Int. J. Devl Neuroscience 34 (2014) 24–32

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

journal homepage: www.elsevier.com/locate/ijdevneu

Maternal micronutrient imbalance alters gene expression of BDNF, NGF, TrkB and CREB in the offspring brain at an adult age

Pratiksha Sable, Anvita Kale, Asmita Joshi, Sadhana Joshi *

Department of Nutritional Medicine, Interactive Research School for Health Affairs, Bharati Vidyapeeth Deemed University, Pune Satara Road, Pune 411043, India

ARTICLE INFO

Article history:
Received 6 December 2013
Received in revised form 7 January 2014
Accepted 13 January 2014

Keywords:
BDNF
NGF
TrkB
CREB
Micronutrient imbalance
Cognition

ABSTRACT

Micronutrients like folate, vitamin B_{12}, and fatty acids which are interlinked in the one carbon cycle play a vital role in mediating epigenetic processes leading to an increased risk for neurodevelopmental disorders in the offspring. Our earlier study demonstrates that a micronutrient imbalanced diet adversely affects docosahexaenoic acid (DHA) and protein levels of neurotrophins like brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the brain and cognition in the offspring by 3 months of age. In this study we attempt to analyze if these effects are a consequence of a change in gene expression of these molecules. Further, we also examined the effect of either a postnatal control diet or a prenatal omega-3 fatty acid supplementation on gene expression in the cortex of the offspring. Pregnant rats were divided into control and five treatment groups at two levels of folic acid (normal and excess folate) in the presence and absence of vitamin B_{12}. Omega-3 fatty acid (eicosapentaenoic acid – EPA + DHA) supplementation was given to vitamin B_{12} deficient groups. Following delivery, 8 dams from each group were shifted to control diet and remaining continued on the same treatment diet. Our results demonstrate that the imbalanced diet caused a marked reduction in the mRNA levels of BDNF, NGF, TrkB, and cAMP response element-binding protein (CREB). Prenatal omega-3 fatty acid supplementation to the maternal imbalanced diet was able to normalize the mRNA levels of all the above genes. This study demonstrates that a maternal diet imbalanced in micronutrients (folic acid, vitamin B_{12}) influences gene expression of neurotrophins and their signalling molecules and thereby adversely affects the brain of the offspring.

1. Introduction

The field of "developmental origins of health and disease" (DOHaD) has developed tremendously over the years and increasing knowledge suggests that maternal diet can have long-lasting effects on the offspring health (Vanhees et al., 2014). Micronutrients like folic acid and vitamin B_{12} are part of the one carbon cycle and are vital for the generation of methyl groups which affect DNA and histone methylation thereby regulating gene expression in the offspring (Dominguez-Salas et al., 2012). Studies by us and others have shown that micronutrients like folate and vitamin B_{12} are interlinked and influence levels of important long chain polyunsaturated fatty acids (LCPUFAs) like docosahexaenoic acid (DHA) (Van Wijk et al., 2012; Kale et al., 2010).

DHA, an important omega-3 LCPUFA in the central nervous system, is located in the synaptic end sites and is required for increasing the fluidity of cell membranes and maturation of synapses (Willatts and Forsyth, 2000). Reports also suggest that DHA supplementation improves behaviour and cognition in children (Milte et al., 2012; Richardson et al., 2012). Brain development and cognition are also known to be influenced by neurotrophins which play a role in development, growth, and differentiation of the nervous system (Stoleru et al., 2013). Studies indicate that neurotrophins can positively be influenced by fatty acids like DHA (Wu et al., 2008; Sharma et al., 2012).

Various neurotrophins are involved in learning, memory and behaviour and their alterations have been linked to the onset of psychiatric disorders (Chao et al., 2006). These neurotrophins are present in abundance in the brain and mediate their function through their membrane tyrosine kinase receptors (Patapoutian and Reichardt, 2001). Further, binding of brain derived neurotrophic factor (BDNF) to its receptor leads to phosphorylation of tyrosine residues thereby activating the transcription factor cAMP response element-binding protein (CREB) (Cunha et al., 2010). A

Abbreviations: BDNF, brain derived neurotrophic factor; CREB, cAMP response element-binding protein; DAG, diacylglycerol; DHA, docosahexaenoic acid; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IP3, inositol 1,4,5-trisphosphate; MAPK, mitogen activated protein kinases; NGF, nerve growth factor; PI3K, phosphatidylinositol 3-kinase pathway; PLCγ, phospholipase.

* Corresponding author. Tel.: +91 020 24366929/020 24366931; fax: +91 020 24366929.
E-mail address: srjoshi62@gmail.com (S. Joshi).

0736-5748/$36.00 © 2014 ISDN. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ijdevneu.2014.01.003

number of studies support the vital role of CREB in signalling mechanisms facilitating learning and memory processes (Kandel, 2012). Apart from BDNF, NGF deficit has also been shown to hamper cognitive processes through inhibition of crucial mechanisms like long term potentiation (Conner et al., 2009) and it has further been suggested that BDNF and NGF may play a vital role in mediating processes associating early life environment with brain development and behaviour (Branchi et al., 2006,2013). Studies report that brain development is sensitive to epigenetic changes like methylation which can cause alterations in gene expression and can affect behaviour in later life (Konycheva et al., 2011).

Our earlier animal studies have adequately demonstrated that an alteration in the maternal micronutrients reduces DHA and neurotrophin levels and impairs cognition in the offspring at 3 months of age. We also demonstrate that these adverse effects were not reversed by a postnatal control diet (Sable et al., 2013a). In addition we have recently reported alterations in global methylation levels in the brain as a consequence of an imbalance in maternal micronutrients (Sable et al., 2013b).

Based on our earlier studies, we hypothesize that an imbalance in maternal micronutrients will adversely affect mRNA levels of molecules involved in the signalling cascade critical for learning and memory. The present study for the first time examines the effect of maternal micronutrient imbalance on mRNA levels of BDNF, NGF, TrkB and CREB in the brain of the adult offspring. The effect of prenatal omega-3 fatty acid supplementation on the above parameters is also examined.

2. Materials and methods

All experimental procedures were in accordance with guidelines of Institutional Animal Ethics Committee (IAEC). The institute is recognized to undertake experiments on animals as per the committee for the purpose of control and supervision of experimental animals, Govt. of India (No. 258/CPCSEA).

The protocol for the study has been recently described by us in detail (Sable et al., 2012, 2013a). Briefly, there were a total of 6 groups, i.e. control and five treatment groups. Each treatment group had 16 dams while the control group had 8 dams. After delivery, the litter size was culled to 8 per dam in each group to maintain nutritional adequacy. From day 1 of lactation 8 dams from each treatment group were shifted to a control diet and the remaining dams continued on the same treatment diet up to 3 months of age (Fig. 1). In this protocol no cross-fostering was undertaken.

The present study examined the dietary effect of maternal folic acid supplementation at two levels, i.e. 2 mg and 8 mg folic acid, both in the presence and absence of vitamin B_{12}. Further, both the vitamin B_{12} deficient groups were supplemented with omega-3 fatty acids. Maxepa (fish oil, Merck) which contained a combination of DHA (120 mg) and eicosapentaenoic acid (EPA) (180 mg) as the source of omega-3 fatty acids. We are now also giving a table incorporating the diet composition (Table 1).

2.1. Extraction of total RNA and cDNA synthesis

Briefly, Total RNA from brain samples was isolated using the Trizol method and quantified using the Eppendorf BioPhotometer plus. The RNA samples were further processed by the DNA-free TM DNase Treatment Kit (Cat No. AM1906, Ambion, Austin, Texas) to remove the contaminating DNA. Using the High-Capacity cDNA reverse transcription Kit (Cat No. 4368814, Applied Biosystems), 2 μg of DNA-free RNA was reverse transcribed to cDNA.

2.2. Real time quantitative polymerase chain reaction (RT-qPCR) assay

RT-qPCR for BDNF, NGF, TrkB and CREB was performed using the TaqMan Universal PCR Master Mix (Cat No. 4324018, Applied Biosystems, California, USA) on the Applied Biosystems 7500 Standard Real Time PCR system. The relative expression level of the gene of interest was examined with respect to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to normalize for variation in the quality of RNA and the amount of cDNA input. The RT-PCR reactions for each gene were performed in duplicate. Relative expression levels of genes were calculated and expressed as $2^{-\Delta\Delta Ct}$. The following TaqMan® assays (Applied Biosystems) were used in this study: GAPDH (Rn99999916_s1); BDNF (Rn01484924_m1), NGF (Rn01533872_m1), TrkB (Rn00820626_m1) and CREB (Rn00578828_g1).

2.3. Statistical analysis

Values are mean ± SE. Mean values of the estimates of various parameters for the treatment groups were compared with those of control group at conventional levels of significance, using least significance difference estimated from a one-way analysis of variance (ANOVA) and the post hoc least significant difference test. The data were analyzed using SPSS/PC + package (Version 20.0, Chicago, IL, USA).

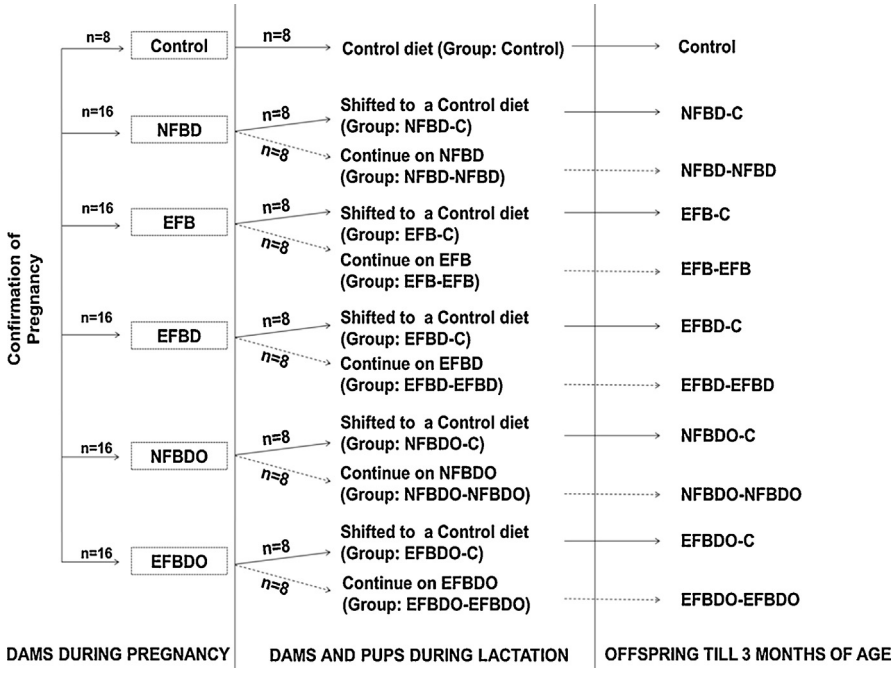

Fig. 1. Study design. Control: normal folic acid, normal vitamin B_{12}, NFBD: normal folic acid, vitamin B_{12} deficient, NFBDO: normal folic acid, vitamin B_{12} deficient, omega-3 fatty acid supplemented, EFB: excess folic acid, normal vitamin B_{12}, EFBD: excess folic acid, vitamin B_{12} deficient, EFBDO: excess folic acid, vitamin B_{12} deficient, omega-3 fatty acid supplemented. NFBD–NFBD (NFBD continuing on NFBD after delivery), NFBD-C (NFBD shifting to control after delivery), NFBDO-C (NFBDO shifting to control after delivery), NFBDO–NFBDO (NFBDO continuing on NFBDO after delivery), EFB–EFB (EFB continuing on EFB after delivery), EFB-C (EFB shifting to control after delivery), EFBD–EFBD (EFBD continuing on EFBD after delivery), EFBD-C (EFBD shifting to control after delivery), EFBDO–EFBDO (EFBDO continuing on EFBDO after delivery), EFBDO-C (EFBDO shifting to control after delivery).