FOCAL ALLOSTICALLY ALTERS HOMEOSTASES CYCLE IN THE COCHLEA AND CAUSES PREMATURE HEARING LOSS IN MICE

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INTRODUCTION
The methionine/homocysteine (Hcy) cycle is modulated by nutritional factors and its alterations are associated to several pathological conditions including deafness (1). A recent study conducted in humans showed that folic acid supplementation slowed down the rate of hearing speech frequencies associated with aging (2). Increased pHe levels could result from a decreased flux through the trans-sulfuration pathway leading to a reduced production of glutathione. Such an effect, together with an increase in the athrogenic and neurotoxic effects of Hcy, constitute a risk factor for the function of the cochlear structure and, as a consequence, hearing function may be compromised. Here, we studied the impact of a dietary-induced folic acid deficiency on cochlear Hcy metabolism and in hearing using C57Bl6 mice, a well-known model of progressive hearing loss due to the Ahi alleles present in its genome (3,4).

HEARING FUNCTION

FOLATE DEFICIENT MICE SHOW EARLY SIGNALS OF SEVERE SENSORINEURAL HEARING LOSS: Increased hearing thresholds and altered latencies

A

B

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(A) ABR thresholds in response to click and tone burst stimuli in NF (a) and FD (b) mice after 8 weeks of diet. (B) The Table shows the click ABR peak and interpeak latencies from NF and FD mice. Data are shown as mean ± SEM. Statistical significances: (a)**p<0.01; (b)**p<0.001.

FOLATE DEFICIENT MICE SHOW ALTERED HOMEOSTASES CYCLE: Changes in systemic levels of metabolites and cochlear expression of enzymes

The scheme showing the folate cycle and Hcy metabolism. Arrows indicate the changes after folate deprivation. SAH,HLS-adenosine/homocysteine hydrolase; MTR, methionine synthase; BHMT,betainehomocysteine methyltransferase;ADDA, Adenosine deaminase;CBS, cystathionine beta synthase;ADK, Adenosine kinase;TTHF, Tetrahydrofolate-5,10-THF-S.

FOLATE DEFICIENT MICE SHOW OXIDATIVE IMBALANCE IN THE COCHLEA

(A) Schematic representation of the role of NADPH oxidases, MnSOD and 3-Nitrosotruxene during oxidative stress. (B) Mitochondria, NOX4 and p22phox protein levels measured by Western blot using p22phox loading control. (C) Immunoperoxidase detection of 3-NIT in the stria vascularis (a,c) and cochlear ganglion (b, d) of NF (a,b) and FD mice (c, d). Intensity level (250 gyc scale) of the 3-NIT expression relative to NF mice (e). Data from at least 3 different experiments involving at least 3 mice per condition are presented as mean ± SEM. Statistical significance: (a)**p<0.005; (b)**p<0.001; (c)**p<0.001.

REFERENCES

CONCLUSIONS
1. Folic acid deficiency causes systemic hyperhomocysteinemia and global alteration in the levels of cochlear Hcy metabolism enzymes, concomitant with cochlear oxidative imbalance.
2. These alterations in the methionine cycle secondary to folic acid deficit cause a premature onset of sensorineural hearing loss and cochlear cellular alterations.
3. Altogether these data suggest that control of Hcy metabolism could be a novel mechanism to prevent age-related hearing loss and that the circulating levels of homocysteine could be a novel prognostic factor for hearing dysfunction.

FOLATE DEFICIENT MICE SHOW OXIDATIVE IMBALANCE IN THE COCHLEA

Normal Folate

Folate Deficient

Material & Methods

Animals: 8-week-old female C57BL6 mice (Harlan ) were divided in two groups receiving Normal folate (NF; Mouse Breeder Diet) or Folate Deficient (FD; TD:9247) diets for an additional eight-week period.
Plasmatic metabolites: Folate and pHcy levels were determined using an IMX System (Abbott Laboratories). B6 levels were obtained by HPLC, essentially as described (5).
Hearing assessment: ABR evaluation was performed in a sound-attenuating chamber by using click and tone burst stimuli generated using TDT equipment (Tucker Davis Technologies), as described (6). One-way analysis of variance (with Bonferroni post hoc test) was performed using PASW 21 statistics software.
Cochlear morphology: Inner ear samples were frozen or prepared for paraffin inclusion. Sections were made following standard procedures (7, 8) and used for hematoxylin–eosin staining. Paraffin sections were used for 3-Nitrosotruxene (3NT) and Myelin protein zero immunohistochemistry, whereas frozen tissues were preferred for potassium channel Kir4.1 and phosphorylation immunolabelling. ImageJ was used for quantifying immunohistochemistry intensity staining.
Western blot and Real-time PCR: Whole cochlear protein and total mRNAs were prepared as previously reported (9) and used for western blot and RT-qPCR determinations.

Cochlear Morphology


Oxidative Imbalance

Normal Folate

Folate Deficient