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REDUCED NERVE GROWTH FACTOR LEVELS IN STRESS-RELATED BRAIN REGIONS OF FOLATE-DEFICIENT MICE

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Abstract—Folate deficiency has been linked to neurodegenerative and stress-related diseases such as stroke, dementia and depression. The role of the neurotrophins nerve growth factor (NGF) and neurotrophin-3 (NT-3) in stress-related disorders and neurodegeneration has garnered increasing attention in recent years. Uracil misincorporation is involved in the neuropsychiatric dysfunction induced by experimental folate deprivation. However, the effects of folate deficiency on the expression of NGF and NT-3 in brain tissue have not yet been investigated. In a 2 × 2 design, aged mice lacking uracil-DNA N-glycosylase ($Ung^{-/-}$) versus wild-type ($Ung^{+/+}$) controls were subjected to a folate-deficient diet versus a regular diet for three months. Independent of genotype, folate deficiency led to decreased NGF protein levels in the frontal cortex and amygdala. In the hippocampus, NGF levels were increased in $UNG^{-/-}$ mice on the normal diet, but not under folate deficiency, while in $UNG^{+/+}$ mice, folate deprivation did not affect hippocampal NGF content. NT-3 protein concentrations were neither affected by genotype nor by folate deficiency. Altogether, the results of our study show that folate deficiency affects NGF levels in the frontal cortex, amygdala and hippocampus. The decrease in NGF content in the hippocampus in response to folate deficiency in $Ung^{-/-}$ mice may contribute to their phenotype of enhanced anxiety and despair-like behavior as well as to selective hippocampal neurodegeneration.

Key words: NGF, NT-3, folate, uracil misincorporation, depression, UNG.

INTRODUCTION

Folate deficiency is related to neuropsychiatric diseases and neurodegenerative conditions such as depression, stroke, dementia and Parkinson's disease (Reynolds, 2002; Mattson and Shea, 2003; D'Anci and Rosenberg, 2004; Irizarry et al., 2005; Kronenberg et al., 2009). The role of folate in the nervous system was first established by studies showing increased risks for neurodevelopmental disorders like neural tube defects in the offspring of folate-deficient pregnant women (Smithells et al., 1976; Blom et al., 2006).

To date, it is not entirely understood by which mechanisms folate deficiency damages the central nervous system. Folate is an important co-factor in one-carbon metabolism promoting the remethylation of homocysteine to methionine. Therefore, folate deficiency results in increased homocysteine levels, which may exert direct neurotoxic effects (Kruman et al., 2000; Ho et al., 2003). Furthermore, folic acid provides the methyl group for the conversion of methionine to S-adenosylmethionine (SAM), which is the major methyl donor in numerous methylation reactions. Low folate levels thereby lead to reduced SAM and thus to reduced methylation of cytosine in DNA. Hydrolytic deamination of cytosine to uracil under conditions of reduced SAM in turn can cause aberrant uracil residues in the form of U:G mispairs (Pogribny et al., 1995; Barnes and Lindahl, 2004). Additionally, disturbances of one-carbon metabolism through folate deficiency lead to reduced methylation of deoxyuridine monophosphate (dUMP) to thymidylate, resulting in the accumulation of deoxyuridine triphosphate and in increased misincorporation of dUMP into DNA generating U:A mispairs (Goulian et al., 1980). To prevent transition mutations, uracil residues are excised by uracil-DNA N-glycosylase (UNG), initiating the base-excision-repair pathway (Lindahl and Barnes, 2000; Kavli et al., 2002).

In order to test the hypothesis that impaired uracil repair is involved in neurodegeneration and

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Abbreviations: BDNF, brain-derived neurotrophic factor; CA, hippocampal cornu ammonis; dUMP, deoxyuridine monophosphate; ELISA, enzyme-linked immunosorbant assay; FD, folate-deficient diet; HPA, hypothalamic–pituitary–adrenocortical; m/s-cortex, motor and somatosensory cortex; ND, normal diet; NGF, nerve growth factor; NT-3, neurotrophin-3; SAM, S-adenosylmethionine; UNG, uracil-DNA N-glycosylase; $Ung^{-/-}$, mice lacking uracil-DNA N-glycosylase; $Ung^{+/+}$, wild-type mice.

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neuropsychiatric dysfunction induced by experimental folate deficiency, we previously compared the effects of folate deficiency in mice lacking uracil-DNA N-glycosylase ($Ung^{-/-}$) and wild-type ($Ung^{+/+}$) mice. While the effects of low folate were partly compensated for in wild-type animals, animals with defective uracil excision repair developed anxious and despair-like behaviors along with selective neurodegeneration in the hippocampus (Kronenberg et al., 2008). The current report is a direct extension of this earlier work.

Nerve growth factor (NGF) and neurotrophin-3 (NT-3) are like brain-derived neurotrophic factor (BDNF) members of the NGF superfamily of neurotrophins. The importance of NGF in the neurobiology of stress-related disorders (von Richthofen et al., 2003; Lang et al., 2004; Cirulli and Alleva, 2009) as well as of neurodegenerative diseases (Hortnagl and Hellweg, 1997; Lang et al., 2004; von Arnim et al., 2005; Williams et al., 2006) has gradually emerged over the past years. Also, NT-3 protein alterations have been implicated in the pathophysiology of stress-related diseases (Amador-Arjona et al., 2010; Fernandes et al., 2010; Mao et al., 2010) and in neurodegenerative processes (Durany et al., 2000; Schulte-Herbruggen et al., 2008). In this study, we therefore investigated NGF and NT-3 protein concentrations in microdissected brain regions of $Ung^{-/-}$ and $Ung^{+/+}$ mice in response to 3 months of folate deprivation.

EXPERIMENTAL PROCEDURES

This paper is part of a larger project examining folate deficiency, uracil repair and central nervous system function (Endres et al., 2004, 2005; Kronenberg et al., 2008, 2009, 2011; Kronenberg and Endres, 2010). The focus of the current study is to assess the effects of folate deficiency and of impaired uracil excision repair on NGF and NT-3 levels in stress-related brain areas.

Animals

The experimental protocol was approved by an official committee. $Ung^{-/-}$ animals have been described in detail previously (Endres et al., 2004). At the age of 12 ± 2 months, $Ung^{-/-}$ and $Ung^{+/+}$ mice were subjected to either a control or an experimental diet for 3 months (Kronenberg et al., 2008). As described previously (Endres et al., 2005), the experimental diet lacked folic acid (< 0.5 mg/kg; Altromin, special diet C1027, Lage, Germany), while the control diet was a standard mouse diet (Altromin control diet C1000, Lage, Germany). The bowel flora produces folate (Hill, 1997). For selective intestinal decontamination (Choi et al., 1998), both diets were supplemented with 1% succinylsulfathiazole (Sigma–Aldrich). Mice were held on a 12-h light–12-h dark cycle with *ad libitum* access to water and their respective chow.

Behavioral endpoints of the animals investigated here have already been published (Kronenberg et al., 2008). The body weight of the relatively old animals after three months of experimental feeding was similar across groups (Kronenberg et al., 2008). The effectiveness of the experimental diet was ascertained by homocysteine measurements, which yielded increased homocysteine levels in the folate-deficient groups (Kronenberg et al., 2008).

Dissection of the brains and homogenization procedure

After decapitation, the brains were rapidly removed, immediately frozen on dry ice and stored at −80 °C until further processing. Frontal cortex, parietal cortex, motor and somatosensory cortex (m/s-cortex), striatum, amygdala and hippocampus were dissected from the frozen right hemisphere on a cold plate (−16 °C) according to Franklin and Paxinos (1997). Each tissue sample was weighed and stored at −80 °C until use. Frozen tissue samples were homogenized by ultrasonication in 20–50 volumes of de-ionized water at 4 °C. For the determination of NGF and NT-3 protein 150 µl of the homogentate was added to 1050 µl of lysis buffer containing 0.1 M Tris–HCl, pH 7.0, 0.4 M NaCl, 0.1% NaN_3, and a variety of protease inhibitors as contained in Protease Inhibitor Cocktail tablets (''complete'', purchased from Roche Diagnostics GmbH, Penzberg, Germany).

Determination of NGF and NT-3 protein levels

Each brain region was consecutively processed for the quantification of each, NGF and NT-3. Determinations of recovery and specific and unspecific neurotrophin binding (the latter against mouse IgG1 obtained from MOPC 21) involved quadruplicate fluorescence determinations for each tissue sample in each neurotrophin assay. Neurotrophin levels were expressed as picograms per milligram of tissue (wet weight). In order to minimize the influence of unavoidable variances between experiments (Hellweg et al., 1989, 2003; Hellweg and Hartung, 1990) neurotrophin levels from all experimental groups were always measured in the same assay. Endogenous NGF protein levels were determined in the rethawed homogenates (again diluted 1:1 with lysing buffer) by a highly specific fluorometric two-site enzyme-linked immunosorbant assay (ELISA) which has been described previously (Korsching and Thoenen, 1987). The assay was further optimized as described in detail in our earlier studies (Hellweg et al., 1989, 1998). NGF content is expressed as equivalents of mouse 2.5 S NGF. Briefly, the detection limit of the assay was 0.37 pg/ml. The individual recoveries were not significantly different between $Ung^{-/-}$ and $Ung^{+/+}$ mice at any time investigated (data not shown). In addition to this, the data of each NGF assay were calculated based on the mean recovery rate. Endogenous levels of NT-3 were measured in the rethawed homogenates using commercial ELISA kits in principle according to the manufacturer's instructions (Promega Inc., Madison, Wisconsin, USA), but improved and adapted to the fluorometric technique used also for NGF determination as described in detail previously (Hellweg et al., 2006). The ELISA values for brain samples were determined from the regression line for the NT-3 standard