Effects of nitrogen and helium on CNS oxygen toxicity in the rat

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Effects of nitrogen and helium on CNS oxygen toxicity in the rat. J Appl Physiol 98: 144–150, 2005. First published August 20, 2004; doi:10.1152/japplphysiol.00506.2004.—The contribution of inert gases to the risk of central nervous system (CNS) oxygen toxicity is a matter of controversy. Therefore, diving regulations apply strict rules regarding permissible oxygen pressures (PO2). We studied the effects of nitrogen and helium (0, 15, 25, 40, 50, and 60%) and different levels of PO2 (507, 557, 608, and 658 kPa) on the latency to the first electrical discharge (FED) in the EEG in rats, with repeated measurements in each animal. Latency as a function of the nitrogen pressure was not homogeneous for each rat. The prolongation of latency observed in some rats at certain nitrogen pressures, mostly in the range 100 to 500 kPa, was superimposed on the general trend for a reduction in latency as nitrogen pressure increased. This pattern was an individual trait. In contrast with nitrogen, no prolongation of latency to CNS oxygen toxicity was observed with helium, where an increase in helium pressure caused a reduction in latency. This bimodal response and the variation in the response between rats, together with a possible effect of ambient temperature on metabolic rate, may explain the conflicting findings reported in the literature. The difference between the two inert gases may be related to the difference in the narcotic effect of nitrogen. Proof through further research of a correlation between individual sensitivity to nitrogen narcosis and protection by N2 against CNS oxygen toxicity in rat may lead to a personal O2 limit in mixed-gas diving based on the diver sensitivity to N2 narcosis.

Hyperbaric oxygen; electroencephalogram; nitrogen narcosis; inert gas; diving

Central Nervous System (CNS) oxygen toxicity can appear in humans on exposure to oxygen pressures above 130 kPa (7, 17, 27, 29), manifesting as convulsions (similar to epileptic seizures, grand mal) and loss of consciousness, without any warning symptoms. It is a risk encountered in diving with mixtures of nitrogen and oxygen (nitrox); nitrogen, oxygen, and helium (trimix); and with oxygen-helium mixtures in sport and professional diving to depths >30 m. The risk of oxygen toxicity is always considered when planning deep dives. The contribution of inert gases to the risk of oxygen toxicity is a matter of controversy: a few studies suggest a protective effect, whereas others claim increased risk. Therefore, diving regulations apply strict rules regarding permissible oxygen pressures (PO2) when diving with mixed gas.

A number of studies suggest that inert gases have a protective effect or no effect at all on the risk of oxygen toxicity. Burns (15) showed that the latency to hyperoxic convulsions in mice at oxygen pressures of 405, 608, and 810 kPa increased as the percentage of oxygen in the gas mixture decreased from 100% to 50% and 25%. Almqvist et al. (1) studied hyperoxic convulsions in mice in 100, 65, and 45% oxygen at a PO2 of 405 kPa. They concluded that nitrogen pressure had no effect on CNS oxygen toxicity. Latency to convulsions in the rat was no different at a PO2 of 537 kPa for exposure to pure oxygen or to an oxygen-helium mixture at a total pressure of 1,884 kPa (11).

Other studies reported an increased risk of oxygen toxicity with increasing pressure of the inert gas. The latency to convulsions in the rat at a PO2 of 537 kPa and a total pressure of 5,370 kPa (90% helium and 10% oxygen) was shorter than with pure oxygen (11), and the latency at a PO2 of 537 kPa decreased at a total pressure of 1,884 kPa when the inert gas was nitrogen or argon (10). The latency to convulsions in mice at a PO2 of 406, 507, and 608 kPa was reduced by the admixture of nitrogen, which reduced the concentration of oxygen to 75 and 55% (8). Latency to convulsions in mice at oxygen pressures of 253, 304, and 405 kPa decreased as the pressure was increased with helium to 1,010, 3,040, and 4,050 kPa, respectively (14).

Other studies (including some of those mentioned above) showed nonhomogeneity of the response and effects related to the inert gas. Latency to the first electrical discharge (FED) that precedes clinical seizures in the rat varied for different mixtures of oxygen-nitrogen or oxygen-helium (13). The latency decreased when the total pressure rose to 810 kPa and increased at pressures above this up to 1,013 kPa. Changing the inert gas from N2O to N2 without changing the PO2 (337 or 400 kPa) resulted in the appearance of CNS oxygen toxicity, whereas changing the inert gases in the opposite direction did not (21). The reduction in latency to CNS oxygen toxicity became more pronounced with an increase in density of the inert gas, from helium to nitrogen and nitrogen to argon (10).

In his analysis of unusual incidents in well-documented series of dives, Leitch (22) suggested that the combination of elevated pressure and the inert gas may be the cause of oxygen toxicity in divers. It has been suggested that other fatalities in diving might also be related to CNS oxygen toxicity (27). Although the effect of inert gas on CNS oxygen toxicity remains an unsettled issue, diving authorities (National Oceanic and Atmospheric Administration, American Nitrox Divers International, National Association of Underwater Instructors, and the US Navy) are cautious and impose strict regulations on permissible levels of PO2 in mixed-gas diving (12, 20, 28, 29).

In our laboratory, we developed a well-controlled experimental system for the study of CNS oxygen toxicity. Repeated measurements from the same rat enable us to overcome the interanimal variability. By temperature control and adjustment of the rat to the high pressure, we prevent any increase in metabolic rate. We suggest that a controlled study of the effects of nitrogen and helium and different levels of PO2 should clear...
up some of the controversial issues regarding the effect of inert gases on CNS oxygen toxicity.

METHODS

Animals

White male white Sprague-Dawley rats (300–350 g) had EEG electrodes implanted under pentobarbital sodium anesthesia (50 mg/kg ip) 3 days before the experiment. The electrodes were stainless steel screws penetrating the skull in the parietal area. Insulated wires attached to a female miniconnector were soldered onto the screws, and the miniconnector was fastened to the skull with dental cement. Forty-seven rats were used for the nitrogen effect and 31 for the helium effect. The experimental procedure was approved by the Israel Ministry of Defense Animal Care Committee, and the rats were handled in accordance with internationally accepted human standards.

Experimental System and Procedure

Experimental cage. The experimental cage was a metal, double-walled cage (25×11×12 cm). One wall for observation of the animal and the top cover, which could be opened, were made of Plexiglas (for details, see Fig. 1 in Ref. 2). Thermoregulated water was pumped through the double wall to control the ambient temperature. The incoming gas flowed through a metal container attached to the cage wall for temperature equilibration before entering the cage. The metal walls of the cage were covered on the outside with thermal insulating material. A cable with a male miniconnector for EEG recording passed through the top cover. A humidity and temperature measuring device (EE20FT, EE Electronics) was inserted through the top of the cage.

Experimental system. The miniconnectors were mated, and the rat was placed in the experimental cage, which was placed in a 150-liter pressure chamber (Roberto Galeazzi, La Spezia, Italy). The mixture of inert gas and oxygen in the cage was controlled by needle valves and by observation of two flowmeters situated inside the pressure chamber, one for the inert gas and the other for the oxygen. The outgoing gas exited via a bypass tube into the atmosphere of the pressure chamber. A small portion of the outgoing gas was directed out of the pressure chamber (this was controlled by another needle valve), passed through a flowmeter, and was sampled for O2 concentrations by an O2 analyzer (Servomex, Sussex, UK). Water hoses were connected to ports in the pressure chamber and to the ports in the experimental cage for recirculation of the thermoregulated water (C/H Temperature Controller Bath and Circulator 2067, Forma Scientific, Marietta, OH). The temperature inside the experimental cage was 27.4±1°C and the humidity was 31±1%. The EEG was recorded on a chart recorder (Gould, Cleveland, OH).

Experimental procedure. The rat was placed in the experimental cage, the EEG miniconnectors were mated, and the cage was placed in the pressure chamber. The rat was unrestrained and could move about freely inside the cage. When the pressure in the chamber was being raised (at 180 kPa/min), the gas mixture flowing through the cage was kept at normoxia (21 kPa O2) by adjusting the two needle valves. When the desired pressure was reached, a period of 5 min was allowed for acclimation to the experimental conditions (pressure and ambient temperature) at normoxia. The high pressure in the present study (up to 1,646 kPa) at normoxia increases the risk of inert gas loading and decompression sickness. Therefore, we shortened the adjustment period we used in previous studies (2–5) from 20 to 5 min. The rat was observed relaxed after the 5-min adjustment. At the end of this period, the composition of the gas mixture was changed to raise the P02 to the predetermined level. The EEG signal was amplified and recorded continuously on chart recorder; ambient temperature was read and recorded. The rat was observed through a window in the pressure chamber for signs of clinical seizure activity. When the first electrical discharge (FED) in the EEG, which precedes classical convulsions, was seen on the recorder, the time was noted and decompression was commenced according to a computer-generated decompression table, whereas the P02 was maintained at 300 kPa. If the FED did not appear within 60 min of exposure, the hyperoxic exposure was terminated to prevent the development of pulmonary oxygen toxicity. The cage was removed from the pressure chamber and the rat was freed from the experimental system.

Decompression. On the reduction of pressure (decompression) after the tissue are loaded with inert gas at high pressure, the evolution of bubbles can cause decompression sickness. The risk of decompression sickness and CNS oxygen toxicity should both be accounted for in the decompression schedule. We wrote a decompression program based on the parameters suggested by Lillo and Parker (23). We selected a 5% risk (about twice the risk used in US Navy Diving Manual) limit for our calculations from the equation:

\[
\text{probability (DCS)} = \frac{\text{dose}^n}{(\text{dose} + P_{50})^n}.
\]

The parameters for the probability equation were:

\[
n = 6.48 \quad \text{and} \quad P_{50} = 4.67.
\]

The dose of nitrogen in ATA was:

\[
\text{dose} = P_{\text{ratN}_2} - P_{\text{ambN}_2},
\]

where \(P_{\text{ratN}_2}\) is the rat tissue nitrogen tension and \(P_{\text{ambN}_2}\) is the ambient nitrogen pressure (both in ATA). The loading and unloading of nitrogen were calculated from Eqs. 3 and 4, respectively:

\[
P_{\text{ratN}_2} = \left( P_{\text{ambN}_2} - P_{0\text{N}_2} \right) \times \left( 1 + e^{-0.006\tau} \right) + P_{0\text{N}_2}
\]

and

\[
P_{\text{ratN}_2} = \left( P_{0\text{N}_2} - P_{\text{ambN}_2} \right) \times e^{-0.006\tau} + P_{\text{ambN}_2},
\]

where \(\tau\) is the time step, \(P_{0\text{N}_2}\) and \(P_{\text{ratN}_2}\) are the nitrogen tension in rat tissue at the beginning and end of the time step, respectively. For helium as the inert gas, the dose was calculated as:

\[
\text{dose} = P_{\text{rHe}} \times 0.91 - P_{\text{ambHe}},
\]

and for the loading and unloading of He the time constant in Eqs. 3 and 4 was changed from 0.0685 to 0.2105 and the symbol \(N_2\) was replaced by \(He\).

During the development of the decompression procedure, we initially selected a P02 of 400 kPa for decompression. In a few cases at the highest pressures tested, clinical seizures developed and did not stop, and the P02 was therefore changed to 300 kPa. The pressure range in the present experiment (up to 1,646 kPa) was higher than the pressure tested for calculation of the parameters by Lillo and Parker (up to 945 kPa) (23). After a rat came out of the experiment seemingly without any symptoms but was found dead 1 day later, we added a half-hour stay at 300 kPa oxygen before the final decompression. No decompression sickness was observed thereafter.

The program was run by the experimenter online. He fed in the bottom pressure and the P02 for the exposure. The program displayed the compression time and the concentration of oxygen for the hyperoxic exposure. The experimenter increased the pressure and adjusted the oxygen concentration while observing a table to keep the rat in normoxia. Inert gas loading was calculated during compression at 180 kPa/min and the 5 min adjustment to pressure in normoxia. On the appearance of the FED, the experimenter fed the latency time into the program and changed the mixture to yield 300 kPa oxygen. The program calculated the load of inert gas at the end of the hyperoxic exposure and presented a table for decompression. The table consisted of pressure reduction at 180 kPa/min and decompression stops of 5 min. The program displayed the concentration of oxygen for each step. When reducing the pressure in steps of 1/20 of the complete decompression schedule led to more than a 5% risk of DCS, the step was extended by another 5 min. When the pressure was reduced to 300 kPa, half-hour stop was made on pure oxygen before decompres-
sion to ambient pressure. After being released, the rat was observed for one-half an hour for signs of decompression sickness.

Experimental protocol. In previous studies, we showed that the sensitivity of CNS oxygen toxicity to different modulators (CO₂, metabolic rate, anti-seasickness medications) decreases with increasing PO₂ (2, 3, 6). In the present study, we therefore used a number of oxygen pressures. Four oxygen pressures were used for the hyperoxic challenge: 507, 557, 608, and 658 kPa. Six combinations of oxygen and inert gas were used for each PO₂: 100, 85, 75, 60, 50, and 40% O₂. Thus the total pressure range used was from 507 to 1,646 kPa.

Each rat was subjected to a different exposure every 2–3 days. In previous studies (4, 5), we showed that the preceding exposure has no effect on the time to the FED in the following one if there is a 2-day interval in between. This procedure enabled repeated measurements to be taken from the same animal, reducing the variability, because we have shown that intra-animal variability is much lower than interanimal variability (4, 5). Each rat was subjected initially to pure oxygen at the selected pressure, and then randomly to the other oxygen-inert gas mixtures yielding the same PO₂. We could not complete all six exposures in all of the rats due to occasional disconnection of the miniconnector. When a rat completed the six exposures at one oxygen pressure, it was used for another oxygen pressure.

Statistical analysis. Test for normal distribution (Shapiro-Wilk) rejected this distribution for the latencies to the CNS oxygen toxicity. However, the same analysis for the logarithmic transformation of the latency times yielded accepted normal distribution. The effect of inert gas pressures and PO₂ on latency to the FED (logarithm of time) were evaluated using three variables (PO₂, N₂, or He, and inert gas pressure) ANOVA with repeated measures on inert gas pressures (Mixed Procedure, SAS Institute, Cary, NC). When the interaction was significant, specific differences of least-square means were tested (Tukey-Kramer).

RESULTS

Decompression

The established experimental procedure was used successfully. In all the multiple exposures of 47 rats to nitrogen-oxygen mixtures, only one animal died. Although no symptoms were observed at the end of the exposure, the rat was found dead the following day. No other observable symptoms of decompression sickness were noted. No decompression sickness was observed in the 31 rats exposed to helium-oxygen mixtures.

Latency to the FED

Latencies to the FED were affected by the species of the inert gas (N₂ and He; P < 0.0001), by the PO₂ (P < 0.0001), and by the inert gas pressure (P < 0.0001). The interaction of PO₂ × inert gas species was significant (P < 0.03), but other interactions, inert gas species × inert gas pressure, PO₂ × inert gas pressure, and PO₂ × inert gas species × inert gas pressure, were not significant (P = 0.20, 0.24, and 0.49, respectively). Latency to the FED was affected by all five inert gas pressures (mixtures 2–6) compared with pure oxygen (P < 0.0004): second mixture was different from third to sixth mixtures (P < 0.002), third was different from fifth to sixth (P < 0.006), fourth was different from sixth (P < 0.003) but not from fifth, and fifth was different from sixth (P < 0.03).

Nitrogen

We discovered that latency to the FED as a function of nitrogen pressure at constant PO₂ was not homogeneous for each rat. The prolongation of latency observed in some rats at certain nitrogen pressures (with respect to latencies at lower PN₂S) was superimposed on the general trend for a reduction in latency as nitrogen pressure increased. This pattern was most probably an individual trait rather than a sign of random variability. For determination whether the nonhomogeneous response was due to measurement variation or represented a specific individual pattern, in a few rats, measurements were repeated twice at few PN₂S. We found that each animal had its own response pattern. Data from three rats with different response patterns are shown in Fig. 1. For example, in rat 19, latency was prolonged at a PN₂ of 89 kPa, and repeated exposures to 0, 89, and 169 kPa nitrogen yielded latencies close to those measured in the first exposures. Repeated exposures in rats 21 and 29 also pointed to a specific individual pattern.

Because of the heterogeneous response of the latency to the FED, we present the results for each PO₂ in a figure with three panels: one for rats without any prolongation of latency at any PN₂, the second for animals in which latency was prolonged only at PN₂S lower than 200 kPa, and the third for rats in which this occurred also at PN₂S above 200 kPa. The term prolongation is used when latency at a specific PN₂ was longer than at a lower PN₂. The purpose of division into three panels is for clear observation and not because there are three distinct groups of responses.

Mean latency to the FED at a PO₂ of 507 kPa did not change up to a PN₂ of 200 kPa, but it shortened as PN₂ increased beyond this value. The high variability is related to the variation in the individual response, which is shown for 13 rats in Fig. 2. Only rats with sufficient data to present the full response are shown in the figure. In some rats (~25%) there was almost no response to increased PN₂ or no prolongation of the latency at a specific PN₂ (Fig. 2, bottom). In others (~50%), latency was prolonged at PN₂S below 200 kPa (Fig. 2, middle). The 60-min latencies represent termination of the hyperoxic exposure after no FED was observed. In a third group of rats (~25%, Fig. 2, top), latency was prolonged at PN₂S above 200 kPa.

Fig. 1. Latency to central nervous system (CNS) oxygen toxicity [first electrical discharge (FED), see Nitrogen] plotted against the nitrogen pressure in 3 rats with repeated exposures in the same conditions. Rat number and the oxygen pressure are shown in the key.
Mean latency to the FED at a PO$_2$ of 557 kPa changed very little up to a PN$_2$ of 200 kPa, but it shortened as PN$_2$ increased beyond this value. The distribution of the individual response of latency to the FED to PN$_2$ (Fig. 3) was similar to that observed for 507 kPa. Of the 12 rats presented, in only one animal (10%) was there no prolongation of latency in response to PN$_2$. In 50%, latency was prolonged below 200 kPa N$_2$. In 40%, latency was prolonged at PN$_2$s above 200 kPa, whereas in some of these animals this was also observed below 200 kPa.

In the smaller range of mean latency to the FED at a PO$_2$ of 608 kPa, the effect of PN$_2$ was small. The distribution of the 13 rats over the three categories was similar to that observed for 507 and 557 kPa (Fig. 4). As PN$_2$ increased at PO$_2$ of 658 kPa, there is a trend for decrease in mean latency to the FED. In ~40% of the 13 rats, there was no prolongation of latency in response to PN$_2$ (Fig. 5). The percentage of rats with a bimodal response of latency to PN$_2$ was lower at 608 and 658 kPa O$_2$ than at the lower PO$_2$s. Mean latencies for the four PO$_2$s are presented in Fig. 6, bottom.

**Helium**

The mean latency to CNS oxygen toxicity in rats at the four oxygen pressures is shown in Fig. 6, top. An increase in PHe caused a reduction in the latency to CNS oxygen toxicity. This reduction was prominent at a PO$_2$ of 507 kPa and reached a small effect at PO$_2$s of 557 and 608 kPa. Because the latency had almost reached a plateau at a PO$_2$ of 658 kPa, we conclude that the PHe would have little or no further effect on latency. In contrast to nitrogen, when helium was the inert gas, no individual prolongation of the latency to CNS oxygen toxicity was
A number of mechanisms may be involved in the effect of inert gas on oxygen toxicity (18). 1) Bennett (9, 11) suggested that hypoventilation while breathing a compressed gas caused elevation of tissue CO2, which enhanced the development of oxygen toxicity. Thus the reduction in latency to CNS oxygen toxicity and the increase in cerebral PCO2 were related to the gas density. 2) Synergism of high helium pressure and hyperoxia in producing the convulsions of oxygen toxicity was demonstrated by Brauer and Beaver (14). High pressure, which may cause the high-pressure nervous syndrome (HPNS), increased the risk of CNS oxygen toxicity. 3) High pressure alone may enhance reactions generating reactive oxygen species (ROS). The generation of superoxide radicals in vitro was enhanced in a similar fashion by the addition of inert gases (He, N2, and Ar) at pressures of 200–6,000 kPa (30). 4) We (and others) demonstrated that increased sensitivity to CNS oxygen toxicity is related to the increase in metabolic rate (2). An ambient temperature lower than the lower critical temperature can raise the metabolic rate. Some of the above mentioned studies report ambient temperatures that may be within the thermoneutral zone (14, 15), others report ambient temperatures below the thermoneutral zone (13, 21), and still others do not mention the ambient temperature (1, 8, 10, 11). Therefore, the involvement of increased metabolic rate cannot be excluded from our interpretation of some of the previous studies. It is also possible that with very high density of the gas, the work of breathing increased the metabolic rate.

DISCUSSION

Fig. 4. Individual latency to CNS oxygen toxicity plotted against PN2 for exposure to 608 kPa O2. Other symbols as in Fig. 2.

Fig. 5. Individual latency to CNS oxygen toxicity plotted against PN2 for exposure to 658 kPa O2. Other symbols as in Fig. 2.

observed at any PHe and the variability was smaller compared with that with nitrogen.
Fig. 6. Mean latencies to CNS oxygen toxicity as a function of PN2 or PHe (abscissa) and PO2 (key to symbols appears at top).

Inert gases have a greater effect on latency to CNS oxygen toxicity in the low range of inert gas pressures and the low range of oxygen pressures (Fig. 6). The greater effect of inert gases at low oxygen pressures is in accordance with other findings from our laboratory that any modulator affecting CNS oxygen toxicity, be it metabolic rate, inspired CO2, or cinna-rizine, will express its effect mostly in the low range of toxic PO2s (2, 3, 6).

With both inert gases, there was a clear reduction of latency to CNS oxygen toxicity at the highest pressures tested. Although it is accepted that HPNS manifests at pressures above 1,500 kPa, the process that leads to HPNS probably contributes to the development of CNS oxygen toxicity as well. Thom (30) proved that inert gas in the pressure range of 200 to 600 kPa enhances the production of superoxide radicals. Pressure below 405 kPa increased membrane conductance and firing rate in neurons of the solitary complex in the brain stem of the rat (26), and Brower and Beaver (14) demonstrated synergism of HPNS and CNS oxygen toxicity at 1,013 kPa and above.

The main finding in the present study is the bidirectional effect of nitrogen pressure and that this is an individual trait. The individual response pattern to PN2 is specific to each rat rather than a random effect, as can be gleaned from Fig. 1. The bimodal response and the variation in response between rats, together with the possible influence of certain experimental conditions on metabolic rate, may explain the conflicting findings reported in the literature and discussed in the introduction.

The bimodal response to PN2 was not found with PHe. The difference between the two inert gases may be related to the difference in their narcotic effect. Various narcotics such as ketamine and N2O have a protective effect against CNS oxygen toxicity (16, 21). It is possible that difference in individual sensitivity to nitrogen narcosis in the rat (similar to the observation in humans) may explain the difference in the protection afforded by PN2 against CNS oxygen toxicity. Because helium is nonnarcotic, the effect of helium is to shorten the latency to CNS oxygen toxicity. Further assessment of the relationship between individual sensitivity of CNS oxygen toxicity to PN2 in the rat and its specific sensitivity to nitrogen narcosis may pave the way for planning individual oxygen limits in mixed-gas diving based on the diver’s personal sensitivity to nitrogen narcosis.

The introduction of trimix diving was aimed at avoiding nitrogen narcosis and obtaining the protective effect of narcosis against HPNS. The present study suggests that trimix may also afford protection against CNS oxygen toxicity. However, both the beneficial and the harmful effect may be related to individual traits. Further evaluation of the effect of inert gas on
CNS oxygen toxicity may improve safety in trimix and nitrox diving.

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