Mammalian CNS barosensitivity: Studied by brain-stem auditory-evoked potential in mice

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ABSTRACT

High pressure nervous syndrome (HPNS) is an instinctive response of mammalian high-class nervous functions to increased hydrostatic pressure [2,5,8]. Electrophysiological activity of mammalian central nervous system (CNS), including brainstem auditory-evoked potential (BAEP), has characteristic changes under pressure. Here we recorded BAEP of 63 mice exposed to 0-4.0 MPa. The results showed that interpeak latencies between wave I and wave IV (IPL1-4) and their changes under pressures (ΔIPL1-4) responded to increasing pressure in a biphase pattern, shortened under pressure from 0 to 0.7MPa, then prolonged later. There were significantly negative correlations between base IPL1-4s and ΔIPL1-4s ($p < 0.01$). Individual IPL1-4s were supposed to respond to increasing pressure in a relative steady pattern in accordance with its base IPL1-4s. Those with shorter-base IPL1-4 presented direct increases in IPL1-4. However, those with longer-base IPL1-4 had a decreased IPL1-4 under small to moderate pressure then rebounded later. Our results suggested that mammalian CNS functions were susceptible to small to moderate pressure, as well as a higher pressure than 1.0MPa. Mice, as a statistical mass, had an "optimum" pressure about 0.7MPa, rather than atmospheric pressure, referred as shortest IPL1-4s. An individual's response to high pressure might be relied on his base biological condition. Our results highlighted a new approach to investigate a practical strategy to medical selecting barotolerant candidates for deep divers. Diversity of individual susceptibility to hydrostatic pressure was under discussed. Underlying mechanisms of the "optimum" pressure for CNS function and its significance to neurophysiology remain open to further exploration.

INTRODUCTION

High pressure (≥1.5MPa) induces a series of symptoms and signs of mammalian central nervous system (CNS) impairment, which is generically termed high-pressure nervous syndrome (HPNS). It is the major obstacle for human beings working under pressure higher than 150msw [2,5,8]. It is clear that the effects of hyperbaric pressure on the CNS are due to the direct effects of increased hydrostatic pressure, which can be mimicked by helium [2].

The effects of increasing pressure are noted instantaneously and throughout the organism. The exact mechanism is unknown. To reveal its underlying mechanism is a great challenge to scientists. Through research, it has been found in the changes of brainstem auditory-evoked potential (BAEP) in latency, interpeak latency (IPL) and amplitude of waves under increasing pressure for human or animal subjects [13,14]. There is a common agreement on the source of BAEP, which is thought to arise principally within the auditory nerve and nuclei of the auditory brainstem [7,10]. The cochlea nuclei output to the ventral nuclei lateral lemniscus produces longer latency field potentials hypothesized for Wave I. The IPL between Wave I and Wave IV (IPL1-4) involved auditory nerve spike potential conducted to the cochlear nuclei and later output to the lateral lemniscus and/or inferior colliculus [10].

Any changes in neuron membrane conductance and synaptic mechanism would exert influence on IPL1-4. The prolongation of IPLs under pressure, representing delayed synaptic transmitting within acoustical nuclei in the CNS, is intrinsic in certain individual responses to pressure with relative reproducibility [12,28]. This makes it possible to semi-quantify HPNS *in vivo* without compression to the utmost high pressure.

In recent work, our primary intent was to probe the relationship between the composition of CNS membranous fatty acids and HPNS [29]. However, we experienced an unexpected phenomenon: the possible correlation between base IPL1-4s and its prolongation under pressure. Therefore, we have conducted a large-sample experiment in normal mice to verify this hypothesis. BAEP recordings were obtained at different stages of compression (0MPa, 0.2MPa, 0.4MPa, 0.7MPa,

1.0MPa, 2.0MPa, 3.0MPa, 4.0MPa) with helium-oxygen. IPL1-4s and their differences between base (0MPa) and increased pressures (ΔIPL1-4s) were chosen as indices of assessment.

METHODS

Animal and surgery preparation

63 male mice (Chumming species of China) had free access to food and water. The details of surgery preparation were described in a previous paper (Xiao *et al.*, 1999). A brief synopsis reads: Before surgery, animals were given pentobarbital and anesthetized. Two small holes were drilled (one hole 3mm from the midline and 1mm behind coronal suture 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 the parietal bone, through which silver wires were implanted, and attached to the dura to record electrocorticography (ECoG). The metal wires were fixed with epoxy. Experiments were started at least three days after surgery. During hyperbaric exposure in the animal chamber, conscious animals were held by an animal fix-up box (fixation device) to keep a continuous record of the BAEP signal and so that animals' behavior changes were not observed.

He-O_2 environment set-up and experimental profile

Partial pressure of oxygen in the chamber was initially raised to 0.05MPa; then, within 120 minutes, pure helium was added to the chamber to attain 4.0MPa. The animals remained at 4.0MPa for 30 minutes. Soda lime was used to absorb CO_2 in the chamber. During hyperbaric exposure, the temperature was kept at 31 ± 0.5°C, PO_2 at 0.04MPa and $CO_2 \leq 0.0005$MPa. The animals were safely decompressed to normal pressure in 120 minutes.

BAEP record

The neuroelectric diagnostic system NDI-200P$^+$, made by the Naval Medical Research Institute of China, was used to record BAEP. The ECoG signal from the animal was delivered through a chamber penetration to be recorded. Rarefaction clicks with a frequency of 10Hz and a stimulus intensity of 120dB were delivered by earphones 10 cm away from the animals. ECoG was amplified with a bandpass filter set in the range 100 Hz~ 5k Hz. 1,000 ECoG segments (each lasting 10 milliseconds) were averaged to acquire a single BAEP profile [4]. BAEP recordings were obtained at different stages of compression (0MPa, 0.2MPa, 0.4MPa, 0.7MPa, 1.0MPa, 2.0MPa, 3.0MPa, 4.0MPa). Latencies of Wave I (L1) and Wave IV (L4), IPL1-4s and their differences between base and under-pressures (dIPL1-4) were chosen as indices of assessment.

Data preparation and statistical analysis

A paired-sample T-test was used to determine the differences between base and under-pressure of the latency of Wave I and Wave IV, IPL1-4 and dIPL1-4; bivariate correlation tested their correlativity. Then data were divided into three groups by K-cluster in accordance with the values of base IPL1-4s to identify differences of base condition. Differences between cluster groups were determined with ANOVA. Among all statistical analysis, when $p < 40.01$ or $p < 0.05$ (two-tailed), it is considered to be significant.

RESULTS

The results showed that, as a mass, the mouse IPL1-4s responded to increasing pressure in a biphase process, with a decreasing trend under pressures lower than 0.7MPa, and then rebounding in higher pressures *(Figure 1, facing page)*. There were no significant differences within the IPL1-4s of 0 - 0.7MPa. However, the IPL1-4s of 1.0MPa were significantly longer than that of 0.4MPa and 0.7MPa ($p < 0.01$ and $p < 0.05$). The IPL1-4s of later depths increased stage by stage ($p < 0.05$ or $p < 0.01$). The IPL1-4s of 2.0MPa were longer than base, but not significant. There were statistically significant differences between IPL1-4 of 4.0MPa and base values ($p < 0.05$) *(shown as Figure 1)*. The IPL1-4s was significantly correlated to pressure ($r = 0.134$, $p < 0.01$). Furthermore, the base IPL1-4s was significantly correlated to the ΔIPL1-4s (from 0.2MPa to 4.0MPa, separately r = -0.491, -0.627, -0.781, -0.804, -0.789, -0.804 and -0.848; all $p < 0.01$) *(linear relationship under 4.0MPa shown as Figure 2, facing page)*.

The IPL1-4s, as an auditory signal transduction process within brainstem, could absolutely reflect the effect of hyperbaric pressure on CNS function. For further detailed analysis of the IPL1-4s changes under pressure, we divided all cases into three groups by quick cluster analysis in accordance with base IPL1-4s, sorted by base IPL1-4s from short to long, and shown as Cluster Groups 1, 2 and 3 *(Figure 4, Page 566)*. Base IPL1-4s figures were significantly different between the three cluster groups. Interestingly, it was found that changes in the IPL1-4s of Cluster Group 3, which had the longest-base IPL1-4s, appeared as a

FIGURE 1

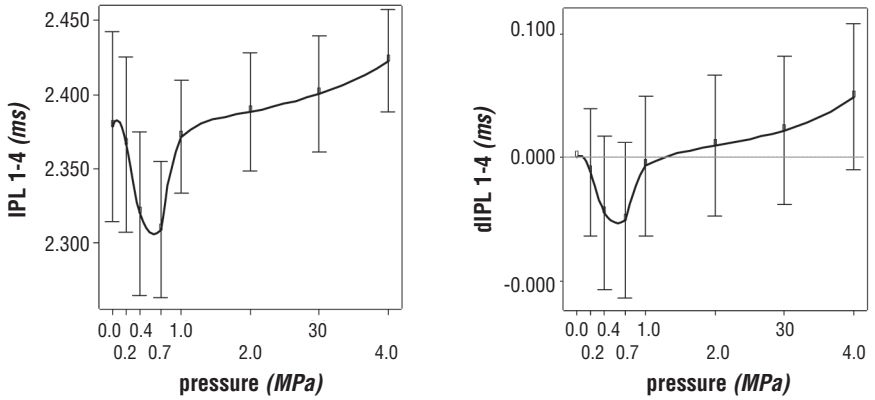

Figure 1. Change of IPL1-4s and ΔIPL1-4s under pressure (mice).

typical biphase process. However, that of Cluster Group 1, with the shortest-base IPL1-4s, appeared to have a direct prolongation according to increasing pressure; Group 2 was in between Group 1 and Group 3. There were significant differences in the IPL1-4s of each pressure between groups (from 0.2 - 4.0MPa, $p < 0.01$ or $p < 0.05$), in the dIPL1-4s of all depths (from 0.2 - 4.0MPa, all $p < 0.01$).

FIGURE 2

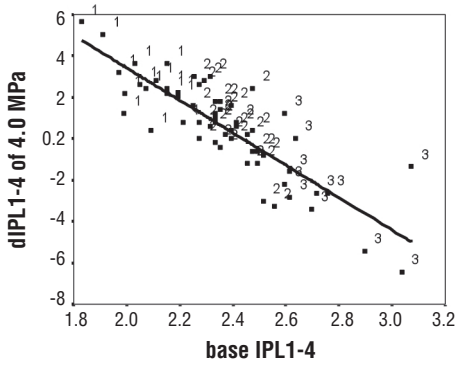

Figure 2. Linear relationships between base IPL1-4 and its changes under pressure of 4.0 MPa. Label number refers to the cluster group. There was significantly linear relation between base L1s and dL1s of 4.0 MPa (r2=0.720, $p < 0.01$), and regular distribution of three cluster groups.

DISCUSSION

It was known early that there is remarkable individual variation of susceptibility to hyperbaric pressure [3,15]. This varied susceptibility was proven to be replicated and intrinsic, which drives diving physicians to find a way to select barotolerant candidates to be deep divers. Rostain *et al.* [23] found there was a relationship between individual susceptibility to HPNS and certain changes of EEG under pressure (1.8MPa). As a strategy for selecting barotolerant individuals to be deep divers, the use of high levels of pressure was questionable because of obvious practical reasons. The results showed that the IPL1-4s prolongation under pressure correlates to the base IPL1-4s, which suggests that individual performance under pressure is partly dependent upon base biological conditions. In other words, individual susceptibility to high pressure can be foreknown before high-pressure exposure by BAEP, by a non-invasive test at atmospheric pressure. It highlights a new approach to investigate a practical strategy for medical selection of deep divers.

Traditional dogma maintains that hydrostatic pressures lower than 1.5MPa have no significant effects on neuronal function [1,26]. Macdonald and Fraser [19] had proposed that many non-neuronal cells respond to micropressures of less than 0.2MPa, but there were no distinctive cellular structures and a practical, specific working hypothesis is lacking. But Hallenbeck's [31] work indicated that increased pressure will cause a deviation of membrane phase-transition temperature (Tm) required by a shift of cell membrane from a disordered liquidlike phase to a relatively immobile gel structure. It was reasonable to extrapolate those effects to neurons. Here, it was the first time for us to identify definite effects of small to moderate – as well as very high – hydrostatic pressures on mammalian CNS function *in vivo*. In particular, these responses represented individual variations due to different base biological conditions, which provided a new approach for us to interpret hydrostatic pressure effects. Plus,

FIGURE 4

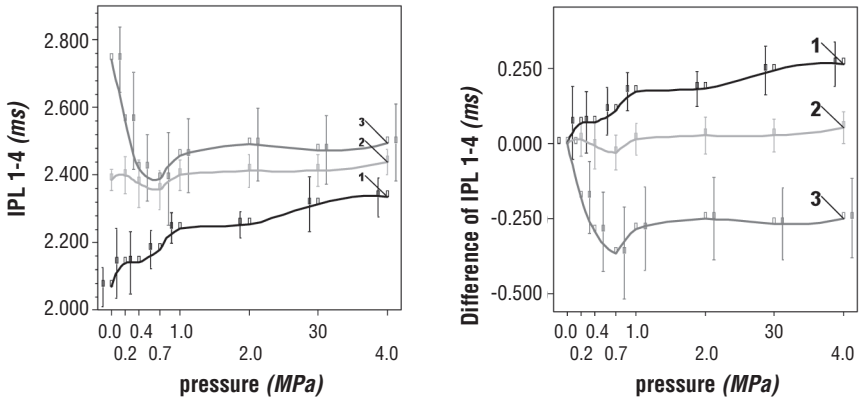

Figure 4. Comparison of the three cluster groups.

FIGURE 3

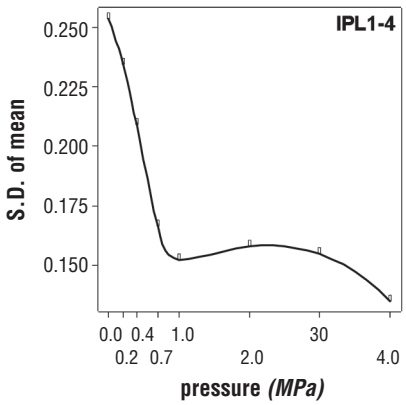

Figure 3. Changes of standard deviation of means of IPL1-4s with increasing pressure.

small to moderate pressures, such as those lower than 0.7MPa, may facilitate synaptic transportation in most individual mice in this study.

For example, we can deduce the effects of pressure on the cellular membrane. Barshtein's [1] study on erythrocytes suggests that the application of pressure of several atmospheres increases the phase order of membrane lipid domains, which, in turn, will alter equilibria between crystalline to liquid crystalline phase, and thus particularly the proximity of proteins. Our recent work [29] has shown that there is individual variation in the ratio of polyunsaturated fatty acids (PUFAs) / saturated fatty acids (SFAs) in brain cell membranes, implying different membrane fluidities. Though chemical properties of proteins alone were relatively resistant to low to moderate pressure based on conventional solute-solvent interactions [4], even a tiny transition of membrane from liquid phase to gel phase induced by low to moderate pressure probably moved this equilibrium to a more suitable condition, which would delicately disturb cellular processes such as ion channels, post-synaptic receptors, etc., and then mounted up a whole effect to facilitate certain membranous functions, such as synaptic function. The results also showed that those individuals with a higher ratio of PUFAs / SFAs had fewer changes of IPL1-4s under pressure, which suggested a stronger ability for animals to counteract the effects of pressure.

However, hyperbaric pressure higher than a certain level – for example, 0.7MPa for mice in accordance to our results – would induce a shift of that equilibrium enough to absolutely deteriorate biological membranous function of all organisms. Shown as Figure 3 *(left)*, the standard deviations of IPL1-4s declined with increasing pressure, which suggested neuronal electrophysiological processes adopted a similar pattern with the increasing pressure. IPL1-4s of all mice were apt to be a coincident value under certain extremely high pressures – which may be the point of no return for mice.

Williams' [30] study on deep-diving marine mammals also suggests that the differences in cell membrane structure, which can affect the order and sensitivity of cell membranes to hydrostatic pressure, is supposed to be an important adaptation that allows repeated and prolonged exposure to great hydrostatic pressures. Although membranous properties are supposed to play an important role in neuronal responses to increasing pressure, our previous experiment failed to identify a significant correlation between ΔIPL1-4s and the composition of membranous fatty acids. It was likely, therefore, to contribute to more complicated mechanisms and multiple structures for further studies.

Based on the IPL1-4s responses to the increasing pressure of three cluster groups and the above hypothesis, it was reasonable to assume that individual IPL1-4s responses to hyperbaric pressure presented a relatively steady curve pattern but were different in the "optimum pressure." The location of the pressure-IPL1-4 curve for certain cases relied on the animals' base biological conditions, represented as base IPL1-4. How and why base biological conditions affected our findings will need more investigation. If a shorter latency (IPL1-4) can be seen as more efficient brain signal processing, these results suggest that the CNS function of mice as a group had an "optimum" pressure of about 0.7MPa rather than atmospheric pressure.

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