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The magnetism responsive gene Ntan1 in mouse brain

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Abstract

We have previously identified Ntan1 as a magnetism response gene by differential display screening in cultured rat hippocampal neurons. Ntan1 mRNA was ubiquitously expressed in all the mouse tissues examined but relatively abundant in brain, retina and testis. Ntan1 mRNA expression was detectable in the embryonic 12-day mouse brain and gradually increased with ageing. In situ hybridization analysis showed high localization of Ntan1 mRNA in pyramidal cell layer of CA region and granular cell layer of dentate gyrus in the hippocampus, and Purkinje and granular cell layers in the cerebellum, respectively. Ntan1 mRNA expression was significantly increased about two-fold 12 h after brief exposure for 15 min to magnetism at 100 mT with a gradual decrease thereafter in cultured mouse hippocampal neurons. When embryonic 12-day-old or newborn mice were successively exposed to magnetic fields at 100 mT for 2 h, four times per day until the postnatal seventh day, Ntan1 mRNA was significantly increased about 1.5–2-fold in the hippocampus in vivo. The mice exposed to magnetic fields under the same condition showed significantly decreased locomotor activity. These results suggest that magnetic exposure affects higher order neural functions through modulation of genes expression.

Keywords: Ntan1; Magnetism; Hippocampus; Ubiquitin-proteasome; RT-PCR; In situ hybridization; Northern blot; Locomotion

1. Introduction

Living organisms are continuously exposed to the natural geomagnetic fields of around 20–70 μT that exists over the surface of the Earth. The development of electromagnets and of superconducting magnets made possible the exposure of humans to intense magnetic fields. With the further development of magnetic resonance imaging for clinical use, such exposure, often to fields some tens of thousands of times the magnitude of the geomagnetic field, has become a routine. However, there have been few studies on the effects of static magnetic fields at animal and cellular levels. Neuronal function is highly influenced by the extracellular environment possibly including exposure of magnetic fields. We have previously reported that brief exposure (15 min) to static magnetic fields at 100 mT leads to a marked but transient increase in binding of a radiolabeled probe for the nuclear transcription factor activator protein-1 (AP-1) in cultured rat hippocampal neurons (Hirai et al., 2002). Exposure to the static magnetic fields increases AP-1 DNA binding through expression of Fra-2, c-Jun and Jun-D proteins in cultured hippocampal neurons (Hirai et al., 2002).

AP-1 is a hetero- and homo-dimer between Jun and Fos family member proteins and modulates transcription of target genes through the specific recognition of the core consensus sequence TGACGTCA. Our findings suggest that static magnetic exposure could lead to long-term consolidation of transient input of magnetic stimuli through modulation of gene

Abbreviations: AP-1, activator protein-1; DIG, digoxigenin; DIV, days in vitro; NMDA, *N*-methyl-D-aspartate; PBS, phosphate-buffered saline; SDS, sodium dodecylsulfate

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transcription in neurons. We have indeed identified Ntan1 as a magnetism response gene with AP-1 site at the promoter region by differential display screening in cultured rat hippocampal neurons, recently, while expression of Ntan1 mRNA is significantly increased about three-fold after 6 h of brief magnetic exposure by Northern blotting (Hirai et al., 2006). Ntan1 encodes N-terminal amidohydrolase (Nt-amidase), which is involved in ubiquitin-proteasome proteolysis by a rule termed as the N-end rule pathway (Grigoryev et al., 1996). Ntan1 is originally identified as the enzyme N-terminal amidase that can deamidate N-terminal asparagine from porcine liver (Stewart et al., 1994, 1995). Ntan1 is considered to be an essential component of a protein degradation signal that destabilizes the N-terminal residue of a protein according to the N-end rule (Grigoryev et al., 1996). The N-end rule pathway has been found in all species examined, including the eubacterium *Escherichia coli*, the yeast *Saccharomyces cerevisiae* and different mammalian cells. In mice, moreover, disruption of Ntan1 gene is shown to lead to reduced spontaneous activity, less effective spatial memory and socially recessive compared to congeneic mice (Kwon et al., 2000).

In this study, therefore, we have further investigated the effects of magnetic exposure on Ntan1 gene expression in vitro and in vivo, in addition to behavioral alterations, in mice.

2. Materials and methods

2.1. Animals

All efforts were made to minimize animal suffering, to reduce the number of animals used and to utilize alternatives to in vivo techniques. Male Std-ddy mice at 4 weeks of age, in addition to Std-ddy and ICR pregnant female mice, were all purchased from Sankyo Laboratories, Toyama, Japan. Animal care was conducted in accordance with the Guidelines of Animal Experimentation of the Japanese Society for Pharmacology and was approved by the Committee for Ethical Use of Experimental Animals at Kanazawa University.

2.2. Magnetic exposure

Embryonic 12-day-old and/or newborn Std-ddy mice were exposed to magnetic fields at 100 mT in a box, whose top and bottom surfaces are made of permanent ferrite magnets, for 2 h, four times per day until the postnatal seventh day as needed. A control group (sham exposure) was also subjected to similar procedures under the same environmental influences as experimental groups. The intensity of magnetic fields was always measured with a Tesla Meter TM-601 (Kanetec), and ranged from 0.05 to 0.3 mT in the sham control group. In contrast to our previous in vitro experiments using cultured neurons exposed to static magnetism for 15 min (Hirai et al., 2002, 2005, 2006), static magnetism was much more often and successively exposed to animals in order to facilitate the transcranial influence on neurons within the brain covered by skull in the present in vivo experiments.

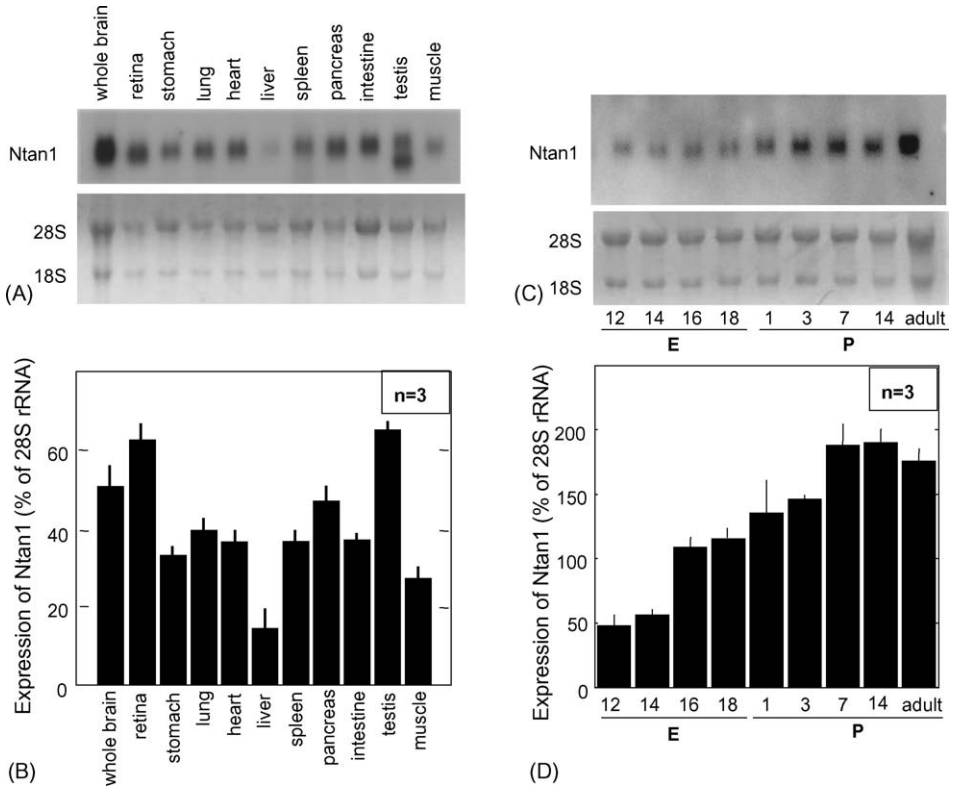

Fig. 1. Expression of Ntan1 mRNA in mice. (A) Expression of Ntan1 mRNA in various mouse organs. Total RNA was extracted from whole brain, retina, stomach, lung, heart, liver, spleen, pancreas, intestine, testis and muscle in adult mice and a single 1.4 kb Ntan1 mRNA was detected by Northern blot (upper panel). 28S and 18S ribosomal RNAs are also shown (lower panel). (B) Quantification of Ntan1 mRNA levels in various mouse organs. Northern blot images were quantified as an integrated density and Ntan1 mRNA levels in various mouse organs were expressed as percentage over 28S ribosomal RNA levels. Each value represents the mean ± S.E. ($n = 3$). (C) Expression of Ntan1 mRNA in developing mouse brain. Total RNA was extracted from E12, E14, E16, E18, P1, P3, P7, P14 and adult mouse brains and Ntan1 mRNA was detected by Northern blot (upper panel). 28S and 18S ribosomal RNAs are also shown (lower panel). (D) Quantification of Ntan1 mRNA levels in developing mouse brain. Northern blot images were quantified as an integrated density and Ntan1 mRNA levels in developing mouse brains were expressed as percentage over 28S ribosomal RNA levels. Each value represents the mean ± S.E. ($n = 3$).