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OVEREXPRESSION OF SERUM RESPONSE FACTOR IN ASTROCYTES IMPROVES NEURONAL PLASTICITY IN A MODEL OF EARLY ALCOHOL EXPOSURE

A. P. PAUL AND A. E. MEDINA*

Department of Anatomy and Neurobiology, Virginia Commonwealth University School of Medicine, Richmond, VA 23298-0709, United States

Abstract—Neuronal plasticity deficits underlie many of the cognitive problems seen in fetal alcohol spectrum disorders (FASD). We have developed a ferret model showing that early alcohol exposure leads to a persistent disruption in ocular dominance (OD) plasticity. Recently, we showed that this deficit could be reversed by overexpression of serum response factor (SRF) in the primary visual cortex during the period of monocular deprivation (MD). Surprisingly, this restoration was observed throughout the extent of visual cortex and most of the cells transfected by the virus were positive for the astrocytic marker GFAP rather than the neuronal marker NeuN. Here we test whether overexpression of SRF exclusively in astrocytes is sufficient to restore OD plasticity in alcohol-exposed ferrets. To accomplish that, first we exposed cultured astrocytes to Sindbis viruses carrying either a constitutively active form of SRF (SRF+), a dominant negative (SRF−) or control Green Fluorescent Protein (GFP). After 24 h, these astrocytes were implanted in the visual cortex of alcohol-exposed animals or saline controls one day before MD. Optical imaging of intrinsic signals showed that alcohol-exposed animals that were implanted with astrocytes expressing SRF, but not SRF− or GFP, showed robust restoration of OD plasticity in all visual cortex. These findings suggest that overexpression of SRF exclusively in astrocytes can improve neuronal plasticity in FASD.

Key words: serum response factor, astrocyte, ocular dominance plasticity, visual development, ferret, fetal alcohol spectrum disorders.

INTRODUCTION

Alcohol consumption during pregnancy can lead to fetal alcohol spectrum disorders (FASD). This condition is characterized by a constellation of morphological, cognitive and behavioral problems with possible lifelong implications (Jones and Smith, 1973). In FASD, the sensory systems are particularly affected and learning and memory problems, as well as visual, somatosensory and auditory deficits are frequently observed (Mattson and Riley, 1998; Coles et al., 2002; Franklin et al., 2008; Manji et al., 2009).

The timing of alcohol exposure can be a determinant factor for the signs and symptoms observed in FASD. During the first-trimester equivalent of human gestation, alcohol exposure can alter normal development of the neural tube and crest, leading to microcephaly (Miller, 1996), hydrocephaly (Ronen and Andrews, 1991; Goez et al., 2011), ocular malformations (Cook et al., 1987; Stromland, 2004), and the facial dysmorphology that characterizes fetal alcohol syndrome (Sulik et al., 1981), a classical type of FASD. In the second trimester, alcohol exposure strongly affects the proliferation of neuronal precursors and the formation of the radial glia leading to abnormal neuronal migration (Miller and Robertson, 1993; Siegenthaler and Miller, 2004; Tateno and Saito, 2008; Vangipuram and Lyman, 2010; Aronne et al., 2011). Finally, during the third-trimester, alcohol can increase cell death, disrupt synaptogenesis and lead to persistent deficits on neuronal plasticity (Hoff, 1988; Rema and Ebner, 1999; Climent et al., 2002; Moulder et al., 2002; Medina et al., 2003; Isayama et al., 2009).

During the last decade, numerous studies have provided evidence that activity-dependent neuronal plasticity, a process crucial for the development of sensory cortices, is disrupted in animal models of FASD. For instance, early alcohol exposure has been shown to disrupt long term potentiation (Puglia and Valenzuela, 2010), eye-blink conditioning (Johnson et al., 2008), barrel cortex plasticity (Rema and Ebner, 1999) and ocular dominance plasticity (ODP) (Medina et al., 2003).

The ODP paradigm is based on the functional and anatomical changes that occur in the visual cortex after closing one eye by eyelid suturing (monocular deprivation, MD). In this paradigm, MD leads to shrinkage and expansion of ocular dominance columns receiving responses from the deprived (closed) eye and experienced (open) eye, respectively (Wiesel And Hubel, 1963; Hubel et al., 1977; Issa et al., 1999). As in the case of other types of activity-dependent plasticity, ODP relies on the integrity of the

*Corresponding author. Address: Department of Pediatrics, University of Maryland, School of Medicine, 655 W Baltimore Road, Room 13-017, Baltimore, MD 21201, United States.
E-mail address: amedina@vcu.edu (A. E. Medina).

Abbreviations: CBI, contralateral bias index; CREB, cAMP response element binding protein; EDTA, ethylene diamine tetra acetic acid; FASD, fetal alcohol spectrum disorders; GDNF, glial cell line-derived neurotrophic factor; GFAP, Glial fibrillary acidic protein; GFP, Green Fluorescent Protein; IP3, inositol triphosphate; LTD, Long term depression; LTP, Long term potentiation; MD, monocular deprivation; OD, ocular dominance; ODP, ocular dominance plasticity; P, postnatal day; PBS, phosphate-buffered saline; ROI, region of interest; SRF, serum response factor.

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transmission of synaptic signals to the nucleus, where plasticity-related genes are regulated by transcription factors (Roberts et al., 1998; Beaver et al., 2001; Di Cristo et al., 2001; Mower et al., 2002; Taha et al., 2002).

Early alcohol exposure has been shown to affect the activity of many molecules that are important for ODP such as the NMDA receptor and many activity-dependent protein kinases (Savage et al., 1992; Davis et al., 2000; Joshi et al., 2006; Naseer et al., 2011). Many of these molecules are part of cascades that converge toward two important transcription factors, namely, cAMP response element binding protein (CREB) and serum response factor (SRF) (Whitmarsh et al., 1995; Chai and Tarnawski, 2002; Knoll et al., 2006). While the role of CREB on activity-dependent plasticity is quite established (Silva et al., 1998; Mower et al., 2002), much less is known about the contribution of SRF. This is surprising since both CREB and SRF can be activated by neuronal activity and both can regulate the expression of plasticity-related genes (Finkbeiner et al., 1997; Lamprecht, 1999; Ramanan et al., 2005; Knoll and Nordheim, 2009).

The transcription factor SRF has three functional domains: the dimerization and DNA binding domain, the transactivation domain and multiple phosphorylation sites (Chai and Tarnawski, 2002). It binds to its serum response element (SRE), which is present in the promoter regions of many genes. Some of the well known SRF target genes are IEGs (*c-fos*, *Egr-1* to *Egr-3*, *Junb*, etc.), actin cytoskeletal genes (*Actb*, *Actg1*, *Gelsolin*, *Arc*, etc.), axonal guidance genes (*Sema3a*, *Epha4*, *Epha7*, etc.), neurotrophin genes (*Bdnf*, *Ngf*) and genes involved in synaptic plasticity (Arc, Psd95) (Etkin et al., 2006; Knoll et al., 2006; Knoll and Nordheim, 2009). SRF has already been demonstrated to be essential for various forms of neuronal plasticity, including Long term potentiation (LTP) (Ramanan et al., 2005), Long term depression (LTD) (Etkin et al., 2006) and learning and memory (Dash et al., 2005; Etkin et al., 2006).

Recently, we used viral mediated gene transfer to test whether overexpression of SRF would reverse the disruption of ODP caused by early alcohol exposure in ferrets (Paul et al., 2010). We observed that during the period of MD, overexpression of a constitutively active form of SRF, but neither its dominant negative form nor GFP, restored ODP in alcohol-exposed animals. Surprisingly, this restoration was observed throughout the extent of the visual cortex instead of being limited to the area of the virus transfection. Moreover, an analysis of the virus expression showed that while only 7% of the SRF-transfected cells expressed the neuronal marker NeuN, more than 60% cells showed expression of the astrocytic marker Glial fibrillary acidic protein (GFAP). Since glial cells can secrete plasticity-related molecules, and because we observed that most of the transfected cells were GFAP positive, we hypothesized that overexpression of SRF in astrocytes can affect neuronal plasticity. However, we could not discard a major contribution of the few SRF-transfected neurons or the unidentified cells. Here we investigate whether overexpression of SRF in astrocytes is sufficient for the restoration of ODP in alcohol-exposed animals. To accomplish this, we overexpressed the constitutively active form of SRF in astrocyte cultures and implanted these modified cells in the visual cortex of ferrets exposed to alcohol during the third trimester equivalent of human gestation.

EXPERIMENTAL PROCEDURES

All procedures that are described in this article were approved by the Institutional Animal Care and use Committee at Virginia Commonwealth University. The protocols for some of the general experimental methods are the same as described in earlier papers (Medina et al., 2003, 2005; Paul et al., 2010). Please refer to Fig. 1 for a summary of our experimental design. Briefly, ferrets were treated with 3.5 g/kg ethanol (25% in saline, i.p.) or saline as control every other day from postnatal day (P) 10 to P30. This alcohol treatment leads to a blood alcohol level of ~250 mg/dl for 1–5 h after injection (Medina et al., 2003). The animals were then left alcohol-free until the peak of the critical period of OD plasticity, which in ferrets is around P40 (Issa et al., 1999). Additional ferrets were sacrificed at P9/P10 and caudal portions of their brains containing the visual area were dissected to make pure astrocyte cultures. The cultures were transfected by Sindbis viruses carrying one of the following, a constitutively active form of SRF (SRF+), a dominant negative form (SRF−) or control GFP.

After 24 h of transfection, the astrocytes were implanted into the left visual cortex of the alcohol-exposed animals. One day after the implantation, the right eye of the animal was monocularly deprived (MD) by eyelid suture for 4 days. Following MD, the animal was anesthetized, the deprived eye was opened and optical imaging of intrinsic signals was conducted on the left primary visual area.

Preparation of astrocyte cultures

The culture procedure is modified from the method established by McCarthy and de Vellis (1980). Ferrets at P9 or P10 are sacrificed and the caudal portion of its brain is immediately cut out, cleaned from its meninges and put in a petri dish containing Hank's Balanced Salt Solution (HBSS) in sterile conditions inside a biosafety hood. The tissue block is minced to smallest possible pieces using a scalpel blade. The minced tissue is collected in a 15-ml falcon tube with 3mls of 0.5% Trypsin–EDTA. The tube is gently agitated for 5–7 min. The tissue pieces are then mechanically triturated using a pipette to finer particles. Then serum media (DMEM/F12 + 10% fetal calf serum) is added to inactivate trypsin. The cell suspension is centrifuged at 3000 rpm for 3 min, resuspended in fresh serum media and plated in T-25 culture flasks (Falcon, BD). The flasks are kept in an incubator at 37 °C and 5% CO_2. The cells become confluent in 8–10 days. At that time, the flasks are agitated on an orbital shaker (250 rpm, 37 °C, 12–15 h) in order to remove the oligodendrocytes and microglia. The supernatant is removed and an astrocyte purity of ~98% is achieved (McCarthy and de Vellis, 1980).

Ex-vivo gene delivery procedure

The Sindbis viral vectors were made and kindly provided by Drs. Amit Etkin, Angel Barco and Eric Kandel (Columbia University, New York, NY). The SRF+ construct was made by fusing the dimerization and DNA binding domains of SRF (aa 90–222) with the transactivation domain of HSV protein VP16 (aa 363–490) (Paul et al., 2010). This construct was then cloned into the pSin-Rep5 vector (Invitrogen, Carlsbad, CA, USA). The SRF− was made by fusing a FLAG epitope sequence to the N terminus of SRF (aa 1-272), which was found to effectively inhibit SRF activity (Etkin et al., 2006). The control GFP construct was made by cloning an EGFP cDNA into the vector.