QUANTITATIVE STUDIES OF THE BRAIN SPECIFIC ANTIGENS S-100, GFA, 14-3-2, D1, D2, D3 AND C1 IN QUAKING MOUSE

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1. Introduction

The Quaking mouse is a recessive autosomal mutant characterized by a myelin deficiency in the central nervous system [23].

Extensive biochemical studies on cerebral lipids have shown drastical deficiencies in long chain fatty acid galactolipids which are myelin specific components [11,13,14], other lipids are less affected [8,12, 24].

More recently electrophoretic analysis have been performed on protein composition of myelin [3,10, 11,15] and other subfractions [3]. Basic encephalitogenic and proteolipid have been shown to be reduced to 70% and 40% of normal respectively. Acidic insoluble proteins seem to be relatively enhanced. Some modifications in myelin associated glycoproteins have also been reported [20]. The microsomal protein pattern on polyacrylamide gel is unaffected. Mitochondrial and nuclear protein patterns are normal too, but some slight electrophoretic changes have been observed in the synaptosomal pattern of Quaking brain proteins [3]. However, these results may not rule out some physiologically important differences.

Crossed immunoelectrophoresis and related techniques have proved to be of great value for identification and quantitation of soluble and membrane proteins [4,5,6]. In the present communication 7 brain specific antigens were determined in the Quaking mutant. The S-100 protein is located in the cytoplasm of glial cells [21]. The glial fibrillary acidic protein (GFA) is located in astroglial cell cytoplasm [9], 14-3-2, is a marker of neuronal cytoplasm [21], D1, D2 and D3 are neuronal membrane antigens [16] and the C1 antigen is predominantly found in the synaptic vesicle. (Bock and Jørgensen, to be published).

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2. Materials and methods

Mutants and littermate controls of the C57/B1 strain were obtained from the breeding of Baumann (Paris). 75 to 90 day old mice have been used. Cerebrum was homogenized at 4°C in a Potter-Elvehjem homogenizer using twenty strokes with 1.5 ml of extraction medium consisting of 2% v/v Triton X-100, 1 mM EDTA 10 mM barbital buffer, 15 mM NaN_3, aprotinin (Trasylol R, Bayer, West Germany) 100 000 units/litre, all adjusted to pH 8.6. The homogenates were then sonicated for 20 sec at 16 000 cycles/sec and centrifuged at 200 000 *g* for 40 min. Estimation of protein content was made on both homogenate and supernatant in order to check the reproducibility of the extraction procedure, using the Lowry method [19].

Antiserum against D1,D2,D3 was raised by immunisation with rat brain synaptosomal membrane. Antiserum against C1 was obtained by immunisation with rat brain synaptic vesicles [6]. Antiserum against ox brain S-100 was kindly supplied by Dr K. G. Haglid, Göteborg, Sweden. Antiserum against GFA was raised using purified human GFA kindly supplied by Dr L. F. Eng, Palo Alto, U.S.A. Antiserum against mouse 14-3-2 was obtained during immunisation with mouse

brain extract. The specificity of the antiserum was determined by comparison to an antiserum against ox brain 14-3-2 kindly supplied by K. G. Haglid, Göteborg, Sweden. The comparison of the antisera was performed as outlined by Axelsen and Bock [1].

S-100 and GFA were determined by means of rockets immunoelectrophoresis (Laurell 18). The neuronal antigens D1, D2 and D3, the synaptic vesicle antigen C1 and 14-3-2 were determined by crossed immunoelectrophoresis [18]. As to 14-3-2 only the acidic component was determined, although the antiserum revealed a component with alpha-mobility cross-reacting with the most anodal component.

Agarose was obtained from Litex A/S, Glostrup, Denmark. The buffer was 20 mM Tris-barbital, 1 mM calciumlactate, pH 8.6. For the determination of S-100 the calcium lactate was substituted by 1 mM EDTA.

2.1. *Statistics*

The comparison between two groups was performed using the Mann-Whitney *U*-test, the probability of difference between the medians due to chance, was obtained from tables to *U*.

3. Results

The protein yield of the Triton extraction procedure was 65%. The median concentration being 13.6 g/litre with no statistical difference between the normal and the Quaking mice. Quantitation of the antigens was performed by measuring either the height of the peaks (for rockets-immunoelectrophoresis) or their area (for crossed immunoelectrophoresis). Table 1 shows the specific concentrations of the individual antigens in the two groups, setting the median value in the control group to 100 arbitrary units. Significant increases have been found in the mutant for 2 antigens of glial origin: GFA (+ 67%) and S-100 (+ 47%) and for the synaptic vesicle antigen C1 (+ 25%). The amounts of the neuronal membrane antigens D1, D2, D3 and of the neuronal cytoplasmic antigen 14-3-2 were not modified in the Quaking.

4. Discussion

The pleiotrophic effect of the Quaking mutation has given rise to several hypotheses tending to correlate histological, biochemical, pharmacological and neurophysiological data.

While most histological studies have focused on myelin and glia, little is known of the aspect of synapses in the mutant. Watanabe [26], however, noted that no alteration of synapses can be recognized. Our results showing a normal level of the synaptic plasma membrane proteins D1, D2 and D3, corroborate this observation and tend to confirm the normal appearance of the synaptic structure. On the

Table 1
Median values and range of brain specific antigens in normal and quaking mouse.

	S-100	GFA	14-3-2	D1	D2	D3	C1
Normal	n = 10	n = 10	n = 10	n = 10	n = 10	n = 10	n = 10
	100	100	100	100	100	100	100
Range	(83–128)	(64–115)	(74–118)	(80–108)	(88–130)	(72–125)	(85–121)
Quaking	n = 10	n = 9	n = 10	n = 9	n = 9	n = 7	n = 7
	147	167	87	107	105	92	125
Range	(126–171)	(127–224)	(64–112)	(85–119)	(93–156)	(61–119)	(116–168)
Significance of differences ($p < 0.01$)	S	S	NS	NS	NS	NS	S

The values are arbitrary specific values with respect to total protein concentration in the brain extracts.

n = number of brains examined.

other hand some abnormalities of the synaptic function may not be ruled out. The increased level of the C1 vesicular antigen could reflect an enhanced activity in this area, associated with chronic stress due to tremor. This finding may be related to the higher level of acetylcholine [25], noradrenaline and dopamine [17,25] observed in the mutant.

The acidic protein 14-3-2 has been shown to be located in the axon [7]. Our data showing an unchanged level for this protein is in agreement with previous histological observations that axis cylinders appear to be normal in number, size and structure [2,22,23,26].

The marked enrichment of two proteins of glial origin (GFA, S-100) may be correlated with the glial alterations observed by several authors. Whereas Berger [2] noted a marked gliosis, dense lamellar inclusions and vacuoles in the oligoglial processes, Watanabe [26] and Wisniewski [27] described dense bodies harbouring degradation products of myelin in the perinuclear cytoplasm of glial cells. Samorajski [22], and Wisniewski [27] pointed out an increased glial density. The higher increase of GFA compared to S-100 might indicate that gliosis is due largely to the prolifereation of astroglial cells.

Our results provide further evidence that both astroglial and oligoglial cells are involved in the pathogenesis of the Quaking mutation. Furthermore increased amounts of the C1 antigen may indicate a changed synaptic function.

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