4 New evidence showing neuronal hyperexcitability and trigeminovascular activation during acute stage of stroke-like episodes in MELAS

Takahiro Iizuka *, Fumihiko Sakai, Toshimitsu Ide, Shintaro Yoshii, Saori Miyakawa, Junichi Hamada

Department of Neurology, Kitasato University School of Medicine, Sagamihara, Kanagawa, Japan

Objective: To examine the hypothesis that the cortical excitability is increased and the trigeminovascular system is activated during stroke-like episodes in MELAS.

Background: We previously reported neuronal hyperexcitability hypothesis (Neurology 2002;59:816), and described a role of trigeminovascular activation in stroke-like episodes (Neurology 2003:61:577), but additional data are necessary to support our speculations.

Methods: We measured motor cortical excitability using transcranial magnetic stimulation (TMS) after the first-ever stroke-like episode in the right cerebral hemisphere in a 47-year-old man with MELAS, who presented with headache and seizure followed by left hemiparesis. TMS study was performed on day 9, 10, 13, 27 and 48 in interictal state under the treatment with antiepileptic drugs. Motor cortical excitability was assessed by measuring resting motor threshold (rMT), MEP/CMAP amplitude ratio, asymmetric index of rMT, intracortical inhibition (ICI) and intracortical facilitation (ICF). TMS was performed in 19 healthy volunteers. Calcitonin gene-related peptide (CGRP), which is a potent vasodilator and secreted from the trigeminal nerve endings surrounding the leptomeningeal vessels, and several cytokines in CSF were measured during acute stage.

Results: Although rMT in the affected cortex was not different from control, increased asymmetric index suggested reduction in rMT in the affected cortex. MEP/CMAP amplitude ratio in the affected cortex gradually increased over a month, suggesting development of neuronal hyperexcitability. No significant ICI or ICF was seen in the affected cortex. Both CGRP and IL6 levels were elevated on day 6, but CGRP became undetectable on day 13 while IL6 returned to normal on day 58. Other cytokines including IL2, IL4, IL10 and TNF were not elevated. Acute brain lesions were accompanied by leptomeningeal vasodilatation, hyperperfusion and focal periodic epileptiform discharges.

Conclusions: This is the first demonstration that motor cortical excitability increases after stroke-like episode, and enhanced release of CGRP could be involved in headache pathogenesis in MELAS.

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5 What is(are) the primary source(s) of the mitochondrial superoxide/hydrogen peroxide production?

Vera G. Grivennikova, Andrei D. Vinogradov *

Department of Biochemistry, School of Biology, Moscow State University, Russia

It is well known that up to 1% of oxygen consumed during respiration is reduced to hydrogen peroxide. Significant part of hydrogen peroxide is produced by mitochondria where superoxide, the precursor of hydrogen peroxide, is formed as a result of univalent oxygen reduction. The respiratory Complex I is believed to be the major source of the mitochondrial superoxide production. Superoxide generation by inside–out coupled bovine heart submitochondrial particles, respiring with succinate or NADH, was measured to get better understanding on the primary source(s) of the mitochondrial hydrogen peroxide. The succinate-supported production was inhibited by rotenone and uncouplers, showing that most part of superoxide produced during succinate oxidation is originated from Complex I. The rate of the superoxide production during respiration at a high concentration of NADH (1 mM) was significantly lower than that with succinate. Moreover, the succinate-supported reaction was significantly decreased in the presence of 1 mM NADH. The titration curves, i.e., initial rates of superoxide production versus NADH concentration, were bell-shaped with the maximal rate (at 50 μM NADH) approaching that seen with succinate. Both NAD+ and acetyl-NAD+ inhibited the succinate-supported reaction with apparent affinities close to those in the Complex I-catalyzed succinate-dependent energy-linked NAD+ reduction (reverse electron transfer) and NADH:acetyl-NAD+ transhydrogenase reaction, respectively. We conclude that: (i) under the artificial experimental conditions the major part of superoxide produced by the respiratory chain is indeed formed by some redox component of Complex I (most likely FMN in its reduced or free radical form); (ii) it is however unlikely that under the physiological conditions Complex I is responsible for the mitochondrial superoxide (and hydrogen peroxide) generation. We propose that the specific NAD(P)H:oxygen superoxide (hydrogen peroxide) producing oxidoreductase(s) poised in equilibrium with NAD(P)H/NAD(P)+ couple is present in the mitochondrial matrix.

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6 ICA stenosis associated with G617A mutation in the mitochondrial tRNA phenylalanine gene: A possible new phenotype

Saori Miyakawa [a,*], Takahiro Iizuka [a], Yu-ichi Goto [b], Mayumi Sato [a], Kosuke Suzuki [a], Junichi Hamada [a], Fumihiko Sakai [a]

[a] *Department of Neurology, School of Medicine, Kitasato University, Japan;* [b] *Department of Mental Retardation and Birth Defect Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan*

Objective: To report a case of mitochondrial encephalomyopathy with de novo mitochondrial tRNA phenylalanine gene mutation presenting with recurrent strokes associated with internal carotid artery (ICA) stenosis.

Background: Only few cases of mitochondrial encephalomyopathy with intracranial artery stenosis have been reported.

Design: Case report. A 36-year-old left-handed Japanese man was admitted with sudden onset of left hemiparesis and aphasia. He had a stroke in the right parietal cortex at age 35 and systemic features including short stature, diabetes, hearing loss, cataract, mental retardation and lactic acidosis. The patient was studied to evaluate its genetic and phenotypic classification.

Results: Neuroimaging studies showed acute ischemic lesion in the vascular territories of the right ICA with focal hypoperfusion and bilateral ICA stenosis. These neuroimaging patterns were different from stroke-like episodes in MELAS. Coagulation study and transesophageal echocardiography were normal. Muscle biopsy showed myopathic changes with COX-negative ragged red fibers, but strongly SDH positive vessels were absent. He was put on aspirin. Fifteen months later he developed embolic stroke in the right ICA territories. Conventional angiography showed the right middle cerebral artery occlusion and progressive ICA narrowing. Intravascular ultrasonography showed no atherosclerotic plaques, suggesting genetic basis for the development of the stenosis.

He underwent stent surgery for both ICAs to prevent artery-to-artery embolism. Whole sequence analysis of the mitochondrial gene showed four mutations, three of which were previously reported polymorphisms, but the G617A substitution in the mitochondrial tRNA phenylalanine gene was shown to be heteroplasmic of 50%. This codon was highly well preserved base pair. The mutation was not detected in 200 Japanese control subjects, and not yet previously described in MITOMAP.

Conclusions: This is the first report of G617A mutation causing mitochondrial encephalomyopathy with ICA stenosis. This case expands phenotypic spectrum of mitochondrial disorder.

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