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

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Mulkey, Daniel K., Richard A. Henderson III, Robert W. Putnam, and Jay B. Dean. Pressure (≤4 ATA) increases membrane conductance and firing rate in the rat solitary complex. J Appl Physiol 95: 922–930, 2003. First published April 18, 2003; 10.1152/japplphysiol.00865.2002.—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+-chemosensitive and -insensitive 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; hyperoxia; neuron.
neuronal firing rate and $R_{in}$ 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 barosensitive to moderate levels of pressure, if it occurred, was mediated by an O$_2$ free radical process. Thus we planned to determine whether our brain stem slice preparation resulted in increased O$_2$ free radical production. Thus we planned to determine whether use of HBHe, similarly, in this setting might alter neuronal excitability, as determined by changes in neuronal CO$_2$/H$^+$ chemosensitivity. The rationale for this study is that neuronal CO$_2$/H$^+$ chemosensitivity 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 \(-2\) ATA/min. Brain slice temperature was maintained at \(37 \pm 0.5^\circ\)C during helium compression and decompression (12).

HBO$_2$ and hypercapnic perfusates. The details for preparation of hyperoxic and hypercapnic solutions were given in the preceding paper (33). Briefly, hyperoxic oxygenated perfusate (i.e., HBO$_2$) with normocapnia was made by equilibrating aCSF with 95 or 98% O$_2$ at 2.2 or 3.3 ATA in separate high-pressure sample cylinders to produce P$_{O2}$ values in the aCSF of \(-1,640\) and \(-2,470\) Torr, respectively. P$_{CO2}$ 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$_{CO2}$ 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 vitamin 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 intracellular microelectrodes. The electrophysiological properties that were measured and method of data collection were as described in the preceding paper (33). Paired-samples t-tests \((P \leq 0.05)\) were used to determine whether the average firing rate, \(R_{in}\), or amplitude of afterhyperpolarization potential (AHP) responses to pressure differed (i.e., mean population differences during HBHe compared with 1 ATA control) significantly from zero. Contingency tables were used to compare tabulated electrophysiological properties, as well as the incidence in SC neurons of CO$_2$/H$^+$ chemosensitivity, HBO$_2$ sensitivity, and barosensitivity. The significance of associations 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$_{O2}$, P$_{CO2}$) 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 A xo-
clamped-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 $R_{in}$ of $110 \pm 4$ MΩ (range $36$–222 MΩ).

**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 $R_{in}$ ($t$-test, change (Δ) in $R_{in} = -7.4 \pm 3.2$ MΩ; $n = 23$ neurons) (Fig. 1C). In addition, pressure decreased the amplitude of the AHP from 22.7 ± 1.1 mV to −1.7 ± 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 $R_{in}$ 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 baroinsensitive, i.e., they did not increase firing rate in response to helium compressions up to 4 ATA (Fig. 3, A and B). Similarly, baroinsensitive neurons did not show a significant change in $R_{in}$ ($t$-test, $P > 0.25$, $\Delta R_{in} = -1.1 \pm 3.5$ MΩ, $n = 44$) or AHP amplitude ($t$-test, $P > 0.25$, $\Delta AHP = 0.08 \pm 0.3$ 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 $R_{in}$. 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 = $1.39 \pm 0.31$ impulses/s) was retained in synaptic block medium ($\Delta$ firing rate = $3.6 \pm 2.25$ 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\textsubscript{2}/H\textsuperscript{+} 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\textsubscript{2}/H\textsuperscript{+} chemosensitivity and barosensitivity. Second, to determine whether pressure affects CO\textsubscript{2}/H\textsuperscript{+} 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\textsubscript{2}/H\textsuperscript{+}-chemosensitive neurons responded to normobaric hypercapnia with increased firing rate and increased \(R_{in}\) (Fig. 4A). We found that, of the 58 neurons exposed to normobaric hypercapnic acidosis, 30 (52\%) were CO\textsubscript{2}/H\textsuperscript{+}-chemosensitive; of these 30 CO\textsubscript{2}/H\textsuperscript{+}-chemosensitive neurons, 10 neurons were also barosensitive (Fig. 4, A and C). The remaining 20 CO\textsubscript{2}/H\textsuperscript{+}-chemosensitive neurons, however, did not respond to pressure. A: traces of PB and jFR (impulses/s) show that compression to 3 ATA with 100\% helium did not significantly affect FR. B: representative \(V_m\) traces show that the recording was healthy and stable during compression and decompression. C: expanded mean \(V_m\) traces \((n = 5)\) during hyperpolarizing current injection of \(-0.2\) nA for 150 ms show that compression to 3 ATA did not affect \(R_{in}\). D: the average of 5–10 expanded spontaneous action potentials measured at 1 and 3 ATA, at the times indicated in A, was superimposed to show that pressure did not affect the AHP in baroinsensitive neurons. B and C: traces 1–3 were taken at the times indicated in A.
not respond to pressure (Fig. 4C). Of the 28 neurons that did not respond to \( \text{CO}_2/\text{H}^+ \), 9 were barosensitive (Fig. 4B), whereas 19 did not respond to either \( \text{CO}_2/\text{H}^+ \) or pressure (Fig. 4C). These results indicate that, whereas some \( \text{CO}_2/\text{H}^+ \)-chemosensitive neurons were also barosensitive, there was not a significant association between barosensitivity and \( \text{CO}_2/\text{H}^+ \)-chemosensitivity (\( \chi^2 \) analysis, \( P = 0.854 \)). In other words, barosensitive neurons included both \( \text{CO}_2/\text{H}^+ \)-chemosensitive and \( \text{CO}_2/\text{H}^+ \)-chemoinsensitive neurons. Similarly, there was not a significant association between \( \text{CO}_2/\text{H}^+ \)-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 PCO\(_2\) 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 \( \text{CO}_2/\text{H}^+ \) chemosensitivity (Fig. 5A), and firing rate increased by the same amount in response to \( \text{CO}_2/\text{H}^+ \) at 1 and 3 ATA (Fig. 5B). These results indicate that \( \text{CO}_2/\text{H}^+ \) chemosensitivity of SC neurons is unaffected by 2–4 ATA of hyperbaric pressure.

**Pressure and HBO\(_2\) sensitivity.** In the preceding paper, we report that increased PO\(_2\) stimulates some SC neurons (i.e., HBO\(_2\) sensitive), specifically, putative \( \text{CO}_2/\text{H}^+ \) chemoreceptors (33). Therefore, to determine whether pressure per se, in addition to increased PO\(_2\),
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 baroinsensitive 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 CO2/H+ chemosensitivity (Fig. 4), and, as already reported (33), HBO2 and CO2/H+ 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 the 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 ~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 CO2/H+ chemosensitive neurons (33), which are thought to function as central CO2/H+ chemoreceptors (10, 11, 18, 35, 40), as well as neurons insensitive to either increased O2 or increased CO2/H+.

**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 = 4 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 O₂ 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 P_O₂, P_CO₂, and/or N₂ 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* pace-maker cells, with a corresponding 30% decrease in R_in (48).

The membrane and synaptic 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 synaptic blockade in high-Mg²⁺-low-Ca²⁺ medium. We did not determine the effects of pressure on synaptic transmission in this study, but we anticipate that it will also be affected by increased pressure. Previous studies (16, 17, 50) have shown that P_b ≥ 35 ATA decreased both excitatory and inhibitory synaptic 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 neurological response to pressure per se that occurs in divers breathing heliox 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 convolution 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 Ca²⁺) 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 Ca²⁺-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 Ca²⁺-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 H2O2 (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 H2O2 sensitivity, a highly oxidative condition (30, 31, 33), and barosensitivity in the SC. In addition, H2O2 and pressure had opposite effects on Rmax; H2O2 stimulated firing rate and increased Rmax (33), whereas HBHe stimulated firing rate but decreased Rmax. 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 ≤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. Neurosci Behav Physiol 26: 241–244, 1996.

43. Southan AP and Wann KT. Effects of high helium pressure on intracellular and field potential responses in the CA1 region of the in vitro rat hippocampus. Eur J Neurosci 8: 2571–2581, 1996.


