Cerebellar Changes of the Female Mice Heterozygous for Brindled Gene*

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Summary. The brindled mutation is an X-linked neurological mutation in mice. Male mice hemizygous for the brindled gene have metabolic defects homologous with kinky hair disease in humans. Neuropathologically, the mutation is characterized by extensive neuronal degeneration associated with pronounced mitochondrial changes in cerebral cortex and abnormal arborization of Purkinje cell dendrites, which are most pronounced in the rostral vermis or anterior lobules.

In the cerebellum of female mice heterozygous for brindled gene, Purkinje cells with abnormal dendritic arborization and with unusually enlarged mitochondria were also observed. Morphological changes in affected Purkinje cells in young heterozygotes were similar to those of young hemizygotes. However, in older heterozygotes, the changes were far less conspicuous, indicating the presence of some extrinsic factor(s) to compensate expression of the mutant gene in heterozygous brains.

Key words: Brindled mouse — Kinky hair disease — Heterozygotes — Purkinje cells — Mitochondria

Introduction

Brindled (MObr) is an X-linked neurological mutation in mouse, which arises spontaneously in the C57BL inbred strain (Fraser et al. 1953). Hemizygous male MObr/y has been shown to have a homologous defect in copper homeostasis with kinky hair disease in humans (Hunt 1974; Prins and Van den Hamer 1979). MObr/y mice are almost devoid of fur pigment except in the ears, develop neurological symptoms and usually die around the 15th postnatal day with extensive neuronal degeneration in the brain (Yajima and Suzuki 1979a, b). Arborization of Purkinje cell dendrites, in particular those in the anterior and middle lobules, has been shown to be abnormal in Golgi preparations of the cerebellum of MObr/y (Yamano and Suzuki 1985).

Copper concentration is low in MObr/y brain (Camakaris et al. 1979; Wenk and Suzuki 1982). The ultrastructural morphology of mitochondria in degenerating neuronal soma and dendrites is closely similar to that of copper-deficient animals (Yajima and Suzuki 1979a, b; Prohaska and Wells 1974). Parenteral administration of the copper salt has been shown to improve the clinical symptoms (Mann et al. 1979). Wenk and Suzuki (1982) reported that intraperitoneal administration of cupric chloride on the 7th and 10th postnatal day induced a transient drastic increase of copper in the brain and neuronal degeneration in the cerebrum has also thus been prevented (Nagara et al. 1981). Neuronal mitochondrial changes could still be detected but were far less conspicuous. These neuronal changes in MObr/y were, therefore, interpreted as the results of copper deficiency in the brain, secondary to an accumulation of copper in systemic organs (Prins and Van den Hamer 1979; Nagara et al. 1981). Copper has been accumulated in the systemic organs in heterozygous brindled mice (MObr/+) and the copper concentration in the MObr/+ brain was less than normal (Camakaris et al. 1979). Yajima and Suzuki (1979b) were able to detect neuronal mitochondrial changes in totally asymptomatic MObr/+ brain.

According to the Lyon hypothesis on random X-chromosomal inactivation, the tissue in which the X-linked gene is active should be a mosaic of normal and abnormal cells in the female (Lyon 1961, 1962,
1972). This mosaicism is well expressed in the fur of MO$^{br}$/+, which showed irregular patches of full colored and very lightly colored fur over the whole coat, indicating significant gene activation on the melanocyte (Holstein et al. 1979). Therefore, we investigated the possible presence of mosaicism in the cerebellum of heterozygotes MO$^{br}$/+, paying particular attention to the chronological changes of mitochondria and dendritic arborization of Purkinje cells.

**Materials and Methods**

Forty-seven female heterozygotes, MO$^{br}$/+, and 24 normal littermates were used for the study. The day of birth was considered as day 1. For light and electron microscopic studies, 29 female heterozygotes were photographed to document coat color and were sacrificed at various ages (Table 1). They were anesthetized with sodium pentobarbital and perfused with 2.5% glutaraldehyde in 0.1 M phosphate buffer. Sagittal sections of the cerebellum were postfixed in osmium and embedded in epoxy resin according to the procedure described previously (Yajima and Suzuki 1979a). As controls, two normal littermates were processed in the same way at days 20, 30, 40, 50, 60 and 70.

One-micron-thick sections of mid-sagittal cerebellum were stained with toluidine blue and examined by light microscopy. Selected areas of the anterior lobules were stained with uranyl acetate and lead citrate, and examined with an electron microscope. For convenience, the cerebellar vermis was divided into three lobules: anterior, middle and posterior; these correspond to I-V, VI-VIII and IX-X vermian lobules, respectively (Inoue and Oda 1980).

For the Golgi study, three heterozygotes and two normal littermates were sacrificed at days 8, 12, 15, 23, 30, and 60. These mice were anesthetized and perfused with physiological saline; the cerebellum was removed immediately and was impregnated by the modified Golgi-Cox method according to Sholl (1953). The 120-$\mu$m-thick sections of midsagittal cerebellum were examined by light microscopy.

**Results**

**Light Microscope Observation**

As noted in MO$^{br}$/y (Yamano and Suzuki 1985), the main pathology of the cerebellum in MO$^{br}$/+ was...