THE BRAIN-SPECIFIC PROTEINS D1, D2 AND D3 IN THE CEREBELLUM OF STAGGERER, REELER AND WEAVER MUTANT MICE

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1. Introduction

The analysis of synaptic organization in mice as modified by the consequences of a single gene mutation offers a useful model for the study of structural plasticity in the cerebellum [1,2]. The mutants investigated in the present study were selected from a pool of spontaneous neurological mutants [3], extensively investigated morphologically [1–3].

In the cerebellum of 'staggerer' mice selective and almost complete absence of synapses between cerebellum parallel fibres and Purkinje cells has been reported [1–6]. According to morphological observations, Purkinje cells are more affected than parallel fibres during the first three postnatal weeks. The progressive disappearance of granule cells (and parallel fibres) has been attributed to transsynaptic retrograde degeneration [5,6]. In the cerebellum of 'reeler' mice the cellular organization is grossly disrupted, with Purkinje cells in abnormal positions [1–3,6,7]. The variation in shape of the Purkinje cell dendrites located in the central agranular mass mimics that described in other agranular cerebella; in particular, they show randomly oriented dendrites devoid of spiny branchlets [7]. The density of climbing fibre varicosities increases in the central cerebellar mass, where Purkinje cells are deprived of parallel fibre afferents. Ectopic synapses (somato-dendritic and dendro-dendritic) may form between the soma and/or the dendrite of the granule cell as a presynaptic element and mainly the Purkinje cell dendrites as a postsynaptic element. Furthermore, heterologous synapses between mossy fibres and Purkinje cell spines are found in the granular layer and within the central mass [7]. In the cerebellum of 'weaver' mice the granule cells degenerate before producing mature parallel fibres [1–3,8] the Purkinje cells do not develop spiny branchlets and have randomly oriented dendritic trees. By contrast, their thick dendrites are studded with spines. By quantitative investigations it has been disclosed that the surface density of climbing varicosities is increased whereas that of mossy rosettes is decreased [6,8].

In the present study we measured the concentration of the synaptosomal membrane proteins D1, D2 and D3 in cerebella from the mutant mice. D1, D2 and D3 were all enriched in the synaptosomal membrane fractions and absent from both synaptic vesicles and cultured astroglial cells [9–11]. D3 was on the inside of the synaptosomal plasma membrane but D1 and D2 were on the outside [12]. In an ontogenic study on mice, the concentration of D1 and D3 in the forebrain rose postnatally to an adult plateau, whereas the concentration of D2 decreased with age, just after the brain growth spurt, to a steady adult level 50% of that level at day 12 [13]. It has been hypothesized that D2 may be involved in intercellular recognition during synaptogenesis [12].

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2. Experimental

The staggerer and reeler mutants were raised on a C57B1 background. Weaver was raised on a B6CBA background. The homozygous mutants were killed 24 days (staggerer and reeler) or 20 days postnatally (weaver). The age controls were Teigler mice. The removed cerebella were stored frozen. The thawn cerebella were weighed and homogenized in 50 vol. (v/w) 73 mM Tris, 24 mM barbitone, 2 mM NaN_3 buffer, at pH 8.6. The membrane proteins were solubilized by mixing the homogenates with equal vol. 4% Triton X-100, 20 mM phosphate, 100 KIE/ml aprotinin, 2 mM EDTA, at pH 8.5 [9].

Crossed immunoelectrophoresis was performed on ~20 μg solubilized proteins as in [9,14]. The polyspecific antisera were raised against rat brain synaptosomal membranes [9,15]; batch anti-SPM 0176 and batch anti-SPM 1077 gave similar results. The crossreacting precipitates, formed with mice cerebella antigens, were correlated to the rat system by sequential addition of the antigens, and the precipitates were quantitated by planimetry of the enclosed areas [14].

The protein concentration in the homogenates was measured on samples solubilized by 2 mg sodium dodecyl sulphate in 0.1 M NaOH at 37°C for 30 min. Bovine serum albumin was used as standard [16].

The statistical analysis was by Student's *t*-test.

3. Results

The specific concentrations of D1, D2 and D3 are given in fig.1. With respect to D1 no change was found in the specific concentration between cerebella from either staggerer, reeler or weaver mice and the cerebella from the corresponding age controls. In contrast, D3 was significantly decreased in reeler and weaver mice compared to the controls, whereas no significant difference was found between the mutants. D2 was highly increased in weaver compared to the control, and it was also increased compared to staggerer ($t = 11.6, P < 0.001$), and reeler ($t = 3.4, P < 0.05$). Furthermore, the specific D2 concentration was increased in reeler mice compared to staggerer ($t = 3.5$, $P < 0.01$).

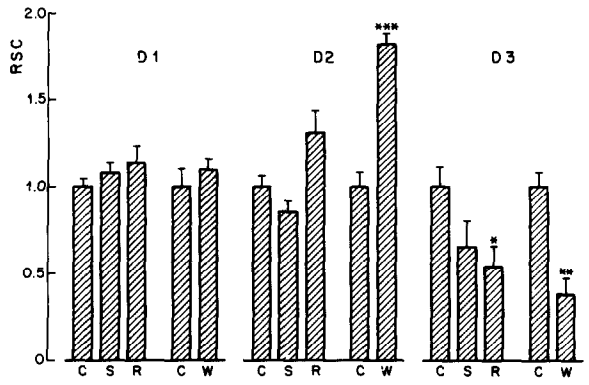

Fig.1. The relative specific concentrations (RSC) of the brain synaptic membrane antigens D1, D2, and D3 in the cerebellum of age controls (C, $n = 3$) and staggerer (S, $n = 5$), reeler (R, $n = 4$), or weaver (W, $n = 4$) mutant mice. The measured amounts of antigens were calculated specifically with respect to the protein concentrations and normalized to 1.00 RSC in the case of the respective age controls. The standard errors of the means are shown by the bars and a significant difference between age controls and mutants is marked by *($P < 0.05$), **($P < 0.01$), or ***($P < 0.001$).

4. Discussion

The concentration of the synaptosomal membrane antigens D1, D2 and D3 has been measured in brains from the mutant mice 'quaking' [13,17] and 'jimpy' [18], both characterized by abnormal myelin production, but no difference was found between mutants and controls with respect to these proteins. The present study comprised the mutants staggerer, reeler and weaver, all showing abnormal interaction among Purkinje cells, granule cells, mossy fibres, and climbing fibres in the cerebellum.

In the cerebellum of staggerer mutants, although no synapses exist between Purkinje cell dendrites and parallel fibres of granule cells, D1, D2, as well as D3 exist, indicating that these antigens cannot be specific of the synapses between Purkinje cells and parallel fibres but must be present elsewhere. The antigens are also present in the weaver mutant which has no parallel fibres and are thus also not specific of parallel fibres. The absence of specificity with respect to types of neurones in the cerebellum and the presence of these antigens in all areas of the cerebrum (O. S. J., unpublished results) is in contrast to the Purkinje cell specific glycoprotein, P400 protein, which has been shown to be enriched in dendritic arborizations of

the Purkinje cells of the cerebellum [19] where its concentration increases from postnatal day 12 [20]. The P400 protein is absent in the cerebellum of the staggerer mutant which has no synapses between Purkinje cell dendrites and parallel fibres of granule cells, indicating that P400 protein may be correlated to the synaptogenesis between Purkinje cells and granule cells [20–23].

We found no difference betweeen the specific concentration of D1 in the mutants compared to their controls, whereas the amount was lowered because of the small cerebella in the mutants [1–3]. D1 has been found exposed to the outside of the synaptosomal membrane [12] but it has also been found in preparations of sciatic nerve axons and in optic nerve axons (O. S. J., in preparation). Thus, as D1 is present in different parts of the neuron, the D1 results possibly indicate an equal concentration of neuronal membranes in the cerebella of the mutants compared to their age controls.

The specific concentration of D3 was decreased in both weaver and reeler cerebella. The synaptosomal membrane protein D3 has been found in all areas of the brain (O. S. J., in preparation) and has not been positively correlated to any known neurotransmitter system. Thus, D3 is probably present in most, if not all, synapses in the cerebellum and the decrease in specific concentration may somehow reflect the decrease of parallel fibres and/or dendrites of granule cells in the cerebella of the mutants.

The D2 protein has been found on synaptosomal membranes [9–12], and it has been postulated that D2 may be involved in intercellular recognition. Studies on primary cell cultures of foetal rat brain have demonstrated the presence of D2 also on the membranes of neurites (Z. Yavin, E. Yavin, E. Bock and O. S. J., in preparation). As mentioned, D2 is not specific of any known type of neurons, and taking into account the high concentration of D2 in cultured neurones containing processes, and in brains of newborn mice before the formation of chemical synapses [13] the D2 concentration may indicate mainly the amount of neuronal processes ready for synapsing or in the process of forming new synapses. Thus, the increased D2 concentration in reeler, and especially in weaver mutant cerebella, may indicate the presence of many free redundant nerve endings, although most connections, at least in the cerebella from the control mice, have probably been selectively stabilized at the ages investigated [6,24]. But since cerebella from the staggerer too contain many free nerve endings of parallel fibres, the reason for the lower concentration of D2 in this than in other mutants remains to be studied.

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