room air was purged from the hyperbaric chamber and replaced with 100% helium. P$_{O_2}$ and P$_{CO_2}$ in the aCSF did not increase, because no additional O$_2$ or CO$_2$, respectively, were present inside the hyperbaric chamber (12, 13, 30, 32, 46). This is our standard test for neuronal barosensitivity in the in vitro brain slice preparation (13, 30, 31). Barosensitive neurons were defined as those that responded to pressure with a $\geq$20% change in firing rate. In all experiments, the rates of compression and decompression were controlled at $\approx$2 ATA/min. Brain slice temperature was maintained at 37 $\pm$ 0.5°C during helium compression and decompression (12).

HBO$_2$ and hypercapnic perfusates. The details for preparation of hyperoxic and hypercapnic solutions are given in the preceding paper (33). Briefly, hyperbaric oxygenated perfusate (i.e., HBO$_2$) with normocapnia was made by equili-
brating aCSF with 95 or 98% O$_2$ at 2.2 or 3.3 ATA in separate high-pressure sample cylinders to produce P$_{O_2}$ values in the aCSF of −1,640 and −2,470 Torr, respectively. P$_{CO_2}$ in the aCSF was kept constant at −40 Torr by reducing the percent CO$_2$ from 5 to 2 and 1.65% at 2.2 and 3.3 ATA, respectively (12, 32, 33). Normobaric and hyperbaric hypercapnic media were made by equilibrating aCSF with 85% O$_2$-15% CO$_2$ at normobaric pressure to produce media P$_{CO_2}$ of 114 Torr (10, 11, 18, 33).

Antioxidant perfusate. The antioxidant used was Trolox C (Sigma-Aldrich). Trolox C is a water-soluble analog of vita-
mnin E, which is thought to cross the lipid bilayer, where it acts as an effective antioxidant (15) and is capable of repairing some types of oxidative damage (3). The concentrations of Trolox C used in this study ranged from 100 to 200 $\mu$M, which have been shown to block the electrophysiological effects of hydrogen peroxide on hippocampal neurons (37) and HBO$_2$ on SC neurons (33).

Analysis and Data Presentation

Electrophysiology was done by using sharp-tipped intra-
cellular microelectrodes. The electrophysiological properties that were measured and method of data collection were as described in our preceding paper (33). Paired-pulse tests ($P \leq 0.05$) were used to determine whether the average firing rate, $R_{\text{in}}$, or amplitude of afterhyperpolarizing potential (AHP) responses to pressure differed (i.e., mean population differences during HBHe compared with 1 ATA control) sig-
nificantly from zero. Contingency tables were used to com-
pare tabulated electrophysiological properties, as well as the incidence in SC neurons of CO$_2$/H$^+$ chemosensitivity, HBO$_2$ sensitivity, and barosensitivity. The significance of associa-
tions between parameters was determined by using $\chi^2$ or Fisher’s exact test with the Yates continuity correction, when appropriate ($P \leq 0.05$). All data are presented as means $\pm$ SE. Pressure was reported in ATA, and specific gas tensions in the aCSF and tissue (e.g., P$_{O_2}$, P$_{CO_2}$) are reported in Torr, where 1 ATA is equal to 760 Torr.

RESULTS

Data were obtained during intracellular recordings made from 102 SC neurons. Specific details regarding the sensitivity of this group of neurons to HBO$_2$, chemical oxidants, and hypercapnic acidosis were reported in the preceding paper (33). A recording was considered to be healthy when it had a membrane potential more negative than −40 mV, as measured from the Axo-
clamping-2A amplifier, and had an action potential amplitude $>50$ mV. These cells had membrane potentials of $-53 \pm 6$ mV (range $-41$ to $-68$ mV) and $R_{\text{in}}$ of $110 \pm 4$ M$\Omega$ (range $36$–$222$ M$\Omega$).

**Barosensitivity**

In a typical experiment, an intracellular recording was established under control conditions at room pressure. After 10–30 min of intracellular recording, the hyperbaric chamber was flushed and compressed to 2–4 ATA (mode = 3 ATA) with 100% helium. In the absence of any changes in PO$_2$ and/or pH of the perfusate (12, 30, 32), this level of pressure caused, at most, a slight depolarization ($\approx 3$ mV), which was not always evident, and significantly ($\approx 20\%$) increased firing rate in 32 of 102 (31%) neurons (Fig. 1, A and B). The barosensitive neurons had a firing rate response that typically occurred in conjunction with a significant decrease in $R_{\text{in}}$ ($t$-test, change (Δ) in $R_{\text{in}} = -7.4 \pm 3.2$ M$\Omega$; $n = 23$ neurons) (Fig. 1C). In addition, pressure decreased the amplitude of the AHP from 22.7 ± 1.1 by $-1.7 \pm 0.6$ mV ($t$-test, $P < 0.05$, $n = 22$, Fig. 1D). This type of barosensitive response was usually reversible on decompression back to 1 ATA. However, 41% of the barosensitive neurons showed an initial firing rate that occurred with decreased $R_{\text{in}}$ and decreased AHP, but that adapted back to control levels within 5 min of reaching the maximum level of pressure (Fig. 2). The remaining 69% of SC neurons tested were barosensitive, i.e., they did not increase firing rate in response to helium compressions up to 4 ATA (Fig. 3, A and B). Similarly, barosensitive neurons did not show a significant change in $R_{\text{in}}$ ($t$-test, $P > 0.25$, $\Delta R_{\text{in}} = -1.1 \pm 3.5$ M$\Omega$, $n = 44$) or AHP amplitude ($t$-test, $P > 0.25$, $\Delta$AHP = 0.08 ± 0.3 mV, $n = 53$) while at pressure (Fig. 3, C and D). These results indicate that PB between 2 and 4 ATA stimulates excitability in a subpopulation of SC neurons.

**Intrinsic Barosensitivity**

To determine whether SC neurons are intrinsically barosensitive or if barosensitivity is mediated by pressure-induced increases in excitatory synaptic transmission and/or decreases in inhibitory synaptic transmission (16, 17, 19, 22, 50), firing rate was measured in response to increased pressure in a solution that blocked chemical synaptic transmission (high Mg$^{2+}$-low-Ca$^{2+}$ medium). Previously, our laboratory has reported that this medium reversibly blocked evoked synaptic potentials in similar brain slice preparations (10). The direct effects of the synaptic block medium on SC neurons include increased firing rate and increased $R_{\text{in}}$. Changes in these parameters were used as an indicator that high-Mg$^{2+}$-low Ca$^{2+}$ medium was effective in our preparation (10). Figure 2, A and B, shows an example of a neuron that was transiently stimulated by 3 and 4 ATA in control medium. We then incubated the slice for ~30 min in synaptic block medium before testing for intrinsic barosensitivity. In synaptic block medium, the cell’s spontaneous firing rate increased at normobaric pressure, presumably due to decreased Ca$^{2+}$-dependent K$^+$ conductance and/or removal of inhibitory input (10). Regardless, exposure to pressure caused an additional increase in firing rate of a similar magnitude and transient nature as that in control medium (Fig. 2). Our results show that the firing rate response of five barosensitive neurons in control medium ($\Delta$ firing rate = 1.39 ± 0.31 impulses/s) was retained in synaptic block medium ($\Delta$ firing rate = 3.6 ± 2.25 impulses/s), thus indicating that these neurons were intrinsically barosensitive. These experiments, however, did not determine the effects of pressure on synaptic activity, which has been reported in other CNS areas at PB ~35 ATA (16, 17, 50).

**Barosensitivity, HBO$_2$ Sensitivity, and CO$_2$/H$^+$ Chemosensitivity**

We reported in the preceding paper (33) that 90% of SC neurons sensitive to HBO$_2$ and/or chemical oxidants were also CO$_2$/H$^+$ chemosensitive, whereas only 19% of HBO$_2$-insensitive neurons were CO$_2$/H$^+$ chemosensitive. Therefore, we wanted to determine whether pressure similarly affected preferentially HBO$_2$- and CO$_2$/H$^+$-chemosensitive neurons in the SC.

**Pressure and CO$_2$/H$^+$ chemosensitivity.** To determine whether 2–4 ATA affected CO$_2$/H$^+$-chemosensi-
tive neurons and CO$_2$/H$^+$ chemosensitivity, two types of experiments were conducted. First, we exposed cells to normobaric hypercapnic acidosis and to pressure separately to see whether there was a relationship between CO$_2$/H$^+$ chemosensitivity and barosensitivity. Second, to determine whether pressure affects CO$_2$/H$^+$ chemosensitivity (45), we compared the firing rate response of SC neurons to normobaric hypercapnic acidosis vs. hyperbaric hypercapnic acidosis.

In the first experiment, we found, as previously reported (10), that CO$_2$/H$^+$-chemosensitive neurons responded to normobaric hypercapnia with increased firing rate and increased $R_{\text{in}}$ (Fig. 4A). We found that, of the 58 neurons exposed to normobaric hypercapnic acidosis, 30 (52%) were CO$_2$/H$^+$-chemosensitive; of these 30 CO$_2$/H$^+$-chemosensitive neurons, 10 neurons were also barosensitive (Fig. 4, A and C). The remaining 20 CO$_2$/H$^+$-chemosensitive neurons, however, did...
not respond to pressure (Fig. 4C). Of the 28 neurons that did not respond to CO₂/H⁻, 9 were barosensitive (Fig. 4B), whereas 19 did not respond to either CO₂/H⁻ or pressure (Fig. 4C). These results indicate that, whereas some CO₂/H⁻-chemosensitive neurons were also barosensitive, there was not a significant association between barosensitivity and CO₂/H⁻-chemosensitivity ($\chi^2$ analysis, $P = 0.854$). In other words, barosensitive neurons included both CO₂/H⁻-chemosensitive and CO₂/H⁻-chemoinsensitive neurons. Similarly, there was not a significant association between CO₂/H⁻ chemosensitivity and either sustained or transient barosensitivity (Fisher's exact test).

In the second experiment, we compared the firing rate response to hypercapnic acidosis at 1 and 3 ATA (aCSF PCO₂ was set external to the hyperbaric chamber, and thus helium compression did not change pH of the aCSF; Refs. 12, 32). We found that pressure had no effect on CO₂/H⁺-chemosensitivity (Fig. 5A), and firing rate increased by the same amount in response to CO₂/H⁺ at 1 and 3 ATA (Fig. 5B). These results indicate that CO₂/H⁺ chemosensitivity of SC neurons is unaffected by 2–4 ATA of hyperbaric pressure.

**Pressure and HBO₂ sensitivity.** In the preceding paper, we report that increased PO₂ stimulates some SC neurons (i.e., HBO₂ sensitive), specifically, putative CO₂/H⁺ chemoreceptors (33). Therefore, to determine whether pressure per se, in addition to increased PO₂,
stimulates HBO₂-sensitive neurons, we compared the firing rate and \( R_m \) responses of SC neurons exposed to pressure and HBO₂. Of the 32 barosensitive neurons, 14 were HBO₂ sensitive. Of the 70 barosensitive neurons, 30 were HBO₂ sensitive. These results indicate that, whereas some barosensitive neurons were also HBO₂ sensitive, not all barosensitive neurons were HBO₂ sensitive. Thus there was not a significant association between barosensitivity and HBO₂ sensitivity (\( \chi^2 \) analysis, \( P = 0.896 \)). Similarly, there was not a significant association between HBO₂ sensitivity and either sustained or transient barosensitivity (Fisher's exact test). This was not surprising, because the preceding analysis had revealed the lack of association between barosensitivity and \( \text{CO}_2/\text{H}_2 \) chemosensitivity (Fig. 4), and, as already reported (33), HBO₂ and \( \text{CO}_2/\text{H}_2 \) sensitivity are strongly associated.

It has been reported that 2.8 ATA helium, and other inert gases, can increase the production of O₂ free radicals in solutions containing either xanthine oxidase-hypoxanthine or phenazine methosulfate-NADH (47). Therefore, our final goal was to determine whether O₂ free radicals contribute to barosensitivity. We tested whether exposure to the antioxidant Trolox C would block barosensitivity as tested by using HBHe. Figure 6 shows that the firing rate (Fig. 6A) and \( R_m \) (Fig. 6B) responses of a barosensitive neuron were retained in the presence of Trolox C. Figure 6 also summarizes the firing rate (Fig. 6C) and \( R_m \) (Fig. 6D) responses of four neurons to HBHe in control and Trolox C media. Notice that exposure to Trolox C had no effect on the increased firing rate response to HBHe. These results indicate that the excitatory effects of HBHe on SC neurons are not mediated by O₂ free radicals.

**DISCUSSION**

We have described, for the first time, the effects of a moderate range of pressures on individual neurons in the mammalian CNS. Previous attempts to do so in isolated mammalian CNS were unsuccessful for reasons already stated elsewhere (13). Our results show that pressure increased firing rate in about one-third of the SC neurons (termed “barosensitive neurons”), usually with decreased \( R_m \) and decreased AHP. This barosensitive response was of two types: one population of barosensitive neurons adapted back to control levels of firing rate within \( \sim 5 \) min of achieving maximum pressure, whereas the other population of barosensitive neurons maintained a sustained increase in firing rate for the duration of the compression. In addition, we showed that barosensitive neurons in the SC represented a heterogeneous group of cells that included HBO₂-sensitive and \( \text{CO}_2/\text{H}_2 \)-chemosensitive neurons (33), which are thought to function as central chemoreceptors (10, 11, 18, 35, 40), as well as neurons insensitive to either increased O₂ or increased \( \text{CO}_2/\text{H}_2 \).

**HBHe as a Compression Medium**

As in previous studies (33, 42–45), helium was used to compress the hyperbaric chamber and tissue bath, thereby effectively pressurizing the brain slice submerged in aCSF. In general, the effects of HBHe on cells, tissues, and intact organisms are believed to be comparable to the effects of hydrostatic pressure over a range of mechanically tolerable pressures (6, 13). Thus we conclude that using helium to compress the tissue bath effectively mimics hydrostatic compression at \( P_B \approx 4 \text{ ATA} \) and that any increase in neuronal excit-
ability observed during exposure to HBHe reflects the effects of pressure per se and not possible narcotic actions of increased tissue helium partial pressure (12, 30). In addition, our results using the antioxidant Trolox C suggest that HBHe does not increase \( \text{O}_2 \) free radical production as previously proposed (47). Dean et al. (13) and others (6, 23) have reviewed the evidence supporting the use of HBHe at low levels of compression to mimic pure hydrostatic compression.

**Barosensitivity of SC Neurons**

Discovering that moderate levels of pressure (2–4 ATA) stimulated approximately one-third of the SC neurons is an exciting finding; previously, it was thought that hyperbaric pressures <15 ATA had no effect on neurons unless accompanied by an increase in tissue \( \text{PO}_2 \), \( \text{PCO}_2 \), and/or \( \text{N}_2 \) partial pressure (23, 26, 43). Moreover, the fact that the remaining two-thirds of SC neurons tested did not show changes in firing rate, \( R_{in} \), or AHP during exposure to =4 ATA pressure indicates that certain SC neurons are more barosensitive than other neurons. We anticipate that compression to pressures >4 ATA, when tested, will stimulate firing rate and decrease \( R_{in} \) to a greater extent and/or in an even greater proportion of SC neurons. This hypothesis is supported by the observation that the effects of pressure on Na\(^+\) current increased linearly in other types of neurons with compression up to 101 ATA (42). Similarly, previous studies showed that 30 ATA increased the number of evoked action potentials in crayfish claw nerves (24, 25), and pressures up to 360 ATA increased the spontaneous activity of *Helix* pacemaker cells, with a corresponding 30% decrease in \( R_{in} \) (48).

The membrane and synapic mechanism(s) by which 2–4 ATA pressure increases excitability of individual neurons remains to be determined. We did determine, however, that neuronal barosensitivity is an intrinsic property of SC neurons that is retained during chemical synapic blockade in high-Mg\(^{2+}\)-low-Ca\(^{2+}\) medium. We did not determine the effects of pressure on synapic transmission in this study, but we anticipate that it will also be affected by increased pressure. Previous studies (16, 17, 50) have shown that \( \text{PB} \geq 35 \) ATA decreased both excitatory and inhibitory synapic transmission in mammalian neurons.

The physiological significance of neuronal barosensitivity to moderate levels of physical pressure is unknown. To speculate, ambient pressure, like ambient temperature, is a normally occurring environmental stimulus, and barosensitivity may represent a normal neuronal response, much like thermosensitivity (4), which contributes to how an organism responds and adapts to its changing environment. It is also possible that barosensitivity is a cellular property that occurs over a continuum of pressure, in which sensitivity to ambient pressures up to 4 ATA represents the early component of what is ultimately known as HPNS. HPNS is a complex neurologcal response to pressure per se that occurs in divers breathing helix and trimix at ambient pressures >15 ATA (1, 13, 38). Symptoms of HPNS have been attributed to increased excitability of the mammalian CNS, but the exact mechanisms involved are unknown (23). Our findings are consistent with a pressure-induced increase in excitability of neurons, however, at hyperbaric pressures well below 15 ATA. It may be that, as ambient pressure increases, neuronal excitability likewise increases, along with recruitment of barosensitive neurons with higher thresholds of sensitivity (42). Once a critical threshold is reached (i.e., stimulation of critical numbers of neural networks), then symptoms of HPNS occur.

We also determined that 41% of the barosensitive responses were transient in nature; i.e., increased firing rate and decreased \( R_{in} \) during compression returned toward normobaric control levels within 5–10 min of sustained pressurization. Transient barosensitivity has been reported previously in *Helix* cells in which 50–156 ATA initially stimulated firing rate, after which the firing rate response adapted to control values within 5 min at pressure (49). Similarly, transient barosensitivity has also been observed in vivo; after 2 wk at 80 ATA, the convulsion threshold of mice increased by 35 ATA (5). The ability of many neurons to adapt to sustained pressurization may explain why previous intracellular recording studies of hippocampal neurons, which compared neurons sampled at different steady-state pressures, did not find a significant effect of pressure on neuronal excitability (43).

**Cellular Mechanism of Barosensitivity**

We anticipate that neuronal barosensitivity will result from the summed effect of pressure on multiple ionic currents, which, overall, are observed as a net increase in membrane conductance (i.e., decreased \( R_{in} \)). Hyperbaric pressure increased excitability and decreased \( R_{in} \), which suggests that pressure increases an inward cation conductance (possibly Na\(^+\) or Ca\(^{2+}\)) or possibly an outward Cl\(^-\) conductance. However, other cellular signaling mechanisms may contribute to barosensitivity. A previous study found that 70 ATA evoked a depolarizing net inward current in vertebrate neurons (20); however, the observed depolarization may have resulted from a partial block of the Na\(^+\)-K\(^-\)-ATPase (21). Therefore, it remains to be determined which specific ion channels or transporters are affected by moderate levels of hyperbaric pressure, or even how pressure alters ion conductance and synaptic transmission in the SC. In addition, our finding that pressure reduced the AHP amplitude suggests that pressure decreases a Ca\(^{2+}\)-dependent K\(^+\) conductance. This finding is consistent with a previous study that showed that the slow AHP measured in rat hippocampal neurons was decreased by pressures up to 100 ATA (43). In contrast, 900 ATA of hydrostatic pressure increased the open probability of large-conductance Ca\(^{2+}\)-dependent K\(^+\) channels in chromaffin cells by 30 times (26).

It is conceivable that small hyperbaric pressures, even fractions of an atmosphere, may affect electrical signaling by neurons. At <4 ATA, it is unlikely that the
changes in neuronal activity are due to changes in thermodynamically driven equilibria (13, 27). Alternatively, Macdonald and Fraser (27) have proposed that various types of cells (nonneuronal) respond to small hyperbaric pressures by a mechanical process, including cytoskeletal rearrangement and/or the development of localized shear forces, resulting from the differential compressibility of various adjoining cellular components. Moderate levels of hyperbaric pressure may similarly affect mammalian neurons. Evidence supporting this hypothesis in the mammalian CNS, however, is presently lacking (13).

**Barosensitivity, CO₂/H⁺ Chemosensitivity, and Cardiorespiratory Control**

We showed that a small proportion of barosensitive neurons in a cardiorespiratory control region of the brain stem was also stimulated by hypercapnic acidosis. CO₂/H⁺-chemosensitive neurons are thought to function as central chemoreceptors for the cardiorespiratory system (10, 11, 18, 35, 40). Although there was a trend for the CO₂/H⁺-chemosensitive responses to decrease during hyperbaric pressure, the firing rate responses to CO₂ at 1 and 3 ATA were not significantly different. However, this negative finding should be interpreted with caution, because larger levels of pressure may have a more pronounced effect on neuronal excitability. For example, Tarasiuk and Grossman (45) have shown that 100 ATA decreased central CO₂/H⁺ chemosensitivity and the neural drive for breathing (44) in the neonatal rat brain stem-spinal cord preparation. Therefore, our finding that some SC neurons were also sensitive to moderate levels of pressure may represent the early effects of pressure on cardiorespiratory control, which precede abnormal function observed in vivo at higher pressures, e.g., high hydrostatic pressure has been shown to decrease heart rate and cause dyspnea in cats (7, 14) and reduce CO₂ chemosensitivity in humans (29).

**Barosensitivity, Hyperoxia, and Oxygen Free Radicals**

A previous study reported that inert gases, including helium, at pressures similar to the level of hyperbaric pressure used in this study, increased production of O₂ free radicals in solutions containing either xanthine oxidase-hypoxanthine or phenazine methosulfate-NADH (47). No study, however, has ever reported that inert gases at hyperbaric pressure increase O₂ free radical production in isolated preparations of the mammalian CNS. Regardless, we were concerned that HBHe may increase neuronal excitability, in part, by increasing the production of O₂ free radicals. This was a concern, because we reported in the preceding paper that certain SC neurons are highly sensitive to oxidative stress (33). However, the antioxidant Trolox C, which blocks sensitivity of SC neurons to HBO₂ (33), did not significantly alter barosensitivity of SC neurons. Thus our results indicate that the excitatory effect of HBHe on firing rate of SC neurons was not dependent on O₂ free radicals. Moreover, we showed that there was no association between HBO₂ sensitivity, a highly oxidative condition (30, 31, 33), and barosensitivity in the SC. In addition, HBO₂ and pressure had opposite effects on Rₚ; HBO₂ stimulated firing rate and increased Rₚ (33), whereas HBHe stimulated firing rate but decreased Rₚ. Collectively, these findings indicate that the cellular effects of HBHe are not mediated by increased levels of O₂ free radicals, as previously suggested (47), but rather are due to the effects of physical pressure (13).

In conclusion, our findings demonstrate, for the first time, that moderate levels of pressure (2–4 ATA) increase excitability of a subpopulation of SC neurons. The specific cellular mechanism by which increased pressure increases neuronal excitability remains to be determined; however, it would appear that more than one ionic current is involved. Our findings indicate that, at least in some SC neurons, the effects of hyperbaric gases at 3–4 ATA, whether it is air or a mixture of O₂ and inert gas, may also include the effects of pressure alone. Thus it will be important in future studies to establish the effects (or lack thereof) of both physical pressure and the chemical effects of high-gas partial pressure.

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**DISCLOSURES**

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**REFERENCES**

10. **Dean JB, Bayliss DA, Erickson JT, Lawing WL, and Millhorn DE.** Depolarization and stimulation of neurons in nucleus


34. Mulkey DK, Reich JL, and Dean JB. Comparison of neuronal barosensitivity in the solitary complex to 1 to 8 atmospheres of helium or air (Abstract). *FASEB J* 13: A825, 1999.


42. Shushakov VV and Demchenko IT. Dynamics of ion currents in the membrane of the isolated mollusk neuron under high pressure. *Respir Physiol* 86: 241–244, 1996.


