A PROTEIN DIFFERENCE ASSOCIATED WITH DEFECTS OF THE PURKINJE CELL IN STAGGERER AND NERVOUS MUTANT MICE

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1. Introduction

One of the most striking features of cerebellum anatomy is the reduced number of its constitutive neuronal classes [1–3]. Such a design makes possible a joint biochemical and genetic analysis of a well-defined mammalian brain center. In the mouse, several neuropathological mutations are known to affect primarily only one, or a few, of these cell-types [4–8]. For instance, in the homozygous weaver mouse, granular cells lack almost completely [5–16]. On the other hand, in the homozygous staggerer [17–21] and nervous [22–25] mice, both anatomical [8] and electrophysiological [26] studies reveal defects at the level of the Purkinje cell. As a consequence of the staggerer mutation, the rate of cell proliferation in the external granular layer is reduced [20], the Purkinje cells have small somas and stunted dendritic arborisations with virtually no tertiary dendritic spines of the type which normally make synaptic contacts with granule cell axons (parallel fibers) [18,19,21]. In the homozygous nervous mouse [22], the Purkinje cells show abnormal mitochondria at about postnatal day 9 [23] and after day 15 most of them die [25].

A previous study [27] by gel electrophoresis in SDS of subcellular fractions from the cerebella of normal and homozygous agranular weaver mice and X-irradiated rats revealed several protein differences associated with the lack of granular cell. The most obvious one consists in a reduction of the histone content which parallels the decrease of the DNA to protein ratio observed in the agranular cerebellum [28]. Using the same technique, we now report a change of protein composition which accompanies the staggerer and the nervous mutation but not the weaver one. Such a change concerns a membrane protein present in purified Purkinje neurons.

2. Material and methods

2.1. *Animals*

'Staggerer' mutant mouse. A stock of mice heterozygous for the staggerer (sg) mutation C57BL (++sg/d se+) established by the Jackson Laboratory (Bar Harbor, Maine, USA) was raised at the Pasteur Institute. Homozygous sg/sg were obtained by intercrossing the heterozygous mice.

'Nervous' mutant mouse. Mice C3HeB/J originating from the Jackson laboratory and either heterozygous or homozygous for the nervous (nr) mutation were intercrossed to produce homozygous nr/nr animals.

'Weaver' mutant mouse. Mice B6CBA/51 (wv/+) originating from the Jackson laboratory and heterozygous forth weaver (wv) mutation were bred as described previously [27].

In all cases, the homozygous animals were easily recognizable by their marked cerebellar syndrome. Control mice were either C57BL or C3HeB/J wild types or, in the case of staggerer, homozygous C57BL (d se+/d se+).

2.2. *Preparation of subcellular fractions from mouse cerebella*

The mice were killed without anesthesia and their cerebellum dissected immediately and mixed with 10^{-3} M Na-phosphate buffer pH 7.0 at 4°C, and homogenized in a motor-driven glass-teflon Potter homogenizer with 50 up and down strokes. The homo-

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genate was then centrifuged at maximum speed for 20 min in a Beckman 152 microfuge. The supernatant was collected and the pellet resuspended with the aforementioned buffer and recentrifuged at maximum speed in the microfuge for 20 min giving the pellet P1. The first supernatant was then centrifuged at 30 000 rev/min in a rotor type 40 for 75 min in a Beckman model L2-65 ultracentrifuge, giving a pellet P2 and a final supernatant S. P1, P2 were dissolved in a 3% solution of sodium dodecylsulphate containing 1% β-mercaptoethanol and brought to boiling for a few minutes.

2.3. *Preparation of Purkinje cells from rat cerebellum*

Purkinje cells were purified from 12–13 days Sprague-Dawley rats by the method of Sellinger et al. [29] with minor modifications. The cell mixture containing Purkinje and granular neurons obtained after the first centrifugation was pushed manually through a 53 μm pore size nylon filter to break the clumps and was then carefully layered on a three-steps gradient of 1.5 ml of 2.3 M sucrose in 1% BSA, 4 ml of 1.9 M sucrose in 1% BSA and 3 ml of 1.75 M sucrose in 1% BSA. The tube was centrifuged in a SW41 Beckman rotor at 124 000 *g* for 2 hr.

The Purkinje cell fraction was diluted 5 times with a 10% sucrose solution and centrifuged 10 min at 1000 *g*. The pellet was then washed 3 times with the sucrose solution, dissolved in a 3% SDS solution containing 1% β-mercaptoethanol and brought to boiling for a few minutes.

3.4. *Slab gel electrophoresis*

Slab gel electrophoresis in 5 to 15% polyacrylamide-SDS was carried out using the procedure of Ames [30]. Each sample contained 20–30 μg of protein. The electrophoresis was run in a cold room at a constant current of 15 mA.

Gels were fixed in isopropanol–acetic acid to remove SDS, stained with Coomassie Blue in methanol–acetic acid–water and destained in methanol–acetic acid–water. The following markers were used for mol. wt calibration: β-galactosidase purified from *E. coli* (a gift of Dr A. Ullman) and phosphorylase b from rabbit muscle (a gift of Dr H. Buc) with various degrees of cross linking with suberimidate.

3. Results and discussion

In Fig.1a, b are shown protein patterns obtained after gel electrophoresis of low speed and high speed particulate fractions from the cerebella of homozygous staggerer, nervous and normal mice. On the same gel, have also been run a preparation of Purkinje cells purified from cerebella of 12–13 day rats (fig. 1 d–f). In all these gels the concentration of acrylamide varied linearly from 5 to 15% from the top to the bottom of the gel and the protein bands were revealed by Coomassie Blue.

Let us first compare low speed pellets from staggerer (fig.1b: S), nervous (fig.1a: R) and normal mice (fig.1a, b: N). Clearly, in the upper part of the gels, a band present in normal mice (arrow) lacks in both staggerer and nervous low speed pellets. This band can also be seen in the high speed pellet from normal mice (fig.1a, b: n) but not in the soluble protein fraction. It lacks as well in the high speed pellet from both staggerer (fig.1b: s) and nervous (fig.1a: r) cerebella. The band disappears after extensive treatment of the low speed pellet from control animals by protease K (1.0 mg/ml for 30 min at 37°C) and therefore contains a protein.

This protein moves particularly slowly. Its R_f in our 5–15% acrylamide gels was estimated by comparison with that of globular protein markers of known mol. wt. It coincides with that of a globular protein of approx. 400 000 mol. wt. We therefore refer to it as the P400 protein. The reasons for this unusually low R_f are not known.

In the protein pattern given by purified rat Purkinje (fig.1 d–f) cells is present a strong band having the same R_f as P400 (fig.1a & b: p). The same band is absent from the patterns given by purified granular cells [27]. Mixing of rat Purkinje cells with low speed pellets from normal and staggerer mouse give a reinforcement of the P400 band in the first case, a pattern similar to the normal mouse in the latter one (fig.2).

The P400 protein is *not* detected in preparations of mouse cerebral cortex but is abundant in the cerebella of the homozygous weaver mouse (fig.1c).

In conclusion, the P400 membrane protein is present in purified rat Purkinje cells and in cerebella of normal and weaver mutant mouse known to have fully developed Purkinje cells but is lacking in cerebella

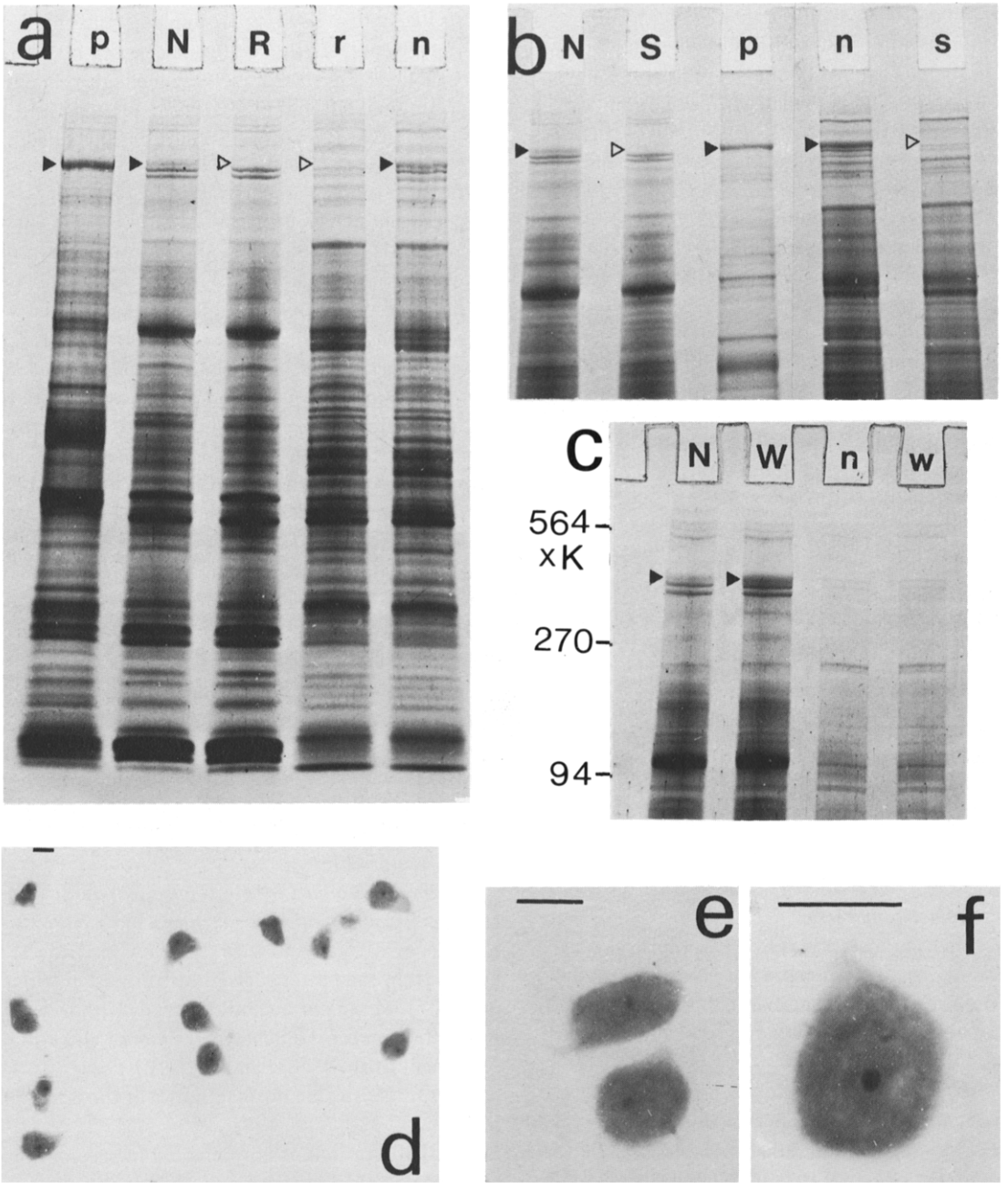

Fig. 1a, b and c. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate of particulate fractions from the cerebella of normal and mutant mice and of purified Purkinje cells (p). The low speed (P_1) and high speed pellet (P_2) were respectively from normal (N and n), nervous (R and r), staggerer (S and s) and weaver (W and w) homozygous mice. The closed arrow indicates the presence of the P400 protein, the open arrow its absence: d, e & f. Purkinje cells after purification. Length of the bar: 10 μ.

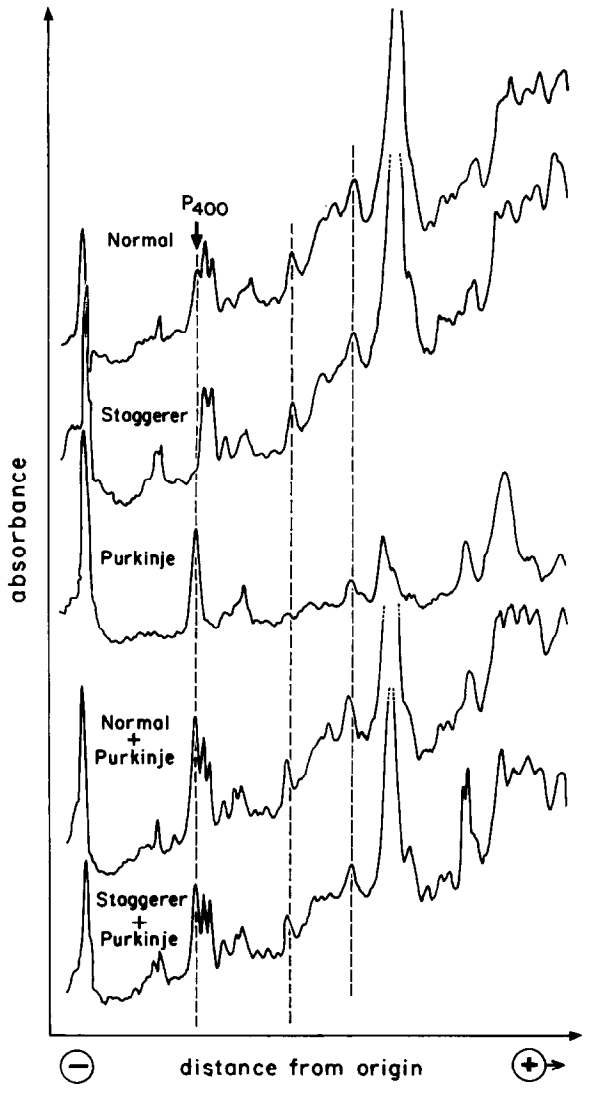

Fig.2. Densitometric profiles of slab electrophoretogram of proteins extracted from low speed (P_1) particulate fractions of the cerebella from normal and staggerer homozygous mice and from purified Purkinje cells. Mixing of Purkinje cells with the particulate cerebellum fraction (P_1) causes a reinforcement of the P400 band in the case of the normal mouse and its apparition at the expected place in the case of the staggerer homozygous mouse.

of two mutants which exhibit a defect in this particular class of neurons.

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