Different activation of ERK1/2 and p38 with hyperbaric oxygen in dorsal root ganglion

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ABSTRACT

Prolonged hyperbaric oxygen exposure causes pulmonary and nervous system toxicity, although hyperbaric oxygen treatment has been used to treat a broad spectrum of ailments. In the current study, animals have been exposed to 100% oxygen at a pressure of 2.3 atmospheres absolute (ATA) for two, six and 10 hours or 0.23 MPa normoxic hyperbaric nitrogen (N2-O2 mixture, oxygen partial pressure=21 kPa) for 10 hours. Then we investigated whether ERK1/2 and p38 had been activated in the dorsal root ganglion (DRG) by hyperbaric conditions. Using Western blot analysis, we found that the phosphorylation levels of ERK1/2 (phospho-ERK1/2) increased significantly (p<0.05, n=3 for each group) in the six-hour treatment of 100% oxygen at a pressure of 2.3 ATA. The phosphorylation levels of p38 (phospho-p38) increased significantly (p<0.05, n=3 for each group) in the 10-hour treatment of 100% oxygen at a pressure of 2.3 ATA – which was consistent with time course changes of an apoptosis marker, cleavage caspase-3 – while the phospho-p38 decreased in the 10 hours of N2-O2 mixture. These results demonstrate that the ERK1/2 and p38 have been differently activated in the DRG by prolonged hyperbaric oxygen exposure.

INTRODUCTION

Hyperbaric oxygen therapy is a non-invasive method that uses 100% oxygen under increased atmospheric pressure in a controlled total-body chamber. Today, it is an approved modality that is most often used as an adjunct or enhancement therapy for a wide variety of medical conditions. Some studies demonstrate that hyperbaric oxygen therapy reduces inflammation and mechanical hypersensitivity in an inflammation pain model [1]. Dorsal root ganglion (DRG) neurons transmit nociceptive signals to spinal cord [2]. Hyperbaric oxygen therapy may change activation of various intracellular signaling pathways in the DRG neurons, which may contribute to behavior change.

On the other hand, prolonged hyperbaric oxygen exposure induces nervous system damage, including degeneration and scarring [3-5]. Recent studies have shown that the effects of prolonged hyperbaric exposure on neurons result in nitrogen narcosis, oxygen toxicity, carbon dioxide toxicity and high-pressure nervous syndrome [6-9].

In this study, we investigated the roles of intracellular signaling pathways such as ERK1/2 and p38 in the DRG after 100% oxygen treatment at a pressure of 2.3 ATA for two, six and 10 hours or 0.23 MPa normoxic hyperbaric nitrogen (N2-O2 mixture, oxygen partial pressure=21 kPa) treatment for 10 hours.

Extracellular signal-regulated kinase (ERK1/2) and p38 have crucial roles in transducing signals from the cell surface to the nucleus and in regulating cell death and survival, neuronal plasticity such as long-term potentiation, learning and memory. A recent study showed there is a decrease in the ERK protein expression in PC12 cells exposed to hyperbaric air at 6 ATA for 30 minutes [10]. Other research showed that hyperbaric oxygen treatment induces phospho-ERK1/2 expression within 15 minutes in the human umbilical vein endothelial cells [11].

However, the roles of ERK1/2 and p38 in the nervous system, especially in the DRG after hyperbaric oxygen exposure, are unclear. So we focused on the time course changes of ERK1/2 and p38 phosphorylation in DRG during hyperbaric oxygen exposure, which may indicate the time window of hyperbaric oxygen-induced DRG damage.

Methods

Experiments were conducted on male Sprague–Dawley rats weighing between 180 and 220 g. The animal protocol was approved by the Institution Animal Care...
and Use Committee of the Naval Medical Research Institute and was consistent with the NIH Guide for the Care and Use of Laboratory Animals. Hyperbaric oxygen exposure was processed to expose animals to 100% oxygen at a pressure of 2.3 atmospheres absolute (ATA) for two, six and 10 hours in a hyperbaric chamber (Groups 2, 6, 10) or expose animals to an N2-O2 mixture for 10 hours in a hyperbaric chamber (N2+O2 Group).

The control group was placed in the hyperbaric chamber but did not receive compression treatment. At the end of the experiments the rats were sacrificed and the DRGs were quickly removed and collected, frozen in liquid nitrogen and kept frozen at -70 °C for later analysis. To determine the phosphorylated and total levels of ERK1/2 and p38, protein extracts were generated from the DRGs. Samples were thawed and homogenized at 4°C in a 400 μl homogenized buffer (50 mM Tris–Cl, pH 7.5, containing 2 mM dithiothreitol, 2 mM EDTA, 2 mM EGTA, 5 mg/ml each of leupeptin, aprotinin, pepstatin A, chymostatin, 50 mM okadaic acid, 5 mM sodium pyrophosphate, 1 mM orthovanadate, and 2% SDS) and sonicated to dissolve the tissue completely.

Then, 50 μg of protein from each sample was loaded per lane for SDS-PAGE (10% SDS gel). At the end of electrophoresis, the proteins were transferred onto the nitrocellulose (NC) membrane (Schleicher and Schell, USA) at 4°C, 400 mA for 2.5 hours. The transferred membrane was washed for 10 minutes in TBST (20 mM Tris, pH 7.5, containing 0.15 M NaCl and 0.05% Tween-20) and then blocked with 10% nonfat milk for one hour. After being washed three times (each wash lasting 10 minutes), the blocked NC membrane was incubated with different primary antibodies against phosphorylated ERK1/2 (Santa Cruz, USA; 1:1000 dilution), phosphorylated p38, cleavage caspase-3, caspase-3 (Cell Signaling Technology, USA; 1:1000 dilution), total ERK1/2, p38 (Santa Cruz, USA; 1:10000 dilution) and GAPDH (Abcam, UK; 1:100000) overnight to determine protein phosphorylated and total levels, respectively.

Next, the membrane was incubated with horseradish peroxidase-conjugated goat anti-rabbit or mouse IgG as second antibodies at 1:8000 dilutions for one hour. Finally, immunoblotting signals were visualized by using the ECL-plus Kit (Perkin-Elmer Life Science Inc., USA). Paired data were evaluated by paired T-test, and difference was considered significant at \( p < 0.05 \).

**RESULTS**

The phosphorylation levels of ERK1 and ERK2 significantly increased at the end of the six-hour treatment of 100% oxygen at a pressure of 2.3 ATA, while phosphorylation levels of ERK1 significantly decreased in the 10-hour treatment of 100% oxygen at a pressure of 2.3 ATA. The signal made no apparent difference within the control group, the two-hour group and the N2+O2 group (Figure 1, facing page). The total ERK1 and ERK2 levels had no differing expression within the five groups compared with the GAPDH expression.

The representative results of the Western blot are depicted in Figure 1A, and quantitative analysis is shown in Figure 1B. The phosphorylation levels of p38 significantly increased in the 10-hour group, and significantly decreased in the N2+O2 group. The signal made no apparent difference within the control group, the two-hour and the six-hour groups (Figure 2, facing page). The total p38 levels had no different expression among the five groups compared with the GAPDH expression. The representative results of Western blot are depicted in Figure 2A, and quantitative analysis is shown in Figure 2B.

We wanted to know the changes of DRG apoptosis during hyperbaric treatment. As we know, caspase-3 is one of the key producers of apoptosis, and cleavage caspase-3 is the activation form of caspase-3, so we examined the changes of cleavage caspase-3 after hyperbaric treatment. We found that the cleavage caspase-3 appeared in the 10-hour group, while there was no signal in the other groups (Figure 3, facing page).

**DISCUSSION**

Hyperbaric oxygen treatment with 100% oxygen at a pressure of 2.4 ATA for 90 minutes in a hyperbaric chamber significantly decreased inflammation and pain following carrageenan injection [1], and phosphorylation of ERK1 and ERK2 in primary afferent neurons occurs in response to noxious stimulation in DRG neurons [12,13]. In our study, DRG treatment with 100% oxygen at a pressure of 2.3 ATA for two hours did not change the phosphorylation level of ERK1 and ERK2. However, after prolonged hyperbaric oxygen exposure to six hours, the animals displayed oxygen toxicity syndrome in the nervous system and lungs [14,15]. Oxygen toxicity may induce inflammatory mediators releasing in the DRG, which can lead to the increase of phosphorylation of ERK1 and ERK2 as showed in our study. In this situation, the activation of ERK is a pro-survival factor to reduce nervous system damage [16,17].
**FIGURE 1**

- The changes of ERK1 and ERK2 phosphorylation levels in DRG following treatment with 100% oxygen at a pressure of 2.3 ATA for 2, 6 and 10 hours or 0.23 MPa normoxic hyperbaric nitrogen (N₂-O₂ mixture oxygen partial pressure=21 kPa) for 10 hours (N₂+O₂).
- The Control Group was placed in the hyperbaric chamber but did not receive treatment.

A – Representative Western blots were labeled with antibodies against phosphorylated and total ERK1/2 and GAPDH.

B – Blots were scanned and the band intensities were quantified using densitometer. Values were normalized to GAPDH and expressed as mean ± S.E.M. (*p < 0.05, compared with the Control Group).

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**FIGURE 2**

- The changes of p38 phosphorylation levels in DRG following treatment with 100% oxygen at a pressure of 2.3 ATA for 2, 6 and 10 hours or 0.23 MPa normoxic hyperbaric nitrogen (N₂-O₂ mixture oxygen partial pressure=21 kPa) for 10 hours (N₂+O₂).
- The Control Group was placed in the hyperbaric chamber but did not receive treatment.

A – Representative Western blots were labeled with antibodies against phosphorylated and total p38 and GAPDH.

B – Blots were scanned and the band intensities were quantified using densitometer. Values were normalized to GAPDH and expressed as mean ± S.E.M. (*p < 0.05, compared with the Control Group).
Longer hyperbaric oxygen exposures (100% oxygen at a pressure of 2.3 ATA for 10-14 hours) could induce animal death and cellular necrosis. In cellular necrosis, the phosphorylation level of ERK1 and ERK2 would be reduced. We also compared the effect of hyperbaric air with 100% oxygen and normal concentrations of oxygen and found that only the 100% oxygen could change the phosphorylation level of ERK1 and ERK2. This condition indicates that hyperbaric oxygen treatment may be a double-edged sword: Normal concentration is necessary, while prolonged hyperbaric 100% oxygen could induce tissue damage and cell apoptosis.

On the other hand, the phosphorylation level of p38 was increased in the 100% oxygen at a pressure of 2.3 ATA for 10 hours, which was consistent with the changes of an apoptosis marker, cleavage caspase-3, in hyperbaric treatment. This indicates that activation of p38 is associated with DRG damage and cellular necrosis. A similar role of p38 has been found as well in DNA damage and oxidative stress [18]. While hyperbaric air with normal concentrations of oxygen reduced phosphorylation levels of p38 in DRG, this indicated that hyperbaric air at 2.3 ATA may be helpful for cell survival or physiological function. The mechanism needs to be further studied.

Our study demonstrates that ERK1/2 and p38 have been differently activated in DRG by prolonged hyperbaric oxygen exposure, which may indicate that ERK1/2 and p38 have different roles in DRG damage and cellular necrosis.

**REFERENCES**


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**FIGURE 3**

- The changes of cleavage caspase-3 and caspase-3 levels in DRG following treatment with 100% oxygen at a pressure of 2.3 ATA for 2, 6, 10 hours or 0.23MPa normoxic hyperbaric nitrogen (N2-O2 mixture oxygen partial pressure=21kPa) for 10 hours (N2+O2). The Control Group was placed in the hyperbaric chamber but did not receive treatment. Representative Western blots were shown by labeled with antibodies against cleavage caspase-3 and caspase-3 and GAPDH (n=3).