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Reduction of nerve growth factor level in the brain of genetically ataxic mice (weaver, reeler)

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By a highly sensitive enzyme immunoassay we measured the level of nerve growth factor (NGF) in the cerebellum and cerebrum of the neurologically mutant mice, weaver, reeler and Purkinje cell degeneration (PCD). A significant decrease in NGF level was observed in both cerebellum and cerebrum of weaver and reeler mutants of either sex. However, there was no such difference between normals and mutants in the case of the PCD mice. These results show that weaver and reeler mice have abnormalities of NGF synthesis and/or degradation not only in the cerebellum but also in the cerebrum.

Nerve growth factor; Weaver; Reeler; Purkinje cell degeneration; Enzyme immunoassay

1. INTRODUCTION

Nerve growth factor (NGF) is a protein that is essential for the development and maintenance of some sensory and sympathetic neurons in the peripheral nervous system [1,2] and basal forebrain magnocellular cholinergic neurons in the central nervous system (CNS) [3-5]. In the CNS, however, NGF and NGF mRNA have been detected in several brain areas other than the target fields of magnocellular cholinergic neurons [6]. In rat cerebellum, mRNAs of both NGF and NGF receptor are co-expressed in the postnatal period, when granule cells proliferate and subsequently migrate to the internal layer [7]. The NGF receptor is present on the surface of Purkinje cells and premigratory granule cells, as judged immunohistochemically [8]. These findings suggest an important role of NGF and/or NGF receptor in the cell differentiation and migration during cerebellar development.

Several mutant mice with hereditary neurological disorders mainly involving the cerebellum have been found. The weaver mutant is characterized by a failure of cerebellar granule cells to migrate from the external to the internal layer and their degeneration during the first postnatal week [9]. The reeler is characterized by an aberrant position of various neurons, including Purkinje cells and cerebral pyramidal cells, and by a relative decrease in the number of granule cells [10,11]. In Purkinje cell degeneration (PCD) mutants, there is a virtually complete loss of Purkinje cells by day 45 [12], which is followed by a progressive loss of granule cells, noticeable by 3 months of age [13].

In the present study, we focused on the NGF level in the brain and its relationship to the pathological alterations in these neurological mutant mice.

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2. MATERIALS AND METHODS

2.1. *Animals*

The mutant mice were originally purchased from the Jackson Laboratory and raised in our animal facility. The background strains of weaver, reeler, and PCD mice were denoted as B6CBA, B6C3Fe-a, and C57BL/4J, respectively. Mice homozygous for the mutation were obtained by intercrossing heterozygous pairs. The homozygous mutants were easily recognized by their ataxic locomotion. Behaviorally normal littermates of each strain were used as controls for each strain.

2.2. *Preparation of samples*

Male and female mice were sacrificed 8 weeks postnatally by inhalation of chloroform. The cerebrum (whole brain except cerebellum) and cerebellum were separated on ice and stored at −80°C until used. The brain samples were made into 5% (w/v) suspensions by 30 repetitive pulses of sonication with a Model 225R sonicator from Heat System-Ultrasonics Inc. The solution used for the sonication was composed of 0.1 M Tris-HCl buffer (pH 7.6), 1.0 M NaCl, 2% gelatin (Bio-Rad), 2% bovine serum albumin (BSA, Fraction V; Sanko Junyaku), 0.02% NaN_3, 0.08 trypsin inhibitor units of aprotinin/ml (Sigma), and 2 mM EDTA (Dojin). The sonicates were centrifuged at 100 000 × *g* for 10 min and their supernatants were used for NGF measurement.

2.3. *Measurement of NGF*

NGF content was measured by a previously described two-site enzyme immunoassay (EIA) [14], with several modifications as follows: (i) anti-NGF antibodies in a small volume (5 μl) were coated on round bottom wells of 96-multiwell plates (Falcon), (ii) volumes of samples and reagents subsequently used were also reduced to 20 μl, (iii) biotinylated NGF-specific antibodies and streptoavidin-β-D-galactosidase (Biogenex Lab.) were used in combination instead of

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antibody Fab'-β-D-galactosidase. Each sample was measured in octuplicate. Recovery of NGF was over 90%, as judged from the NGF concentrations in the supernatants following homogenization of samples with or without exogenously added NGF. The detection limit was as low as 1 pg/ml.

3. RESULTS AND DISCUSSION

We used 8-week-old mutants for our experiments, because the morphological characteristics of their cerebella are well documented at this age. The wet weights of cerebellum and cerebrum in the mutants were 30–60% and 80–90% of those of normals, respectively, in conformity with other reports [15–17]. Therefore, we evaluated NGF level as an amount per g wet tissue weight rather than as absolute content per tissue.

We observed sex-dependent differences in both cerebellum and cerebrum of all normals and mutants. Males contained higher levels of NGF than respective females in both cerebrum and cerebellum (Fig. 1). This finding is in agreement with the recent report by Katoh-Semba et al. [18], who detected higher levels of mouse brain NGF in males than in females, but found no difference in peripheral tissues. This sex-dependent difference may be peculiar to the mouse, because it is not the case in the rat [3]. Since NGF synthesis and accumulation in the mouse submaxillary gland is known to be regulated by testosterone [19], it is expected that some kinds of sex-specific hormones may influence NGF synthesis and/or degradation in the mouse brain. Eventually, normals and mutants of each sex must be examined for possible hormonal involvement in these differences.

As shown in Fig. 1a,b, a marked decrease in NGF content was observed in mutants of reeler and weaver of both sexes. NGF levels were reduced to 30–40% of control levels in the cerebellum and to 30–70% of control levels in the cerebrum. In contrast, no difference in NGF level was apparent between control and PCD mutant in cerebellum and cerebrum of males or females (Fig. 1c). These results indicate that weaver and reeler have abnormalities in NGF synthesis and/or degradation not only in the cerebellum, but also in the cerebrum. An outstanding aspect of these results is the reduction of NGF levels in cerebrum as well as cerebellum of weaver and reeler mutants. It is difficult to interpret these findings in relation to previous knowledge, because these mutants are well documented as models of cerebellar abnormalities, especially in the lack of cerebellar granule cells, and there have been few pathological studies on the cerebrum of these mutants [20–22]. It is possible that the changes of NGF levels both in the cerebellum and the cerebrum may be due to a secondary or compensatory reaction as the result of some specific mutations in the cerebellum. However, it is likely that cerebellar granule cells are involved in the regulation of the cerebellar NGF level. Namely, granule

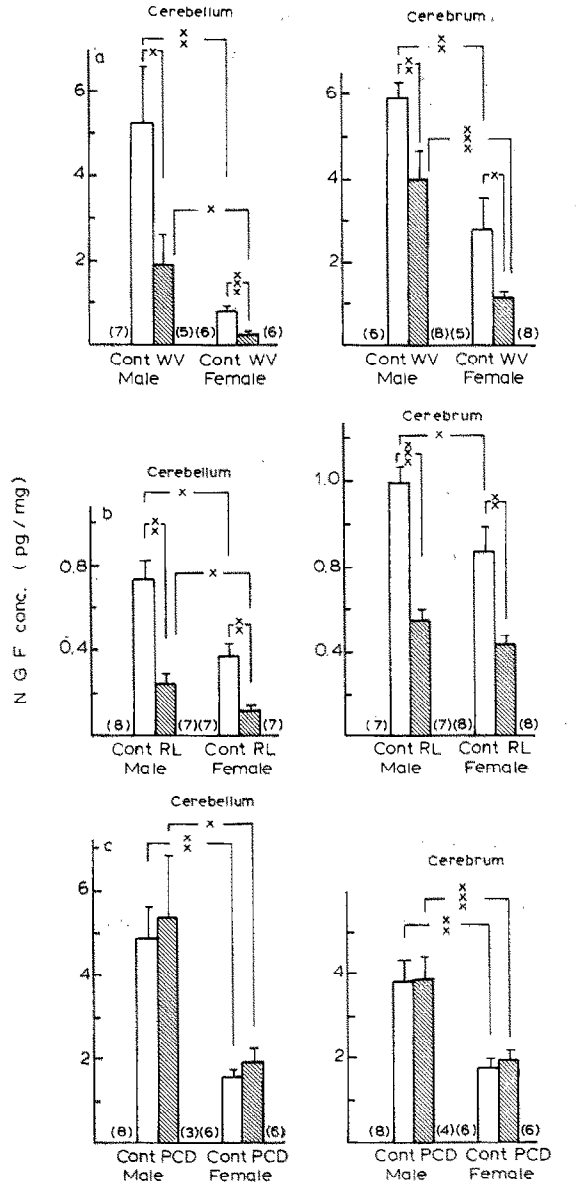

Fig. 1. NGF levels in the cerebellum and cerebrum of weaver (WV) (a), reeler (RL) (b), PCD (c), and respective control (Cont) mice of both sexes.

Vertical lines on the top of each bar are S.E.M. Parentheses indicate number of mice. $*P<0.05$, $**P<0.01$, $***P<0.001$ (Significant difference between mutants and control or between male and female by Student's t-test).

cells themselves may synthesize NGF or modulate NGF synthesis by other cells such as astroglial cells [23]. At the age of animals used in this work, a marked reduction in granule cell numbers is apparent in weaver and reeler mutants, however, granule cells in the cerebellum of PCD mice are still normal in numbers [9–13]. This may further indicate that a progressive reduction in the number of Purkinje cells has little to do with the NGF level in the cerebellum. If NGF plays an important role in supporting the mitotic proliferation of granule cells and their migration process from the external to inter-

nal layers of the mouse cerebellum, severe damage to the granule neurons would occur when the synthesis and transport system of NGF are impaired during brain development. This view is supported by a report [8] suggesting that the granule cells dividing at the external granule layer express NGF receptors and that the expression becomes progressively weaker during the progress of the migration process. To confirm the alternative possibilities, we are now investigating developmental changes in the NGF level of these mutants.

We previously observed that the degree of ataxic gait is least in PCD among all kinds of mutant mice [24]. Taken together, there may be some relationship between the degree of ataxic gait, cerebellar atrophy and NGF levels in the brain.

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