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CNS oxygen toxicity in oxygen-inert gas mixtures

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Bitterman N, Laor A, Melamed Y. CNS oxygen toxicity in oxygen-inert gas mixtures. Undersea Biomed Res 1987; 14(6):477–483.—Central nervous system oxygen toxicity in hyperbaric oxygen-inert gas mixtures was studied by exposing male rats to various gas mixtures having the same oxygen partial pressure and varying pressures of inert gases. The duration of the latent period until the appearance of electrical discharges in the electroencephalogram was used as the criterion for the sensitivity to CNS oxygen toxicity. Two hundred and twenty rats with chronically implanted cortical electrodes were subjected to a single exposure to 1 of 11 different gas mixtures at ambient pressures ranging from 5 to 10 ATA. All gas mixtures had a constant oxygen partial pressure of 5 ATA and varying pressures of inert gas (helium or nitrogen). The duration of the latent period was found to be significantly different in the 11 experimental groups ($P < 0.001$). While increasing the inert gas pressure, the latency gradually shortened, reaching the lowest values in mixtures of 5 ATA oxygen and 3 ATA of either inert gas. On further increase in the inert gas pressure up to a total ambient pressure of 10 ATA, the latency returned to control (pure 5 ATA oxygen) values. No difference was found between nitrogen and helium of equal partial pressures. These findings suggest that the risk of CNS oxygen toxicity in gas mixtures is not determined solely by the P_{O_2}, and even a relatively low pressure of inert gas can contribute to the imminence of hyperbaric-oxygen-induced seizures.

hyperbaric oxygen
hyperoxia
inert gas
electroencephalogram
rat

Central nervous system oxygen toxicity has long been assumed to be determined merely by the oxygen partial pressure (P_{O_2}) or as Bert (1) stated a century ago: "La tension d'oxygene est tout" (the tension of oxygen is all that matters). However, the increasing use of compressed gas mixtures containing high percentages of oxygen raised doubts as to whether the risk of CNS O_2 toxicity is indeed determined solely by the P_{O_2}, regardless of the inert gas with it.

While trying to construct limits for CNS O_2 toxicity in divers using oxygen-nitrogen mixtures, Lanphier (2) found, unexpectedly, that a given partial pressure of oxygen was more toxic in the presence of inert gas. Animal studies provided contradictory information. Although Bennett (3), Bartelson et al. (4), Brauer and Beaver (5), and Hills (6) found increased sensitivity to CNS oxygen toxicity while breathing compressed oxygen-inert gas mixtures, Burns (7) found a decrease in the susceptibility

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to the toxic effects of oxygen with an increase in absolute pressure and a fixed P_{O_2}. In contrast, a report by Almqvist et al. (8) supports the classic concept that CNS O_2 toxicity depends entirely on the P_{O_2}.

A recent retrospective analysis of diving accidents (9) presents an increased incidence of high oxygen pressure (HOP) convulsions among divers using various oxygen-inert gas mixtures, although the calculated P_{O_2} of their breathing mixture was known to be safe when breathing pure hyperbaric oxygen.

Our purpose was to examine the imminence of CNS oxygen toxicity in oxygen-inert gas mixtures. We monitored well-defined electrical discharges in the EEG, which express CNS oxygen toxicity (10) in unanesthetized, free-moving rats exposed to various oxygen-inert gas mixtures, all having the same P_{O_2}.

METHODS

Experiments were carried out on male rats of Charles River strain weighing 220–280 g, fed on commercial food ad libitum, and kept in a normal day-night cycle.

Under short-term anesthesia, the animal's skull was exposed and four stainless steel screws were introduced into the bone bilaterally over the parietal and posterior cortex. Soldered wires connected the screws to a miniature connector fixed to the bone with dental cement. After a recovery period of 4 d, a male plug with cable was inserted into the connector and a continuous two-channel bipolar recording of the EEG could be obtained from the free-moving rat.

The rats were exposed to the various gas mixtures in a 3-liter Plexiglas cage, which was placed inside a 150-liter animal hyperbaric chamber (T.C.A.M.O., Roberto Galleazi, Italy) compressed with air. This double-cage system enables economic use of gas mixtures. A constant gas flow of 2 liter/min was employed to prevent accumulation of carbon dioxide in the Plexiglas cage. Chamber temperature was maintained at 22°C with a transient rise of temperature up to 2°C during and shortly after the compression.

Two hundred and twenty rats were independently assigned to the different gas mixtures by a completely randomized design. Each rat was exposed only once to one of the oxygen-inert gas mixtures.

The ambient pressure of the gas mixtures ranged from 5 to 10 ATA. All gas mixtures had a constant oxygen partial pressure of 5 ATA and a varying pressure of inert gas (helium or nitrogen). A single identification number (ID) was used to name each of the oxygen-inert gas mixtures. The number describes the partial pressures of different gases as well as the total ambient pressure of the gas mixture. The rightmost digit identifies the P_{O_2}, the tens place is for the P_{N_2}, and the hundreds place identifies the P_{He} of the gas mixture. The sum of the ID digits yields the total pressure of the mixture, i.e., ID 305 describes a gas mixture composed of 5 ATA oxygen, no nitrogen, and 3 ATA helium adding to a total pressure of 8 ATA. Figure 1 is a graphic presentation of the 11 gas mixtures employed in this study.

The duration of the latent period measured from the time the desired pressure was reached until the appearance of electrical discharges in the EEG was used as the criterion for the sensitivity to CNS oxygen toxicity. Additionally, the rats were observed for behavioral changes (e.g., drowsiness, breathing difficulties, irregular pattern of clinical seizures).

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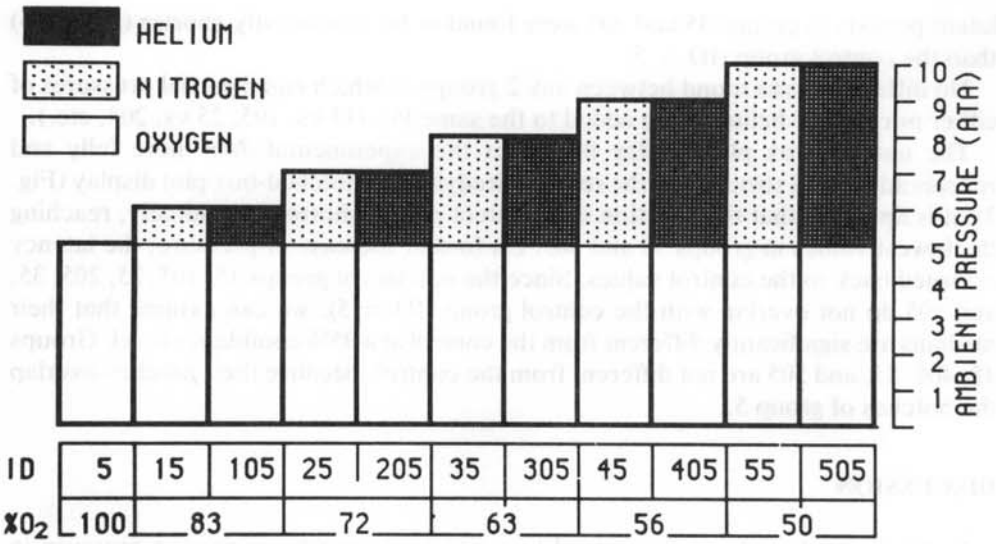

Fig. 1. Composition of oxygen and inert gas pressures in the 11 experimental gas mixtures. ID and percentage of oxygen are marked below each mixture.

Because the dispersion of the experimental values deviated from normal distribution (mostly skewed to the right), we used distribution-free, nonparametric statistical tests. The data are presented by the notched-box plot display (11) showing the extremes, the upper and lower quartiles (75 and 25 percentile, respectively), and the medians. The notches add information by presenting confidence intervals around the medians. In the notched-box display, when the notches about two medians do not overlap at all, the medians are significantly different at about a 95% confidence level (11). A more formal one-way analysis of variance on ranks was performed by the Kruskal-Wallis test (12), testing the equality between the duration of the latent period for convulsions in the 11 groups. Distribution-free, multiple comparisons of independent samples were performed at a 0.05 level of significance by Nemenyi pairwise comparisons of all possible pairs of treatment (13).

RESULTS

The electrical discharges appearing during exposure to high oxygen pressure, both pure and in combination with inert gases, were similar and had the typical spike and wave pattern known for CNS oxygen toxicity. In groups 45 and 55 exposed to 4 and 5 ATA of nitrogen, respectively, several rats appeared slightly drowsy. However, when the electrical discharges started, they lifted themselves upright, stirred, and entered the typical phase of electrical and clinical seizures. The duration of the latent period did not correlate with the severity of the epileptic seizures.

Duration of the latent period was significantly different in the 11 experimental groups, as proved by the Kruskal-Wallis test ($P < 0.001$). To identify the source of inequality of the latent period, a more stringent test by Nemenyi, comparing all possible pairs of treatments was performed. According to the Nemenyi test only the

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latent periods in groups 35 and 305 were found to be significantly shorter ($P < 0.05$) than the control group (ID = 5).

No difference was found between any 2 groups in which equal partial pressures of either nitrogen or helium were added to the same P_{O_2} (15 vs. 105, 25 vs. 205, etc.).

The notched-box plot display illustrates the experimental data more fully and reveals additional structure in the results. Studying the notched-box plot display (Fig. 2), it is apparent that the duration of the latent period shortened gradually, reaching the lowest values in groups 35 and 305. On further increase of pressure, the latency returned back to the control values. Since the notches of groups 15, 105, 25, 205, 35, and 305 do not overlap with the control group (ID = 5), we can assume that their medians are significantly different from the control at a 95% confidence level. Groups 45, 405, 55, and 505 are not different from the control, because their notches overlap the notches of group 5.

DISCUSSION

In the present study, rats exposed to a constant hyperbaric oxygen pressure in combination with various pressures of inert gases exhibited different sensitivity to CNS oxygen toxicity.

Our results, analyzed by the stringent Nemenyi test which compares every possible pair of experimental groups, show that only groups 35 and 305 are significantly different from the control. In these 2 groups, rats were exposed to gas mixtures composed of 5 ATA oxygen and 3 ATA nitrogen or helium, respectively (thus the oxygen concentration in these mixtures was 63%).

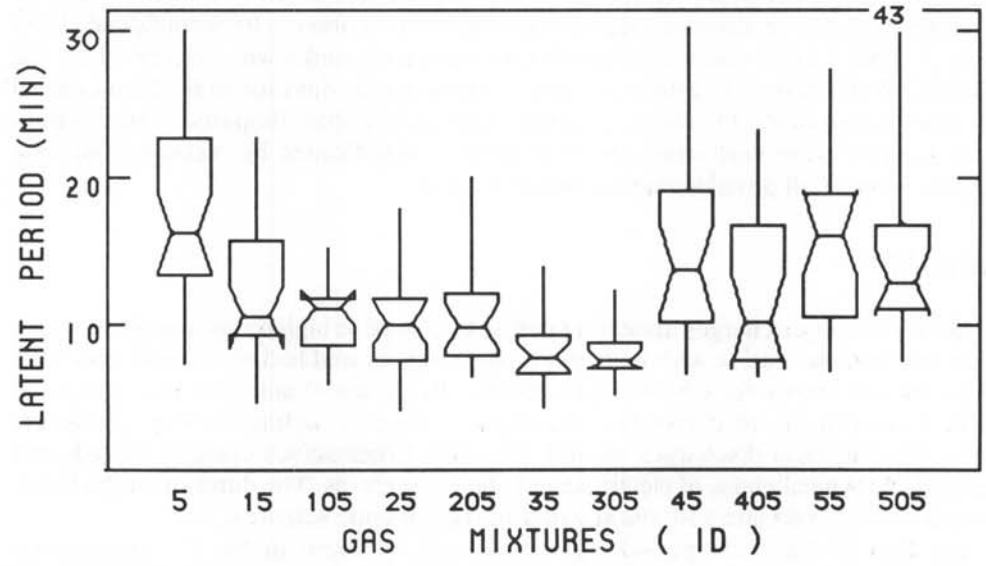

Fig. 2. Duration of the latent period for CNS oxygen convulsions in different oxygen-inert gas mixtures ($n = 20$ for each group). Data are presented by notched-box plot (11) demonstrating extremes, interquartile range (25–75%), medians, and confidence intervals.

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The notched-box plot display allows us to follow the stepwise pattern of the change in the duration of the latent period in the different experiment groups. It can be seen that latency shortens gradually to the lowest values in groups 35 to 305 and then returns to the control values. According to this display not only groups 35 and 305 but groups 15, 105, 25, and 205 are significantly more sensitive to CNS oxygen toxicity. This is in contrast to groups 45, 405, 55, and 505 which exhibit the same sensitivity to CNS oxygen toxicity as the control group, although 4 and 5 atmospheres of inert gas are added to the gas mixture.

If Bert's classical "P_{O_2} concept" is valid (1), the expected sensitivity to oxygen toxicity, expressed by the duration of the latent period, would have been the same in all the exposures to oxygen-inert gas mixtures employed in our study. However, our results present a nonuniform relationship between the development of CNS oxygen toxicity and the pressure of the inert gas added to oxygen. Furthermore, these findings can partially explain the contradictory data concerning the P_{O_2} concept in the literature. It is suggested that different combinations of oxygen and inert gas pressures chosen by various investigators could contribute to the conflicting results (1, 8 vs. 3–6, vs. 7). Another possible source for the conflicting conclusions in the literature is the different criteria for CNS oxygen toxicity that were used in these studies.

The pressure range that we used (5 to 10 ATA) was determined by two restricting factors. Pure oxygen of 5 ATA was used as the control mixture because in the rat this was found to be the lowest oxygen pressure causing CNS toxicity within a plausible time limit (mean latency = 17.8 min) without development of pulmonary oxygen toxicity. Ten ATA ambient pressure was selected as the highest pressure attainable without the appearance of overt nitrogen narcosis or breathing difficulties due to increased gas density. However, this relatively narrow pressure range was sufficient to demonstrate the invalidity of the P_{O_2} concept. Further systematic studies of different oxygen-inert gas pressure combinations are needed to construct tables for the safe use of hyperbaric gas mixtures. Such a study could isolate the most important factor contributing to the increased sensitivity of CNS oxygen toxicity in oxygen-inert gas mixtures. This might elucidate the relative importance of the absolute pressure of the inert gas added, compared to the significance of the ratio between the pressures of oxygen and the inert gas.

Nitrogen and helium were chosen by us and are the most common inert gases used in mixed gas diving. Within the pressure range we studied, both gases had the same effect of CNS oxygen toxicity, although it is well known that they differ in their narcotic potential (14). We suggest, therefore, that the observed effect of these inert gases is of a physical nature, rather than identifying their chemical qualities.

Physical effects of high pressures on neural functions are known (15), but they were reported to be evident at much higher pressures than those used in our study. The mechanism for augmentation of toxicity by low pressures of inert gases, which disappears by further increase of the pressure, is yet unclear.

Bradley (16) has recently suggested that the maximum permitted P_{O_2} used in mixed gas diving should be limited to 1.3 ATA or less (although 1.8 ATA oxygen is considered to be safe in pure oxygen diving). His suggestion referred to Leitch's survey (9), which reported increased incidence of CNS oxygen toxicity among mixed gas divers. Our findings provide additional experimental support for this current practice in commercial diving. From the practical point of view, we suggest that since the P_{O_2}

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concept is valid only within a narrow range of pressures in oxygen-inert gases mixtures, automatic calculation of the P_{O_2} in the breathing mixture should not be considered the only factor predicting the danger of CNS oxygen toxicity.

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Bitterman N, Laor A, Melamed Y. Toxicité du système nerveux central dans des mélanges oxygène-gaz inertes. Undersea Biomed Res 1987; 14(6):477–483.—La toxicité de l'oxygène sur le système nerveux central (SNC) dans des mélanges hyperbares d'oxygène-gaz inertes fut étudiée en soumettant des rats mâles à divers mélanges gazeux ayant une même pression partielle d'oxygène et des pressions de gaz inertes variables. La durée du temps de latence jusqu'à l'apparition de décharges électriques dans l'électroencéphalogramme fut utilisée comme critère pour la sensibilité du SNC à la toxicité de l'oxygène. Des rats (n = 220) avec des électrodes corticales implantées chroniquement furent soumis à une seule exposition à l de 11 mélanges gazeux différents à des pressions ambiantes variant de 5 à 10 ATA. Tous les mélanges gazeux avaient une pression partielle d'oxygéne constante de 5 ATA et des pressions de gaz inertes (azote ou hélium) variables. Il fut trouvé que la durée du temps de latence était significativement différente ($P < 0.001$) chez les 11 groupes expérimentaux. La latence diminua graduellement avec l'augmentation de la pression du gaz inerte, atteignant des valeurs minimales dans des mélanges d'oxygène à 5 ATA et de gaz inerte (l'un ou l'autre) à 3 ATA. Avec l'augmentation additionnelle de la pression du gaz inerte jusqu'à une pression ambiante totale de 10 ATA, la latence retourna aux valeurs témoins (oxygène pure à 5 ATA). Aucune différence ne fut observée entre l'azote et l'hélium à des pressions partielles égales. Ces résultats suggèrent que le risque du SNC à la toxicité de l'oxygène dans des mélanges gazeux n'est pas déterminé uniquement par la P_{O_2}, et que même une pression partielle de gaz inerte relativement faible peut contribuer à l'émergence de convulsions induites par l'oxygène hyperbare.

oxygène hyperbare
hyperoxie
gaz inerte
électroencéphalogramme
rat

Bitterman N, Laor A, Melamer Y. Toxicidad del SNC en mezclas de gas inerte-oxígeno. Undersea Biomed Res 1987;14(6):477–483.—Se estudió la intoxicación por oxígeno del SNC en mezclas de gas inerte y oxígeno en ambiente hiperbárico, mediante la exposición de ratas machos a varias mezclas de gases con la misma presión parcial de oxígeno y variando la presión del gas inerte. La duración del periódo latente hasta la aparición de descargas electricas en el electronecefalograma, se empleó como parámetro de sensibilidad para la intoxicación por oxígeno del SNC. Se sometió a doscientas veinte ratas con implantación crónica de electrodos corticales a una sola exposición de 1 de las 11 diferentes mezclas de gases a presiones ambientales que variaban de 5 a 10 ATA. Todas las mezclas de gases tenían una presión parcial de oxígeno constante de 5 ATA y presiones variables de gas inerte (hélio o nitrógeno). Se encontró que la duración del periódo latente fue significativamente diferente en los 11 grupos experimentales ($P < 0.001$). Al aumentar la presión del gas inerte, se disminuyó gradualmente la latencia, hasta alcanzar los valores más bajos en las mezclas de oxígeno a 5 ATA y 3 ATA de cualquier gas inerte. Al aumentar la presión del gas inerte hasta una presión de 10 ATA, la latencia regresó a los valores del control (oxígeno puro a 5 ATA). No se encontró diferencia entre el nitrógeno y el helio a la misma presión parcial. Estos hallazgos sugieren que le riesgo de la toxicidad del oxígeno para el SNC en mezclas de gases, no se encuentra determinada solamente por la P_{O_2}, y hasta una presión relativamente baja del gas inerte puede contribuir a la aparición de convulsiones inducidas por el oxígeno hiperbárico.

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oxígeno hiperbárico
hiperoxia
electroencefalograma
gas inerte
rata

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