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EFFECTS OF ELECTROACUPUNCTURE ON THE LEVELS OF RETINAL GAMMA-AMINOBUTYRIC ACID AND ITS RECEPTORS IN A GUINEA PIG MODEL OF LENS-INDUCED MYOPIA

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Abstract—Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter of the retina and affects myopic development. Electroacupuncture (EA) is widely utilized to treat myopia in clinical settings. However, there are few reports on whether EA affects the level of retinal GABA during myopic development. To study this issue, in the present study, we explored the changes of retinal GABA content and the expression of its receptor subtypes, and the effects of EA stimulation on them in a guinea pig model with lens-induced myopia (LIM). Our results showed that the content of GABA and the expression of $GABA_A$ and $GABA_C$ receptors of retina were up-regulated during the development of myopia, and this up-regulation was inhibited by applying EA to Hegu (LI4) and Taiyang (EX-HN5) acupoints. Moreover, these effects of EA show a positional specificity. While applying EA at a sham acupoint, no apparent change of myopic retinal GABA and its receptor subtypes was observed. Taken together, our findings suggest that LIM is effective to up-regulate the level of retinal GABA, $GABA_A$ and $GABA_C$ receptors in guinea pigs and the effect may be inhibited by EA stimulation at LI4 and EX-HN5 acupoints.

Key words: electroacupuncture, gamma-aminobutyric acid, lens-induced myopia, retina, guinea pig.

INTRODUCTION

Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter of the retina and central nervous system (CNS); it acts at many different sites via various postsynaptic receptors, $GABA_A$, $GABA_B$ and $GABA_C$ (Messersmith and Redburn, 1993; Koulen et al., 1997; Barker et al., 1998; Wassle et al., 1998; Macri et al., 2000; Represa and Ben-Ari, 2005; Zucker et al., 2005; Delgado et al., 2009). $GABA_A$ receptors are protein complexes that consist of several subunits (α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, θ and π) which form a pentameric chloride channel (Carter et al., 2010); $GABA_C$ receptors encompass $\rho1$, $\rho2$ and $\rho3$ subunits (Wegelius et al., 1998); $GABA_B$ receptor is a dimer comprising $GABA_BR_1$ and $GABA_BR_2$ components (Benke et al., 2002). Although GABA receptors are mainly expressed in retina, some studies reveal that the GABA signaling machinery exists in the development of non-retinal ocular tissues, including cornea, lens, and sclera (Kwakowsky et al., 2008; Cheng et al., 2011, 2012).

GABA is correlated with visual development. Some studies have demonstrated that a reduction of GABAergic inhibition would be able to restore plasticity in the adult visual system (Harauzov et al., 2010), and bicuculline (a $GABA_A$ receptor antagonist) could reduce the suppression of amblyopia (Burchfiel and Duffy, 1981). Moreover, in clinic, GABA is used for some ocular diseases. Vigabatrin, a GABA transaminase inhibitor, causes permanent peripheral visual field deficits, visual electrophysiological abnormalities and other visual disturbances (Hosking and Hilton, 2002; Wild et al., 2006; Sergott, 2010). In the treatment of nystagmus, several GABAergic drugs have been proven to be effective in clinical application (Tegetmeyer, 2014).

Currently, although it is confirmed that GABA agents influence eye growth; antagonists of GABA inhibit myopia development in the chick myopic model (Stone et al., 2003; Chebib et al., 2009), there are few reports of studying directly the expression change of GABA or

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Abbreviations: EA, electroacupuncture; ELISA, enzyme-linked immunosorbent assay; GABA, gamma-aminobutyric acid; GCL, ganglion cell layer; HPLC, high performance liquid chromatography; INL, inner nuclear layer; IPL, inner plexiform layer; LIM, lens-induced myopia; ONL, outer nuclear layer; OPL, outer plexiform layer.

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GABA receptors in myopic eyes. Following consideration of the important role of the retina in modulating eye growth, here we investigate whether GABA and its receptors vary in the retina of myopic eyes.

Electroacupuncture (EA), a reformative form of the manual acupuncture, is a therapeutic method in Traditional Chinese Medicine. Acupuncture or EA is often utilized to treat various diseases such as Parkinson's disease (Kim et al., 2005, 2011), headache (Ahn et al., 2011; Dietzel et al., 2013; Fofi et al., 2014), and cardiovascular dysfunction (Gan et al., 2005; Tjen et al., 2007). EA has also been shown to be an effective treatment of some optical disorders including amblyopia (Wu et al., 2011), glaucoma (Chan et al., 2005) and retinitis pigmentosa (Pagani et al., 2006; Bittner et al., 2014). Most notably, as a popular, traditional and side-effect free treatment, acupuncture or EA is widely used in adolescent myopia (Val'kova and Niurenberg, 1989; Tao et al., 2008; Wei et al., 2011; Yang et al., 2012), yet the underlying mechanisms are not well understood.

Some neurotransmitters play a role in the therapeutic effect of EA, such as glutamate, acetylcholine, and dopamine (Zhou et al., 2007a; Sun et al., 2012; Chavan and Tracey, 2014), especially GABA (Gan et al., 2005; Fusumada et al., 2007; Du et al., 2011) in the nervous system. It is suggested that EA would improve neural diseases and exert a neuroprotective effect by regulating the level of GABA. In the search for underlying visual mechanisms of myopia, persuasive evidences now support a notion that the retina was central to the mechanisms (Ganesan and Wildsoet, 2010; Stone and Khurana, 2010; Stone et al., 2013). Therefore, in the present study, the role of retinal GABA and its receptors in the eye growth of lens-induced myopia (LIM), the dynamic changes of retinal GABA and its receptors in the LIM eyes at 1 week, 2 weeks, and 4 weeks scales were investigated. To clarify whether EA inhibits the changes of GABA and its receptors in myopic eyes, visual-related acupoints Hegu (LI4) and Taiyang (EX-HN5), which had been extensively applied in clinical settings (Dabov et al., 1985; Li et al., 2007; Ding and Zuo, 2008; Tao et al., 2008), were selected in the present study.

EXPERIMENTAL PROCEDURES

Experimental design

Animals. All pigmented guinea pigs (Cavia porcellus, 3-weeks old) were obtained from the Henan Kangda Laboratory Animal Ltd. (Henan, China) and reared in the animal lab of Eye Institute of the Shandong University of Traditional Chinese Medicine. Water and food were available *ad libitum* and the room temperature was maintained at 22 °C. Three or four guinea pigs were raised together in a plastic cage (15 × 26 × 32 cm) under a 12-h light–dark cycle. The average light intensity in the cage was approximately 300 lux. All experimental protocols and animal handling procedures were approved by the Animal Care and Use Committee of the Shandong University of traditional Chinese medicine, and were in accordance with the statement of the Association for Research in Vision and Ophthalmology for the use of animals in vision and ophthalmological research.

Our study contained two experiments. Experiment 1: Observing the change of retinal GABA and/or its receptors during myopic development. In this experiment, three time-points of myopic development were chosen, and guinea pigs were randomly divided into 3 groups: 1-week, 2-weeks and 4-weeks lens-induced myopia according to these time-points. Experiment 2: Effects of EA on the retinal GABA and its receptors after 4 weeks. In this experiment, influence of EA was assessed in myopic guinea pigs which consisted of lens-induced myopia group (LIM), lens-induced myopia + electroacupuncture group (LIM + EA) and lens-induced myopia + sham acupoints group (LIM + sham). All 3-weeks-old animals were raised with a −10 D lens attached to the right eyes. The fellow eyes (left eyes) were untreated and served as the self-control group. Meanwhile, animals of LIM + EA group were treated accompanied by EA stimulation at acupoints Hegu (LI4) and Taiyang (EX-HN5) at the same time for 4 consecutive weeks, and sham acupuncture at a LIM + sham group.

Lens. Concave lenses purchased from Shanghai million new optical glasses Co., LTD (Shanghai, China) were modified mechanically using human polymethylmethacrylate (PMMA) lenses with 11 mm diameters and large optic zones (10 mm). Lenses were mounted onto a self-made frame using surgical tapes and glued onto the right eyes of guinea pigs.

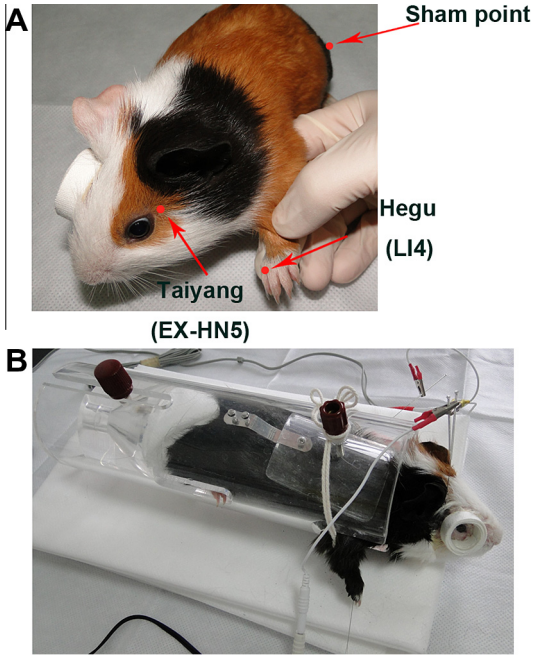

Fig. 1. Electroacupuncture treatment experiment. (A) The point location of acupuncture treatment (red circle). (B) Photograph of electroacupuncture treatment at LI4 and EX-HN5 points. A guinea pig was lightly immobilized using a holder made by our laboratory to minimize the restraint stress during acupuncture treatment.