Effect of 2.1 MPa and 4.1 MPa \( \text{H}_2\text{-O}_2 \) exposure on auditory brain stem evoked potential in mice.

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Weibing X, Jun L, Zheng M, Fengtao Y, Hexiang Z. Effect of 2.1 MPa and 4.1 MPa \( \text{H}_2\text{-O}_2 \) exposure on auditory brain stem evoked potential in mice. Undersea Hyperb Med; 32(5):391-396. Brain auditory evoked potential (BAEP) in mice exposed to hyperbaric \( \text{H}_2\text{-O}_2 \) pressure was monitored to reveal the correlation between altered synaptic transmission and hydrogen narcosis or isobaric HPNS. Inter peak latencies and wave amplitudes were selected as indices of assessment. The animals were exposed either to He-O\(_2\) or \( \text{H}_2\text{-O}_2 \) at 2.1 MPa and 4.1 MPa. Results showed that synaptic transmission was inhibited to various extents. The inhibition was partly due to the narcotic effect of hydrogen, which was added to the effect caused by hydrostatic pressure. On the other hand, asymmetrical reaction of each segment in the neuro-network might be responsible for the occurrence of HPNS.

INTRODUCTION

Hydrogen-oxygen (\( \text{H}_2\text{-O}_2 \)) diving is a research area encompassing new diving techniques and diving medicine. Hydrogen, when used as a high-pressure breathing medium, has drawn much attention from the diving community the world over, because it has great potential to substitute for helium which is both expensive and scarce in its natural reserve. Though literature review has shown that \( \text{H}_2\text{-O}_2 \) diving within its tolerance limit does not significantly impact negatively on life processes of mammals, there are still many problems before it can be put to practical use.

Like any other inert gases, hydrogen also has some narcotic effect which emerges at 1.6 MPa–2 MPa (1). During simulated experimental dives at a pressure greater than 2.5 MPa, divers will experience symptoms of HPNS, which usually involve mental function, motor and autonomic nervous systems, and bioelectricity also changes accordingly. Changes in the motor system are usually expressed as inaccurate control and over excitability (e.g. tremors, jerks and epilepsy). Mental function deficiency displayed its signs as somnolence, damaged judgment and amnesia (1,2,3).

Due to low ignition point of hydrogen, the percentage of oxygen in \( \text{H}_2\text{-O}_2 \) mixture should not exceed 4%. This means that \( \text{H}_2\text{-O}_2 \) mixture as a breathing medium, could only be used in diving beyond 60m. Shallower than this depth, other inert gases have to be used instead. For this reason, switching of different gases will be unavoidable in a \( \text{H}_2\text{-O}_2 \) dive, and so-called gas counter diffusion and isobaric HPNS will thus be encountered. These problems are dealt with in our \( \text{H}_2\text{-O}_2 \) diving research projects.

Brain auditory evoked potential (BAEP) can be used to explore the mechanisms involved. It is generally recognized that BAEP reflects graded PSP (post synaptic potential), BAEP waves reflect functionally separable substrate systems and BAEP latency is a function of fiber transmission time plus time added to synaptic threshold of a target cell population (4). Lorenz and his colleagues found that increased latency was correlated in a certain degree with HPNS (5), so BAEP might be used to assess quantitatively the effect of high pressure on synaptic events. In our present hyperbaric \( \text{H}_2\text{-O}_2 \) research using mice, BAEP monitoring was used to reveal correlations between altered synaptic transmission and hydrogen narcosis or isobaric HPNS.
METHOD

Experimental animals
8 healthy male mice (Kunming species of China) age of 6 weeks were used for the experiments. In pentobarbital anesthetized animals, two small holes were drilled (one, 3mm from the midline and 1mm behind Coronal suture, was used to induce the signal; the other, 4mm from the midline and 1mm in front of the lambdoidal suture was used as a reference with the metal wire grounded at the nasal bone) to the left side of parietal bone, through which silver wires were implanted, and attached to the dura to record ECoG (electrocorticalgraphy). The metal wires were fixed with epoxy. Experiments were started at least five days after surgery. During the experiment, the conscious animal was held tightly by a screw, which had been fixed previously on top of the skull with epoxy.

The H₂-O₂ environment set-up and experimental profile
Partial pressure of oxygen in the chamber was initially raised to 0.05 MPa, and then pure helium was added to the chamber to attain the desired depth (2.1 MPa or 4.1 MPa) in 60 min. The animals stayed at He-O₂ pressure for 1 h, then a 98 % H₂-2 % O₂ pre-mixed gas (H₂ purity>99.999%) was used to ventilate the chamber for 10 min (consequently more than 95% of the gas would be replaced), and then 2 groups of animals underwent high pressure exposures at 2.1 MPa/6h or 4.1 MPa/2.5h, respectively. Sodalime was used to absorb CO₂ in the chamber. During hyperbaric exposure, temperature was kept at 31±0.5 °C, PO₂ at 0.04 MPa and CO₂≤0.0005 MPa. Upon completion of H₂-O₂ exposures, a He-O₂ mixture was used to ventilate the chamber for 10 min to wash out the hydrogen. In the second stage of He-O₂ exposure, the animals stayed at 4.1 MPa for another 40 min. A second group of animals did not experience the second stage of He-O₂ exposure, but were decompressed directly from 2.1 MPa, following termination of H₂-O₂ exposure. Decompression did not start until less than 1% hydrogen concentration was achieved. The animals were brought to the surface safely on linear decompression profile at a rate of 2 m/min(6).

Instruments and statistics
The neuro-electric diagnostic system NDI-200P+ made by the Naval Medical Research Institute of China was used to record BAEP. ECoG signal from the animal was delivered through a chamber penetration to be recorded. Rarefaction clicks with a frequency of 10 Hz and a stimulus intensity of 120 dB were delivered by earphones 10 cm away from the animal. ECoG was amplified with a band pass filter set in the range of 100 Hz~5k Hz. 1000 ECoG segments (each lasting 10 ms) were averaged to acquire a single BAEP profile(4).

BAEP recordings were obtained at different stages of the hyperbaric exposure. Inter peak latencies (IPL₁-₄ and IPL₁₋₅) and wave amplitudes were chosen as indices of assessment. Variations and deficiencies of wave forms were sometimes present in actual recordings, so t test was used for statistical analyses of two population means.

RESULTS

At a 2.1MPa He-O₂ pressure, IPL seemed to lengthen slightly when compared with the normal pressure control, but a lack of statistical significance was likely due to small sample size. At the initial stage when the experimental animals were switched from the He-O₂ into the H₂-O₂ environment, no alteration in IPL could be observed. It was 3 hours later that IPL lengthened to an extent that it became statistically different from the normal pressure control. Upon completion of decompression, IPL was seen to return to its pre-exposure control level (Table 1, Figure 3). As shown by the experiment, the H₂-O₂ environment had little effect on the amplitude of the BAEP at 2.1 MPa (Figure 1).

He-O₂ exposure at 4.1MPa caused a significant increase in IPL, when compared with the normal pressure control. IPL increments were more pronounced when the breathing gas
DISCUSSION

It is generally postulated that generator potentials in the unmyelinated terminal dendrites of the acoustic nerve are the substrate of wave 1, while PSP in the CN (cochlear nuclei) are taken as the generator of wave 2. The CN output to the VNLL (ventral nuclei lateral lemniscus) produces longer latency field potentials hypothesized for wave 4, and PSP field potentials in the inferior colliculus are hypothesized as the generator of wave 5 (7). The VNLL is also suggested as an essential relay in the production of wave 5. This additional synaptic relay for wave 5, not postulated in the generator systems of wave 4, shows some physiologic differences between wave 5 and the earlier BAEPs in terms of its greater variability (7). That is why IPL\textsubscript{1-4} and IPL\textsubscript{1-5} were chosen as indices in the experiment. On the other hand, Lorenz and his colleagues believed that increased IPL\textsubscript{1-5} was correlated with tremor (one of the typical symptoms of HPNS) particularly (5). On the other hand, following an increase in the density of the gas mixture, the characteristics of sound transmission would change, this would mainly influence the latency of wave 1 and leave the IPL unaffected.

There are literature reports that hyperbaric helium environments will cause inhibition of synaptic transmission. This presynaptic effect occurs both to inhibitory and excitatory synapses (8). As a result, hyperbaric helium environment would cause IPL of BAEP to lengthen, and amplitude to diminish, as proved by Lorenz, whose experiment showed that BAEP latency began to prolong at a pressure greater than 2.3 MPa (5).

In the present experiment at 2.1 MPa He\textsubscript{2}-O\textsubscript{2} pressure, changes in the IPL seemed insignificant until the breathing gas was switched from He-O\textsubscript{2} to H\textsubscript{2}-O\textsubscript{2}, and then IPL appeared to be lengthening, but with obvious individual differences. As there was a certain correspondence between HPNS and synaptic transmission(5), we speculated that subjects would only experience slight HPNS at this pressure. With further pressurization and finally at 4.1 MPa He-O\textsubscript{2} pressure, IPL obviously lengthened, which was also reported previously at an identical pressure (2). It could be clearly seen that switching the breathing gas from He-O\textsubscript{2} to H\textsubscript{2}-O\textsubscript{2} mixture caused further lengthening of IPL, which suggests that hydrogen and hydrostatic pressure might have a cumulative effect on synaptic transmission.

Since there was no evidence to prove that helium at 4 MPa would produce any narcotic effect, the lengthening of IPL at He-O\textsubscript{2} might solely be the result of hydrostatic pressure. Hydrogen will show its narcotic effect as pressure reaches 1.6 MPa. For this reason, it can be speculated that the further lengthening of IPL is caused by hydrogen, after the breathing gas is switched from He-O\textsubscript{2} to H\textsubscript{2}-O\textsubscript{2} and this narcotic effect of hydrogen will explain deterioration of mental function observed by others (9). Soon after the gas switch, adaptation to the hydrogen-induced narcosis was seen from IPL recovery, though the effect of H\textsubscript{2}-O\textsubscript{2} on synaptic transmission seemed to be slightly stronger than that at He-O\textsubscript{2} exposure. This finding is of some utility in estimating how long the time course would be for the subjects to gain adaptation.
Table 1. Changes of mice BAEP IPL (IPL1-4, IPL1-5) during 2.1MPa He₂-O₂ and H₂-O₂ exposure (ms)

<table>
<thead>
<tr>
<th>Pressure/mixture</th>
<th>Time course (min)</th>
<th>n</th>
<th>IPL1-4</th>
<th>IPL1-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-com</td>
<td></td>
<td>7</td>
<td>2.38±0.28</td>
<td>3.47±0.35</td>
</tr>
<tr>
<td>2.1 Mpa/ He-O₂</td>
<td>0</td>
<td>6</td>
<td>2.42±0.25</td>
<td>3.70±0.23</td>
</tr>
<tr>
<td>2.1 Mpa/ He-O₂</td>
<td>60</td>
<td>7</td>
<td>2.67±0.42</td>
<td>3.86±0.58</td>
</tr>
<tr>
<td>2.1 MPa/H₂-O₂</td>
<td>120</td>
<td>6</td>
<td>2.79±0.52</td>
<td>3.98±0.66</td>
</tr>
<tr>
<td>2.1 MPa/ H₂-O₂</td>
<td>180</td>
<td>7</td>
<td>2.62±0.57</td>
<td>3.88±0.73</td>
</tr>
<tr>
<td>2.1 MPa/ H₂-O₂</td>
<td>240</td>
<td>6</td>
<td>2.79±0.61</td>
<td>3.91±0.67</td>
</tr>
<tr>
<td>2.1 MPa/ H₂-O₂</td>
<td>300</td>
<td>7</td>
<td>2.84±0.59</td>
<td>4.07±0.58*</td>
</tr>
<tr>
<td>2.1 MPa /H₂-O₂</td>
<td>360</td>
<td>7</td>
<td>2.81±0.58</td>
<td>4.03±0.69*</td>
</tr>
<tr>
<td>Post-dec</td>
<td></td>
<td>6</td>
<td>2.56±0.43</td>
<td>3.60±0.60</td>
</tr>
</tbody>
</table>

*represents significant change compared with pre-compression control, # represents significant change compared with the first He-O₂ exposure, * and # refers to p<0.05, ** and ## refers to p<0.01.

Fig. 1. Typical BAEP monitoring during 2.1 MPa H₂-O₂ exposure in a mouse. 1000 ECoG segments (each lasting for 10 ms) were averaged to obtain a single BAEP profile.

Fig. 2. Typical BAEP monitoring during 4.1 MPa H₂-O₂ exposure in a mouse. 1000 ECoG segments (each lasting for 10 ms) were averaged to obtain a single BAEP profile.
Table 2: Changes of mice BAEP IPL (IPL\textsubscript{1-4}, IPL\textsubscript{1-5}) during 4.1 MPa H\textsubscript{2}O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2} exposure (ms)

<table>
<thead>
<tr>
<th>Pressure/mixture</th>
<th>Time course (min)</th>
<th>n</th>
<th>IPL\textsubscript{1-4}</th>
<th>IPL\textsubscript{1-5}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-com</td>
<td></td>
<td>8</td>
<td>2.58±0.13</td>
<td>3.66±0.18</td>
</tr>
<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>0</td>
<td>7</td>
<td>2.61±0.17</td>
<td>3.79±0.10</td>
</tr>
<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>12</td>
<td>7</td>
<td>2.66±0.18</td>
<td>3.90±0.24*</td>
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<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>24</td>
<td>7</td>
<td>2.64±0.17</td>
<td>3.86±0.25</td>
</tr>
<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>36</td>
<td>8</td>
<td>2.79±0.24</td>
<td>3.98±0.20** #</td>
</tr>
<tr>
<td>4.1 MPa/H\textsubscript{2}O\textsubscript{2}</td>
<td>60</td>
<td>8</td>
<td>2.97±0.37* #</td>
<td>4.10±0.30** #</td>
</tr>
<tr>
<td>4.1 MPa/H\textsubscript{2}O\textsubscript{2}</td>
<td>72</td>
<td>8</td>
<td>2.92±0.30* #</td>
<td>3.98±0.35*</td>
</tr>
<tr>
<td>4.1 MPa/H\textsubscript{2}O\textsubscript{2}</td>
<td>84</td>
<td>8</td>
<td>2.91±0.18** ###</td>
<td>3.97±0.22**</td>
</tr>
<tr>
<td>4.1 MPa/H\textsubscript{2}O\textsubscript{2}</td>
<td>96</td>
<td>8</td>
<td>2.87±0.30*</td>
<td>3.97±0.26*</td>
</tr>
<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>108</td>
<td>8</td>
<td>2.77±0.16*</td>
<td>3.95±0.15**</td>
</tr>
<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>120</td>
<td>8</td>
<td>2.68±0.10</td>
<td>3.96±0.16** #</td>
</tr>
<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>132</td>
<td>8</td>
<td>2.69±0.14</td>
<td>3.99±0.10** ###</td>
</tr>
<tr>
<td>4.1 MPa/He-O\textsubscript{2}</td>
<td>144</td>
<td>8</td>
<td>2.71±0.15</td>
<td>4.01±0.16** ###</td>
</tr>
<tr>
<td>Post-dec</td>
<td></td>
<td>8</td>
<td>2.55±0.10</td>
<td>3.68±0.16</td>
</tr>
</tbody>
</table>

*represents significant change when compared with pre-compression control, # represents significant change when compared with the first value of He-O\textsubscript{2} exposure, * or # refers to p<0.05, ** or ### refers to p<0.01.

Fig. 3. Results of statistical analyses of BAEP IPL\textsubscript{1-4} (lower curve) and IPL\textsubscript{1-5} (upper curve) during 2.1 MPa H\textsubscript{2}O\textsubscript{2} exposure (with empty triangles or empty squares). * represents significant change compared with pre-compression control, # represents significant change compared with the first value of He-O\textsubscript{2} exposure. * and # p<0.05, ** or ### p<0.01.

Fig. 4. Results of statistical analyses of BAEP IPL\textsubscript{1-4} (lower curve) and IPL\textsubscript{1-5} (upper curve) during 4.1 MPa H\textsubscript{2}O\textsubscript{2} exposure (with empty triangle or empty squares). * represents significant change when compared with the normal pressure control, # represents significant change when compared with that of He-O\textsubscript{2} exposure. * and # p<0.05, ** and ### p<0.01.
However, after switching back to He-O₂ from H₂-O₂ at 4.1 MPa, it was found unpredictably that the recovery of IPL₁₋₄ and IPL₁₋₅ was asymmetrical. This finding might give some clue for the mechanism of HPNS. It has been reported that quick switch from H₂-O₂ to He-O₂ would induce a re-bounce of HPNS called isobaric HPNS(10). The occurrence of isobaric HPNS coincided with that of differentiated IPL₁₋₄ and IPL₁₋₅ recovery, which might indicate that certain symptoms of HPNS were not just due to inhibited synaptic transmission, but might be the result of asymmetrical reaction of neural pathways under high pressure.

The sensitivity of the nervous system to high pressure can be explained in the complexity of neural network. Since major effects of hydrostatic pressure and H₂-O₂ exposure at synaptic level are displayed in the form of inhibition and assumed asymmetrical reaction, certain approaches might be appropriate to facilitate synaptic transmission and restore the imbalance in neuro function, in order to alleviate symptoms of HPNS. Actually, procedures, such as stops in the process of compression, might be considered as countermeasures to maintain the symmetrical reaction of neural pathways.

CONCLUSION

During exposure of mice to either He-O₂ or H₂-O₂ at 2.1 MPa and 4.1 MPa, the monitoring of BAEP showed that synaptic transmission was inhibited to various extents. The inhibition was partly due to the narcotic effect of hydrogen, which was added to the effect caused by hydrostatic pressure. On the other hand, pressure-induced asymmetrical reaction of each segment in neural network might be responsible for the occurrence of HPNS, when the breathing gas was switched from H₂-O₂ to He-O₂.

REFERENCES