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Research report

GABA and GABA receptors alterations in the primary visual cortex of concave lens-induced myopic model

Wen Zhao[b,1], Ai-Ling Bi[a,1], Chao-Li Xu[b,c,1], Xiang Ye[a,1], Mei-Qing Chen[d], Xin-Ting Wang[e], Xiao-Yan Zhang[c], Jun-Guo Guo[a], Wen-Jun Jiang[a], Jin Zhang[c], Hong-Sheng Bi[a,c,*]

[a] Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Therapy of Ocular Diseases of Shandong, Eye Institute of Shandong University of Traditional Chinese Medicine, Jinan, Shandong, People's Republic of China
[b] Shandong University of Traditional Chinese Medicine, Jinan, Shandong, People's Republic of China
[c] Affiliated Eye Hospital of Shandong University of Traditional Chinese Medicine, Jinan, Shandong, People's Republic of China
[d] Graduate student of grade 2015, the Second clinical medicine school, Shandong University of Traditional Chinese Medicine, People's Republic of China
[e] School of Public Health, Shandong University, Jinan, Shandong, People's Republic of China

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ABSTRACT

Until recently most researches on myopia mechanisms have mainly been focused on the eye ball and few investigations were explored on the upper visual pathway, such as the visual cortex. The roles of gamma-aminobutyric acid (GABA) in the retinal and in the upper visual pathway are inter-correlated. As the retinal glutamate decarboxylase (GAD), GABA, and the mRNA levels of GABA receptors increased during the concave lens induced myopia formation, however, whether GABA alterations also occurred in the visual cortex during the concave lens induction is still unknown. In the present study, using HPLC, Enzyme-Linked Immunosorbent Assay (ELISA) and Real-Time Quantitative-PCR (RT-PCR) methods, we observed the changing trends of GABA, glutamate decarboxylase (GAD), and GABA receptors in the visual cortex of concave lens-induced myopic guinea pigs. Similar to the changing patterns of retinal GABA, the concentrations of GAD, GABA and the mRNA levels of GABA receptors in the visual cortex also increased. These results indicate that the exploration on myopia mechanisms should possibly be investigated on the whole visual pathway and the detailed significance of cortical GABA alterations needs further investigation.

1. Introduction

The characteristics of myopia are the extension of the axial length of the eyes and the decreased refractive error. Until recently most researches on myopia mechanisms have mainly focused on the eye ball, including the retina. Few investigations were explored on the upper visual pathway, such as the visual cortex. Gamma-aminobutyric acid (GABA), an important inhibitory neurotransmitter in the retina, played especially important roles in the myopia development (Stone et al., 2003). A recent animal research of our lab also reported that the concentrations of glutamate decarboxylase (GAD), GABA, and the mRNA levers of type-A and type-C GABA receptors were increased in the retina of a concave lens induced myopic model (Sha et al., 2015).

GABA is also expressed in the visual cortex of all vertebrate in comparable concentrations to the retina (Elder, 1992) and the maturation of GABA interneuron plays critical roles to initiate the critical period plasticity during visual development (Berardi et al., 2003). In particular, the roles of GABA in the retinal and in the upper visual pathway are inter-correlated, which not only occurred in visual development process but also in retinal or cortical injury process. During visual development, a retina-derived protein, OTX2 could be transferred from the retinal into the primary visual cortex via a visual experience-dependent mechanism and then nurtures GABA interneurons maturation (Huang and Di Cristo, 2008; Sugiyama et al., 2008). For another example, the retina injury or removal of retinal visual input could decrease GABA levels in the visual cortex of the mice or cat (Arckens et al., 2000; Keck et al., 2008; Massie et al., 2003). On the contrary, the GABA levels in the retinas also decreased significantly after the superior colliculus, pretectum and optic tract were lesioned (Yamasaki et al., 1997).

* Corresponding author at: Key Laboratory of Integrated Traditional Chinese and Western Medicine for Prevention and Therapy of Ocular Diseases of Shandong, Eye Institute of Shandong University of Traditional Chinese Medicine, Affiliated Eye Hospital of Shandong University of Traditional Chinese Medicine, No. 48 Yingxiongshan Road, Jinan, 250002, Shandong, People's Republic of China.
E-mail address: hongshengbi1@163.com (H.-S. Bi).
[1] These authors contributed equally to this work.

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As the retinal GAD, GABA, and the mRNA levers of GABA receptors altered during the concave lens induced myopic formation, however, whether GABA alterations also occurred in the visual cortex during the concave lens-induction is still unknown. In the present study, using the concave lens-induced guinea pigs as a myopia model, we observed the changing trends of GABA and its receptors in the visual cortex of concave lens induction.

2. Experimental procedures

2.1. Preparation

Three-week-old pigmented guinea pigs (males) were obtained from the Laboratory Animal Co., LTD of Henan Kang-da and were housed 2 per cage in a constant temperature room (26 °C) in open-topped cages, on a cycle of 12 h light (provided by 36 W white fluorescent tubes) and 12 h dark, with free access to food (guinea pig feed and fresh vegetables) and water. All experiments conformed to the statement of the Association for Research in Vision and Ophthalmology for the use of animals in vision and ophthalmological research and were approved by the Institutional Animal Ethics Committee of Eye Institute of Shandong University of Traditional Chinese Medicine.

To observe the dynamic changes in GABA and its receptors during lens induction, guinea pigs were randomly assigned to six groups, including lens-induced groups at 1, 2, and 4 weeks and their corresponding control, respectively. Myopia was induced using the out-of-focus method with a −10 D (spherical power) concave lens over the right eye of the three-week-old pigmented guinea pigs for four weeks. Control animals were from the same litters as the respective experimental groups but did not wear lenses. Data were collected at those sample times for which the retinal GABA concentration had been determined in a previous investigation (Sha et al., 2015). During the experimental period, measures were taken to prevent the form-sense deprival effect, such as keeping the lens clean at all times.

2.2. Localization of the guinea pig's primary visual cortex

Since few previous reports have showed the three-dimensional coordinates of guinea pig's primary visual cortex, we searched for the coordinates according to the rats' brain stereotaxic atlas. Guinea pigs were deeply anesthetized and perfused with 0.9% NaCl, followed by phosphate-buffered fixative containing 4% paraformaldehyde. Next the guinea pigs' brains were taken out, postfixed, immersed in 30% sucrose solution. After being embedded by optimal cutting temperature compound (OCT), 40 μm-serial coronal sections were cut throughout the brain using a freezing sliding microtome (SLEE, Germany). Every sixth serially obtained section (200 μm interval) was processed for Nissl staining. According to the values determined from analysis of 8 guinea pigs, the primary visual cortex was identified as the coordinates relative to bregma: anteroposterior (AP), −8.18 to −10.34 mm; lateral (L), ± 4.0–4.5 mm; and ventral (V), −2.0 mm (Fig. 1A).

Next, using flash visual evoked action potential (FVEP) detection combined with cortical chemical destruction method, we further testified whether the visual cortex we locate above had its function. The FVEP changes were detected before and after ibotenic acid, a chemical neurotoxicity substance microinjection into the central location of the above coordinates. Briefly, guinea pigs were anesthetized, restrained in a stereotaxic apparatus (Reward 8001, Reward Life Science, Shenzhen, Guangdong, China), and implanted with a microsyringe aimed at the V1 region of the visual cortex bilaterally, respectively (coordinates relative to bregma: AP, −9.0 mm; L, ± 4.2 mm; and V, −2.0 mm). Ibotenic acid (10 μg in 1 μL/hemisphere) was microinjected into the binocular visual cortex. To minimize backflow of the injected liquid up the injection tract, an additional 2 min was allowed for diffusion before the microsyringe was removed. FVEP were detected using Roland Electrophysiological Test Unit (Germany) before ibotenic acid microinjection and 48 h after chemical microinjection. The tissue used for HPLC, ELISA and RT-PCR were derived from different set of guinea pigs. All tissues were taken out at the same coordinates (Fig. 1B).

2.3. HPLC

Ultimate 3000 HPLC (including the Ultimate 3000 Pump, Ultimate 3000 Autosampler, Ultimate 3000 RS Column Compartment, Ultimate 3000 Diode Array Detector, and 6.80 SR9a Chromeleon Software, Dionex, Germany) was used to quantify the levels of amino acid. GABA was purchased from Sigma-Aldrich (Sigma, USA). Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from TEDIA (Tedia, USA). All other chemicals were of analytical grade. Chromatographic-grade water was produced by an Option-Q 7/15 system (ELGA, England).

The visual cortices were dissected at the indicated time point. Based on a published protocol (Nishimura and Song, 2012), the left visual cortex (V1) was transferred into previously weighed Eppendorf tubes and stored in liquid nitrogen, This procedure was performed by a single investigator. GABA was measured immediately after tissue was removed. For each sample, 0.4 moL/L $HClO_4$ was added according to a mass volume ratio (mg: μL) of 1:10 and the tissue was fully ground. Samples were then centrifuged at 4 °C, 12000*g* for 30 min, and the supernatant was reserved.

The mobile phase A was composed of sodium acetate (100 mM/L) with 7% acetonitrile (pH 6.5), and the mobile phase B was methanol/acetonitrile/Chromatographic-grade water (20: 60: 20, v/v/v). The elution was facilitated by the following gradient program: initial, 0% B; 6.0 min, 6% B; 15.0 min, 9% B; 20.0 min, 45% B. The mobile-phase solution was then passed through a 0.22-μm filter under vacuum and degassed by passing under an ultrasonic washing unit for 10 min. Amino acid separation was performed on an Atlantis ® d C18 reverse phase column, (4.6 × 150 mm, 3 μm; Waters, USA). Each 1 μL sample was injected for HPLC analysis at a flow rate of 0.8 mL/min and an operature temperature of 30 °C. UV detection was set at 254 nm. Concentration of GABA in visual cortex = concentration of sample (ug/mL) × volume of sample (mL)/weight of cortex (g)/molecular mass of GABA. Each experimental group contains 6–10 samples.

2.4. Enzyme-linked immunosorbent assay (ELISA)

The left visual cortex was dissected at the indicated time point and stored in microcentrifuge tubes at −80 °C until analysis. Brain tissue samples were homogenized in ice-cold 1 × PBS lysis buffer (pH 7.4) according to a mass volume ratio (mg:μL) of 1:5. The tissue homogenates were centrifuged at 3000*g* for 20 min at 4 °C. The supernatants were collected and used for quantification of glutamate decarboxylase (GAD) levels. The GAD levels in each sample were assessed using a commercially available assay kit for guinea pig from Ji-Yin-Mei (Wuhan, China). All steps were performed according to the protocol provided in the kit and color change was measured in an ELISA plate reader at 450 nm. There are 6–10 tissue samples per group.

2.5. Real-time quantitative-PCR (RT-PCR)

Samples were analyzed in triplicate using gene-specific guinea pig visual cortex primers together with SYBR Green (Roche Applied Science, Indianapolis, IN) using a Real-time PCR Detection System,