Hyperbaric oxygen pretreatment according to the gas micronuclei denucleation hypothesis reduces neurologic deficit in decompression sickness in rats

Katsenelson K, Arieli R, Arieli Y, Abramovich A, Feinsod M, Tal D. Hyperbaric oxygen pretreatment according to the gas micronuclei denucleation hypothesis reduces neurologic deficit in decompression sickness in rats. J Appl Physiol 107: 558–563, 2009. First published May 21, 2009; doi:10.1152/japplphysiol.91557.2008.—During sudden or too rapid decompression, gas is released within supersaturated tissues in the form of bubbles, the cause of decompression sickness. It is widely accepted that these bubbles originate in the tissue from preexisting gas micronuclei. Pretreatment with hyperbaric oxygen (HBO) has been hypothesized to shrink the gas micronuclei, thus reducing the number of emerging bubbles. The effectiveness of a new HBO pretreatment protocol on neurologic outcome was studied in rats. This protocol was found to carry the least danger of oxygen toxicity. Somatosensory evoked potentials (SSEPs) were chosen to serve as a measure of neurologic damage. SSEPs in rats given HBO pretreatment before a dive were compared with SSEPs from rats not given HBO pretreatment and SSEPs from non-dived rats. The incidence of abnormal SSEPs in the animals subjected to decompression without pretreatment (1,013 kPa for 32 min followed by decompression) was 78%. In the pretreatment group (HBO at 304 kPa for 20 min followed by exposure to 1,013 kPa for 33 min and decompression) was 78%. In the pretreatment group (HBO at 304 kPa for 20 min followed by exposure to 1,013 kPa for 33 min and decompression) this was significantly reduced to 44%. These results call for further study of the pretreatment protocol in higher animals.

A sudden or excessively rapid reduction in ambient environmental pressure, such as during escape from a disabled submarine or the ascent from an aborted underwater dive, or even in high-altitude flight (5), can entail a very serious risk of decompression sickness (DCS). It is widely accepted that DCS is caused by the formation of gas bubbles in body tissues during decompression (12). The most widely accepted theory for the appearance of bubbles in tissue claims that bubbles cannot emerge ex nihilo, but must grow from preexisting gas micronuclei (10, 28, 29). The severity of the symptoms of DCS depends on the size of the bubbles and their location in the body. The most common symptom of DCS is joint pain, which causes a distorted gait known as “the bends.” Bubbles in the brain can cause loss of consciousness and even death, bubbles in the lungs can cause breathing abnormalities known as “chokes,” and bubbles in the spinal cord can result in paralysis (DCS Type II) (8).

It has previously been hypothesized that exposure to hyperbaric oxygen (HBO) at the beginning of a dive would result in replacement of the inert gas in the micronuclei by oxygen, with subsequent consumption of the oxygen by the mitochondria. This would shrink gas micronuclei having the potential to grow into bubbles and thus reduce the risk of DCS. The preoxygenation hypothesis was supported by experiments conducted on the transparent prawn (3). Katsenelson et al. (16) took this one step further and attempted to elucidate whether the hypothesis might be applicable to higher life forms that have a circulatory system and express DCS at the clinical level. We evaluated the injury to the efferent pathway and the protective effect of preoxygenation in rats using a rough assessment of motor performance in a rotating cage. This technique cannot reveal all of the damage induced by DCS or demonstrate the full extent to which it has been prevented, because it is limited to detecting severe injuries that have motor expression. To gain a clear understanding of the phenomenon, it might be better to use more sensitive methods of measuring somatosensory spinal or cortical evoked potentials that are capable of detecting electrical conduction insults that do not always evoke motor performance expression.

Somatosensory evoked potentials (SSEPs) may serve as a valid and objective method for measuring neurologic signals and have been used in the past as a means of evaluating spinal cord injury, including that incurred in DCS Type II (14, 17, 23). SSEPs result from action potentials and synaptic potentials within the dorsal column-lemniscus-thalamocortical sensory system in the central nervous system (30). The stimulus employed excites the somatesthetic and proprioceptive fibers, which lie in the dorsal root ganglion neurons. Sensory information from the trunk and limbs enters the spinal cord. The gray matter of the dorsal horn contains the sensory nuclei, whose axons receive stimulus information from the body’s surface. On entry to the spinal cord, the central axons of dorsal root ganglion neurons branch extensively and project to nuclei in the spinal gray matter and brain stem. Touch and proprioception are transmitted by axons to the dorsal horn of the spinal cord and then to the brain stem and thalamus through the dorsal column-medial lemniscal system. Pain and temperature sense are conveyed by nerves that terminate in the most superficial layers of the spinal or trigeminal dorsal horn. These modalities are conveyed directly, and through multisynaptic networks, to the thalamus via the contralateral anterolateral pathway (15).

This procedure can be carried out under anesthesia and is, therefore, also an accepted method for use in a pain model. SSEPs are characterized by positive tracking of negative deflections in response to sensory stimuli. The interlatencies represent the travel time via the neural pathway, and the amplitude reflects the degree of neural activity.

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In the present study, SSEPs were used to evaluate the severity of DCS and the protective effect of the new HBO pretreatment protocol in rats.

METHODS

Animals. Seventy-four male albino rats (Sprague-Dawley strain), weighing 247–305 g, were used for the experiments. The experimental procedures were approved by the Israel Ministry of Defense Animal Care Committee, and the rats were handled in accordance with the principles of laboratory animal care.

Experimental system. Exposures were conducted in a double-walled metal cage (16). The ambient temperature was kept in the range 24–28°C. This exposure cage was placed in a 150-liter hyperbaric chamber (Roberto Galeazzi, La Spezia, Italy), as described previously (16).

To ensure the normal state of the animal before the beginning of each trial, a pneumatically operated cylindrical cage, which could be rotated at a speed of 3 m/min (16), was used to observe the animals’ gait and behavior before the exposure. For DCS assessment by SSEPs, recording amplification (×7,500) and analog-to-digital conversion were carried out by a biologic evoked potentials system (Bio-logic Systems, Mundelein, IL) using a bandpass filter setting of 30 to 3,000 Hz, as described previously (27).

Hyperbaric exposure. An animal was put in the exposure cage, which was placed inside the experimental hyperbaric chamber. Pressure was increased linearly (at 101 kPa/min) to 304 kPa on oxygen (Pretreatment group), where the animal remained for a period of 20 min to allow the hypothesized gas exchange from the micronecules to take place. The oxygen was then switched to air, and pressure was increased linearly (at 101 kPa/min) to 1,013 kPa. For the Decompression group, pressure was increased directly to 1,013 kPa on air. Rats remained at this pressure on air for 33 or 32 min (Pretreatment or Decompression groups, respectively) to achieve a 65% risk of DCS (16) in the Decompression group. The purpose of the additional 1 min at high pressure in the oxygen-pretreated rats was to obtain the same nitrogen loading in the two groups before decompression. The parameters for this calculation were taken from the study of Lillo and Parker (20). Immediately thereafter, rats were subjected to rapid decompression at 202 kPa/min.

DCS assessment by SSEPs. About 15 min after decompression, rats were anesthetized with IP ketamine (80 mg/kg) and xylazine (8 mg/kg). Ketamine-xylazine has no significant effect on peripheral conduction (13) and has been used previously in rats for this type of study (27). The 15-min waiting period is based on preliminary trials that showed that shorter periods of time resulted in a higher mortality rate, probably due to the combination of DCS and anesthesia. Animals from the reference group were anesthetized without having undergone any of the experimental procedures. The animal’s body temperature was maintained at 37°C by means of a thermostatically heated pad to prevent alterations in body temperature affecting SSEP wave latency and amplitude (24). During recording, the heating pad was disconnected to eliminate electrical interference.

For the SSEP recordings, stimuli of electrical square wave pulses of 100-μs duration were employed using a disposable monopolar needle with cable electrodes at a repetition rate of 4 pulses/s, as described previously (27). The electrodes were positioned subcutaneously between the medial malleolus and the Achilles tendon for hindpaw peroneal nerve stimulation and in the palmar aspect of the wrist for forepaw median nerve stimulation, as described previously (27). Alternate bilateral stimulation of the upper and lower limbs was delivered at supra-threshold intensity sufficient to cause moderate contraction of the digits and the whole limb. Recording electrodes were placed transcutaneously over the C2-3 and L1-2 intervertebral spaces and on the skull vertex over the motor cortex (Pz). References for the Pz, C2-3, and L1-2 recordings were placed subcutaneously 1.5 cm below the Pz recording, between the scapulae, and between the iliac crests, respectively. A common ground electrode was placed below the tail root (Fig. 1). Impedance of the recording and reference electrodes was maintained below 5 kΩ. Analysis time was 21 ms, and 500 repetitions per trial were averaged. To avoid possible side or limb bias, stimulation and recording order were random among the animals. Each averaged recording was replicated and superimposed to verify reproducibility of waveform, amplitude, and latency.

Experimental protocol. Three protocols were used for the assessment of DCS by means of SSEPs, as presented in Fig. 2. The hyperbaric exposure protocols were chosen according to the results of a previous study (16). The decompression protocol we chose was designed to cause 65% DCS in rats, and the HBO pretreatment was selected as representing the least risk for oxygen toxicity, but with maximum effect on DCS reduction. In the Decompression group, 28 rats were compressed to 1,013 kPa on air for 32 min without HBO pretreatment and then decompressed as described above. In the Pretreatment group, 28 rats were pretreated with oxygen at 304 kPa for 20 min, compressed to 1,013 kPa for 33 min, and then decompressed as described above. The 18 rats in the reference group were not subjected to any of the procedures. All three groups of rats were assessed for DCS using SSEPs, as described in the experimental procedure above. Each rat was assigned to one exposure only. Before any exposure, the animal was placed in the rotating cage to ensure a normal motion pattern.

Data analysis and statistics. The Mann-Whitney and χ2 tests were employed to compare SSEP abnormalities in the Decompression and Pretreatment groups.

RESULTS

Although oxygen toxicity was not specifically monitored by recording electrical discharges in the EEG, no obvious symptoms of oxygen toxicity, which usually follow the first electrical discharge in the rat, were observed in any of the animals exposed to HBO.

SSEP recordings were successfully obtained from animals that survived the experimental conditions. The percentage of dead animals was similar in the Decompression and Pretreatment groups, 36% of rats in either group. The number of animals from which SSEP recordings could be obtained was therefore 18 in each group. The SSEP montage selected was based on a previous, similar study (27) and is illustrated in Fig. 1.
Waveform morphology of the evoked potentials was consistent among the reference group, the majority of rats in the Pretreatment group, and some of the rats in the Decompression group, and was similar to the findings of previous studies (6, 26, 27). At lumbar intervertebral space (L1-2), biphasic wave forms P1L1, N1L1 or N1L1, P2L1 were present in 28% of the animals and triphasic wave forms P1L1, N1L1, P2L1 in 72%. An initial sharp positive deflection was noted (P1L1), followed by a higher amplitude negative peak (N1L1), and then a lower amplitude positive wave (P2L1), as can be seen in Fig. 3, A and B. The SSEPs recorded from cervical intervertebral space (C2-3) exhibited a positive sharp deflection (P1C2) followed by a narrow negative peak (N1C2), as can be seen in Fig. 4. The SSEPs recorded from the cortex (Pz) exhibited a late slow positive deflection (P1Pz), followed by a symmetrical negative wave (N1Pz), as can be seen in Fig. 5A. There was great variability of amplitude between the animals.

All SSEP components were present in the reference group. In the Decompression and Pretreatment groups, some of the peaks could not be recognized, but whenever SSEP peaks were identified, no significant difference could be found between any of these groups for latency, amplitude, or conduction time (results not shown). Both subjective and objective analyses of SSEPs were carried out. For the objective analysis, peaks were selected when the statistical correlation between the same peaks in two subsequent recordings was 0.7 or greater. A peak latency more than two standard deviations from the mean latency of the reference group was defined as abnormal. The results were also analyzed by two observers, who judged whether the SSEPs were abnormal or not. For the purpose of their analysis, the observers were blinded to the origin of the results. The severity of the injury was scored from 0 to 3 for each animal. A normal recording, when all peaks could be identified and their latency was within two standard deviations of the mean of the reference group, was scored 0. A score of 1 indicates that one peak could not be identified and/or it was more than two standard deviations from the mean latency of the reference group. This could have happened when the L1-2 or Pz peaks could not be recognized. A score of 2 indicates that two peaks could not be identified and/or they were more than two standard deviations from the mean of the reference group. This could have happened when the L1-2 and Pz peaks could not be recognized or when both the C2-3 and Pz peaks could not be recognized. A score of 3 was the worst case, indicating that three peaks could not be identified and/or they were more than two standard deviations from the mean of the reference group. This could have happened when none of the Pz, C2-3, and L1-2 peaks could be recognized. Examples of normal and abnormal waves can be seen in Figs. 3 and 5.

In the Decompression group (animals that received no HBO pretreatment), 78% of the rats that survived had at least one abnormality in their SSEP recordings. This was significantly
lower in the Pretreatment group, in which only 44% had abnormal SSEPs (Fig. 6; \( P < 0.05 \), \( \chi^2 \), a 2×2 table). In addition, the severity of DCS in each group was calculated by dividing the average number of abnormal waves in a group by the number of animals in that group. In the Decompression group, there was an average of 1.38 abnormal waves per animal, whereas in the Pretreatment group this was significantly lower, 0.44 abnormal waves per animal (Fig. 7; \( P < 0.009 \), Mann-Whitney).

DISCUSSION

This study demonstrates that neurologic abnormalities in rats decompressed from 1,013 kPa can be significantly reduced by pretreatment with HBO at 304 kPa. In addition, oxygen pretreatment reduced the severity of the neurologic deficit compared with the group that received no HBO pretreatment. The main finding of the present study was a significantly lower degree of wave abnormality in the Pretreatment group compared with the Decompression group.

Abnormal waves were classified according to the study by Feinsod and Hoyt (11), which states that a single component of the evoked response is the sum of electrical changes generated by impulses of varying conduction velocity. Loss of different axons with different conducting velocities would deteriorate the normal morphology of the evoked potential components, leading to the production of multipeaked evoked potentials as seen in Figs. 3 and 5. There are a number of possible reasons for abnormal SSEPs: dysfunction at the level of the peripheral nerve, plexus, spinal root, spinal cord, brain stem, thalamocortical projections, or primary somatosensory cortex. Diseases of the dorsal column, such as DCS Type II, in which joint position sense and proprioception are impaired, are also associated with abnormal SSEPs.

No differences were found in mortality rate between the Decompression and Pretreatment groups. This was not in agreement with the findings of our previous study (16), in...
which animals were assessed by clinical observation alone. Those results demonstrated a lower mortality rate in the HBO pretreatment groups compared with the non-pretreatment group. This discrepancy may be explained by the combination of the anesthetic procedure and decompression. In the clinical study (16) the animals were not anesthetized, whereas in this SSEP study animals underwent an anesthesia procedure. All of the animals in the reference group survived. Although it has previously been shown that ketamine-xylazine has no significant effect on peripheral conduction (13, 27), it may have a fatal effect nonetheless in combination with DCS. Conkin and Schuhmann (7) showed that rats that developed DCS lost their resistance to anesthesia by diethyl ether and even died. This loss of resistance to anesthetic drugs, like diethyl ether, may be explained by alterations in the blood-brain barrier caused by DCS, which may enhance the influence of the drug in the brain. Blood perfusion inequalities, or changes in cardiopulmonary mechanics due to the presence of gaseous emboli, may also bring a greater amount of the drug to the brain in a shorter time. All of these might cause loss of resistance to anesthesia, which can be fatal.

No evidence could be found of prolongation or shortening of latency in the Decompression and Pretreatment groups compared with the reference group. In addition, there was great variability of amplitude between all the groups in the study. SSEP recordings revealed a much higher percentage of rats with neurologic abnormalities most likely connected to DCS (78% of animals that received no HBO pretreatment, compared with 44% of those pretreated with HBO at 304 kPa) than did the rotating wheel method (65 and 40%, respectively; Ref. 16). It would therefore appear that SSEPs are more sensitive for the assessment of DCS in the rat than clinical evaluation using the rotating wheel. However, humans have multiple parallel afferent somatosensory pathways (i.e., the anterior spinthalamic tract and dorsal column tracts within the spinal cord), some of which may be tested clinically and others neurologically. Therefore SSEPs may sometimes be normal in patients with a significant sensory deficit. In the case of DCS, a combination of the two methods, clinical and sensory, might well provide a better diagnosis of the patient’s condition. In addition, on the basis of the demonstration by Allison and Hume (1) of similarities between scalp recordings in humans and other mammals, these findings in the rat can be extrapolated to humans. It was not possible to conduct both clinical and sensory tests on the rats in the present study. The clinical test in our previous study (16) had to be run immediately after decompression and lasted for half an hour. This is the critical time in rats, which tend to recover from DCS faster than humans. Beginning the sensory test after a delay of half an hour in the present study would have been of no use and might have resulted in our missing the neurologic abnormalities we observed. It may have been useful to conduct a Doppler examination, for example, as a supplementary test to assess the efficacy of HBO pretreatment on DCS. However, the need to anesthetize the rat for the SSEPs and the time-consuming process involved are not conducive to carrying out neurologic tests.

This study supports the hypothesis of hyperoxia-induced denucleation, according to which bubbles originate from pre-existing micronuclei that can be reduced by hyperoxic pretreatment. Although bubbles were not measured directly in this study, their reduction has been measured previously in the prawn, and we assume that the same mechanism of bubble reduction operates in mammals (2–4, 9, 16, 18). The procedure used in the present study has proven to be a useful method of reducing the neurologic risks of DCS. It is not the same as denitrogenation, i.e., oxygen pretreatment just before decompression to flush the inert gas out of the tissues after it has already been loaded, as used in previous studies (19, 21, 25, 31). In contrast to denitrogenation, in our procedure oxygen washes the tissues before hyperbaric air exposure and any loading of inert gas. The purpose of the additional 1 min at high pressure in the oxygen-pretreated rats was to obtain the same nitrogen loading in the two groups before decompression, assuming a single compartment in the rat (20). It may be suggested that in a multiple-compartment rat, some compartments may remain unloaded due to the oxygen pretreatment. However, this seems unlikely, because the loading time (33 min at high pressure) was much longer than the preceding unloading time (20 min at lower pressure). There are other beneficial effects of oxygen on the outcome of DCS. Oxygen pretreatment in rats for 45 min at 2.8 ATA followed by 2 h exposure to air at 6 ATA reduced white cell adhesion (22) and improved running ability after decompression compared with control. The exposure duration in that study was longer than the effective exposure in our protocol, and a denucleation effect may well have been present. However, beneficial effects of oxygen other than denucleation cannot be excluded.

The methods employed for denitrogenation necessitate a very long exposure time in humans, hours rather than the minutes required by the method under examination here. One potential use of this HBO pretreatment is in enabling divers to make a fast ascent to the surface when faced with a problem or serious danger during a dive. It may also be used as a preparatory measure before escape from a disabled submarine or before high-altitude flight. The HBO pretreatment procedure for denucleation presented here may be a major step toward reducing the risk of DCS.

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REFERENCES


