Original Article

Cranial neurotransmitter alteration in newborn piglets exposed to oxygen

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Abstract

Background: To evaluate the influence of hyperoxia on neurotransmitters in the developing brain of newborn piglets.

Methods: Ten newborn piglets were randomly assigned to hyperoxia (inhaled 100% oxygen) or control (inhaled room air) groups and ventilated for 4 hours. Blood samples were obtained at 0, 15, 30, and then every 30 minutes for 4 hours. Extracts of whole brain tissue were assayed for dopamine, serotonin, and their metabolites using high-performance liquid chromatography.

Results: In comparison with the control group, there was a general trend of lower neurotransmitter content in the brains of the hyperoxia group. In addition, the levels of dopamine and 3,4-dihydroxyphenylacetic acid in the left frontal lobe, and serotonin in the right occipital lobe and left frontal lobe, of the hyperoxia group were significantly lower compared with the control group (p < 0.05).

Conclusion: The results indicate that hyperoxia may alter the production or metabolism of dopamine and serotonin in some cortical areas of the neonatal central nervous system, and it tended to have some inhibitory effects. Therefore, pediatricians should be very judicious in using high oxygen on the developing brain.

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1. Introduction

Dopamine is a monoamine neurotransmitter formed in the brain by the decarboxylation of dopa and is essential to the normal functioning of the central nervous system (CNS). Dopamine has many functions in the brain, including important roles in behavior and cognition, voluntary movement, motivation, punishment and reward, inhibition of prolactin production, sleep, mood, attention, working memory, and learning. Serotonin, or 5-hydroxytryptamine, is also a monoamine neurotransmitter, biochemically derived from tryptophan. Serotonin has various functions, including the regulation of mood, appetite, sleep, muscle contraction, and some cognitive functions involving memory and learning. Therefore, any influence on the production or metabolism of dopamine or serotonin may have adverse effects, especially in the developing CNS.

Oxygen supplementation is of vital importance in the treatment of neonates in critical circumstances. Oxygen is a ubiquitous molecule in the CNS and is known to influence the synthesis and oxidation of essentially all neurotransmitters. From previous studies, it has been postulated that hypoxia-induced increases in extracellular dopamine result from inhibition of reuptake or an increase in the releasable dopamine pool. The increase in the striatal synthesis rate of dopamine is also associated with reduced brain tissue oxygen
The dopaminergic system in the immature brain is thus clearly sensitive to changes in tissue oxygen tension. Therefore, interest in and research that further scrutinizes the influence of oxygen on neonatal cranial neurotransmitters is substantial.

On the other hand, researchers have noted potentially harmful effects of hyperoxia as it might impact the neonate brain. One foreseeable adverse effect of aerobic metabolism is oxygen radical generation. Oxygen radicals have been known to be powerful oxidizing agents and may cause structural damage to proteins and nucleic acids in human cells. Oxygen supplementation is commonly given to newborns in numerous countries throughout the world. However, diseases such as prematurity and bronchopulmonary dysplasia remind us to exercise restraint during oxygen therapy, especially in developing neonates. Research is limited concerning the influence of hyperoxia on excitatory neurotransmitters and their metabolites, and both over- and underproduction of excitatory neurotransmitters may be harmful to the developing brain.

Our hypothesis is that hyperoxia may be harmful to the CNS by way of stimulation or depression of excitatory neurotransmitters in the neonatal brain. Thus, the purpose of our study was to investigate the influence of 100% inspired oxygen on the production of dopamine, serotonin, and their metabolites in the developing brain of newborn piglets.

2. Methods

All animals were managed according to the National Institutes of Health guidelines for the care of animal subjects. All procedures were approved by the local Institutional Review Board.

2.1. Animal preparation and physiological monitoring

Ten newborn piglets (less than 2 weeks of age) were anesthetized, instrumented, and ventilated with a volume-controlled animal ventilator as described previously. Throughout the experiment, the electrocardiograph, mean arterial blood pressure, peripheral capillary oxygen saturation, and anal temperature were continuously monitored. The initial settings for mechanical ventilation were as follows: 10 mL/kg tidal volume, ventilator rate of 30 breaths/min, inspiration to expiration ratio of 1:1, positive end-expiratory pressure of 5 cm H2O, and fractional concentration of inspired oxygen of 0.21. Throughout the experiments, the ventilator settings were not changed except for the fractional concentration of inspired oxygen, which was adjusted after randomization according to their grouping. All signals for respiratory flow and airway pressure were recorded. The flow signal was integrated to give tidal volume, and lung compliance of respiratory system was measured.

2.2. Experiment protocol

After stabilization and the baseline measurement, the newborn piglets were randomly and evenly divided into two study groups. In the hyperoxia group, the fractional concentration of inspired oxygen was adjusted to 1.0 until the end of the experiments. In the control group, animals were continuously ventilated with room air (fractional concentration of inspired oxygen 0.21). Arterial blood samples were drawn for blood gas analysis at baseline, 15, 30, and then every 30 minutes until 4 hours using an automatic blood gas system (Chiron; Ciba Corning Diagnostics, E. Walpole, MA, USA). After completion of the procedure, the animals were euthanized by injection of a high dose of 15% potassium chloride. Within 10 minutes of the end of the experiment, the scalp was opened and the total brain tissue, including the brainstem, was removed.

2.3. Brain tissue assessment

The brains were first examined grossly and then dissected into the cerebrum, striatum, and brainstem based on brain anatomy. In advance, the cerebrum was dissected to the right/ left frontal, parietal, and occipital cortices. Block samples (approximately 0.5 cm3) were obtained from each site. Each sample was weighed and homogenized in a stock solution containing 0.1 N perchloric acid to prevent oxidation of the monoamines (1 g tissue: 5 ml stock solution). The homogenate tubes were centrifuged (10,000 rpm at 4 °C for 10 minutes), and then the supernatant was aspirated, filtered with a 0.45 µm pore-size filter, and stored at −80 °C until analysis. The concentrations of dopamine, serotonin, and their metabolites, such as 3,4-dihydroxyphenylacetic acid, homovanillic acid, and 5-hydroxyindoleacetic acid, were determined by high-performance liquid chromatography (HPLC) with electrochemical colorimetric detection.

2.4. High-performance liquid chromatography assay for neurotransmitter content

The HPLC system consisted of a pump (BAS PM-92E; Bioanalytical Systems, West Lafayette, IN, USA), a refrigerated microsampler (CMA/200) and a sample injector (CMA/240) with a 20 µL loop (CMA, Stockholm, Sweden), and a digital amperometric electrochemical detector (Decade II; Antec Leyden BV, Zoeterwoude, The Netherlands). The mobile phase consisted of 900 mL phosphate solution (14.7 mM NaH2PO4, 2.2 mM sodium-1-octanesulfonate, 0.027 mM EDTA, 2 mM KCl, and 30 mM sodium citrate), 1 mL diethylamine, and 100 mL acetonitrile. The mobile phase was adjusted to pH 3.3 using H3PO4 and filtered with a 0.22 µm membrane filter before use. The mobile phase was delivered at 50 µL/min. A reversed-phase C-18 column (150 × 1.0 mm, 5 µm internal diameter) was used for sample separation. The applied potential of the glassy carbon electrode was +0.65 V relative to the reference electrode (Ag/AgCl), the filter setting was 0.005 Hz, and the range setting was 20 nA for the determination of neurotransmitters. EZChrom software (Scientific Software, San Ramon, CA, USA) was used for data processing.
2.5. Statistical analyses

Values are presented as means ± standard errors of the mean (SEM). Student t tests were performed to determine statistical differences between groups for basic characteristics and neurotransmitter content. The Mann–Whitney U test was used to analyze the pre- and post-hypoxic levels of neurotransmitters. Two-way repeated measures analysis of variance was used to compare the serial cardiopulmonary data during the experimental period between the two study groups. Statistical significance was defined as p < 0.05.

3. Results

There was no significant difference in age, weight, or any of the baseline hemodynamic parameters between the two study groups (Table 1). Comparing the hemodynamic parameter data after initiating 100% oxygen in the hyperoxia group, there was no significant difference in lung compliance, heart rates, mean arterial blood pressure, pH, or arterial carbon dioxide tension during the experimental period between the two groups. The only difference observed was in the arterial oxygenation parameters between the hyperoxia and control groups (Fig. 1). As shown, arterial oxygen tension and alveolar—arterial oxygen tension difference were both significantly higher in the hyperoxia group compared with the control group (p < 0.05). In the comparison of the ratio of arterial to alveolar oxygen tension, this ratio was significantly lower in the hyperoxia group compared with the control group at 15 and 30 minutes after initiating the experiments (p < 0.05); however, there was no significant difference in these parameters at later time intervals.

Comparing the contents of dopamine, serotonin, and their metabolites, there was a general trend of lower neurotransmitter content in the hyperoxia group compared with the control group (Fig. 2). Furthermore, we found significant decreases in the levels of dopamine in the hyperoxia group compared with the control group (controls 23.5 ± 5.8 ng/mL; hyperoxia group 7.1 ± 2.0 ng/mL; p = 0.028) (Fig. 2A) and 3,4-dihydroxyphenylacetic acid (controls 117.7 ± 29.5 ng/mL; hyperoxia group 10.4 ± 4.6 ng/mL; p = 0.007) (Fig. 2B) in the left frontal lobe, and serotonin in the right occipital lobe (controls 10.8 ± 2.6 ng/mL; hyperoxia group 4.2 ± 1.3 ng/mL; p = 0.038) and left parietal lobe (controls 27.4 ± 5.5 ng/mL; hyperoxia group 8.4 ± 3.8 ng/mL; p = 0.023) (Fig. 2D). However, there was no significant difference in homovanillic acid or 5-hydroxyindoleacetic acid at any of the cranial sites between the two groups (Fig. 2C and E).

Table 1

Characteristics of baseline physiological variables in both study groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control (n = 5)</th>
<th>Hyperoxia (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (d)</td>
<td>8.6 ± 1.1</td>
<td>9.8 ± 1.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>2.1 ± 0.1</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>193 ± 19</td>
<td>204 ± 31</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>83 ± 10</td>
<td>88 ± 15</td>
</tr>
<tr>
<td>pH</td>
<td>7.38 ± 0.05</td>
<td>7.39 ± 0.06</td>
</tr>
<tr>
<td>PaO2 (mmHg)</td>
<td>85.5 ± 2.1</td>
<td>89.6 ± 7.0</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>38.3 ± 2.5</td>
<td>37.9 ± 1.7</td>
</tr>
</tbody>
</table>

PaO2 = arterial oxygen tension; PaCO2 = arterial carbon dioxide tension.

Our study demonstrated a significant reduction of dopamine and 3,4-dihydroxyphenylacetic acid in the left frontal lobe and of serotonin in the right occipital and left parietal lobes in animals receiving 100% oxygen for 4 hours compared with those piglets receiving room air. Therefore, dopamine, 3,4-dihydroxyphenylacetic acid, and serotonin levels were altered in some cortical areas of neonatal developing brain in the piglets after exposure to 100% oxygen for a period of time.

It is well known that aerobic metabolism possesses recognized advantages for human life. On the other hand, the accompanying disadvantage of aerobic metabolism is the generation of oxygen radicals. Excess reactive oxygen species along with impaired intracellular redox status leads to aging and DNA damage. Any signal or environmental stimulus (such as too much oxygen) that may trigger overproduction of reactive oxygen species can lead a cell to undergo apoptosis.
Compared with adults, the developing organs of neonates are more sensitive to differences in oxygen tension. As early as the 1940s, Comroe et al. reported on the adverse effects of oxygen on the lungs, respiratory control, and mental status. Inappropriate oxygen use is a health hazard to neonates and is associated with aging, DNA damage, cancer, retinopathy of prematurity, bronchopulmonary dysplasia, infection, and potential injury to the developing brain. However, ongoing studies are still required to define the ideal or optimal oxygen doses under different situations.

Oxygen constitutes one possible contributing neurotoxic factor. The developing brain is rich in free iron, has a limited antioxidant capacity, and shows an increased vulnerability of the immature neurons to hydrogen peroxide. Recent adult animal model studies have reported better neurological outcomes when using normoxic compared with hyperoxic resuscitation following cardiac arrest. The disadvantage of hyperoxia might come from the increased oxidation of brain lipids, increased hippocampal oxidative stress, metabolic dysfunction, and neuronal death. Hyperoxia has been demonstrated to alter cerebral blood flow, especially in the immature brain, with its impaired capability of autoregulation, and to trigger diffuse apoptosis in the immature rodent brain with a peak at 1 week of postnatal age. Even following a short period of exposure to hyperoxia, these changes have been found in the cortex and ventricular zone of newborn mice. Our experiments further highlighted the influence of hyperoxia on neurotransmitters in the neonatal CNS. Therefore, the oxygen should be used in neonates carefully to prevent its adverse influence on their developing CNS.

In the present study, we demonstrated that hyperoxia had a negative influence on the levels of cranial dopamine, serotonin, and 5-hydroxyindoleacetic acid (5-HIAA) in different parts of the brain. RF = right frontal; RP = right parietal; RO = right occipital; LF = left frontal; LP = left parietal; LO = left occipital, S = striatum, BS = brainstem. *p < 0.05 versus control group at the same cranial site.
A reasonable explanation for these findings is that hyperoxia may cause some kind of inhibitory effect in the neurotransmitter production and their catabolic enzyme activities. Furthermore, individual variations may influence the responses of cranial transmitters, which is why we noted markedly individual difference in the results for 5-hydroxyindoleacetic acid and homovanillic acid. Therefore, although the alteration in cranial neurotransmitters exists in the cortical area of hyperoxia-exposed subjects, further studies are crucial to elucidate the mechanisms, clinical significance, and influence of individual difference.

There are some limitations to this study. First, it was an animal study, and the results may not truly reflect the influence of hyperoxia on a human neonatal brain. Second, the sample size of this study may not have been large enough to demonstrate certain differences such as the trend of a decrease in 5-HIAA level. Third, the oxygen exposure period in our experiment may have been too short to induce enough damage in some subjects. Fourth, the study subjects were normal hosts who had not encountered any hypoxic insult or asphyxic event before hyperoxia treatment. Therefore, the response of neurotransmitters to hyperoxia in an uninjured brain may not be as severe as that occurring in an injured brain. Further studies may be necessary to clarify the influence of hyperoxia on the CNS in an injured neonatal brain.

In conclusion, hyperoxia may alter the production or metabolism of dopamine and serotonin in some cortical areas of the neonatal CNS, and it tended to have some inhibitory effects. Therefore, pediatricians should be very judicious in using high levels of oxygen on the developing brain. Further studies will be necessary to elucidate the related mechanisms and clinical significance in neonatal subjects with different brain conditions.

Acknowledgments

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