BUTANOL EXTRACTS FROM MYELIN FRAGMENTS: TRYPTAMINE BINDING TO "QUAKING" MICE-MYELIN BUTANOL EXTRACTS

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In previous studies (1), we demonstrated that the myelin butanol extracts from rat brain stems (i.e., myelin proteolipids) had a specific binding capacity for tryptamine. The apparent Kd for 14C-tryptamine binding to these extracts was found to be 1.14X10^-7 M, and only tryptamine and 5-methoxytryptamine inhibited the tryptamine binding, but indole analogues and other neurotransmitters had no effect. However, the physiological relevancy of these binding components was obscure. The Quaking mouse is an autosomal recessive neurological mutant with a characteristic body tremor and a deficit in the formation of central nervous system myelin (2). As a first step in the investigation of the physiological importance of myelin proteolipids, several kinds of myelin butanol extracts were prepared from Quaking mice, and 14C-tryptamine binding properties of these extracts were examined.

The mutants and littermate controls of strain B6C3HF1-OK originated from the Jackson Laboratory. We used 12- and 26-day-old control mice in this study. The Quaking mutants were 26 days of age. The brains, except the cerebellum, were removed, washed, weighed and stored at -80°C until use. The myelin fragments-rich fraction was isolated from frozen brain by the method of Matthieu et al. (3), but the light and heavy myelin fraction was combined because of the lower yield of the former. The details for preparation of the myelin butanol extracts were as reported previously (4). Briefly, the resultant myelin pellet was resuspended in 50% sucrose (0.1 g pellet/3 ml) and extracted with 10 vol. of water-saturated butanol. Several myelin butanol extracts were treated with water (14%, v/v), and aliquots of each sample (0.3 ml) were incubated at room temperature for 20 min with 14C-tryptamine. After incubation, the mixtures were loaded onto a Sephadex LH20 column (0.6 x 19 cm), and stepwise elution was carried out with the following solvents: 10 ml each of chloroform and chloroform-methanol (CM) 10:1 and then 16 ml of CM 9:1. After chromatography, the radioactivity of each of the collected fractions was counted in a liquid scintillation counter. In this elution system, a minute amount of the free ligand (approx. 5%) appeared in the binding peak. Thus, the amount of bound tryptamine was calculated by subtracting the free portion from the total binding. Lipid analysis was performed as described previously (5).

The yields of myelin recovered from littermate controls, pups and mutants were 54.8±2.4, 9.5±0.6 and 9.4±0.7 mg/g brain (mean±S.E.M. of 10 determinations), respectively. Morphological examinations of the myelin fractions isolated from mutants revealed the appearance of loosely packed myelin, in comparison with the compact
myelin (i.e., multilamellar structure) in those of littermate controls. On the other hand, myelin fractions of pups showed the presence of a large number of single membraneous vesicular structures (electronmicrographs are not presented). All these observations are in good agreement with the previous reports (6, 7). We examined the 14C-tryptamine binding capacities of the butanol extracts obtained from these three myelin fractions. In previous studies (8), we found that the tryptamine binding components present in the myelin butanol extracts of rat brains are lipid in nature, and its binding entity is mainly phosphatidylserine (PS). Thus, to obtain the corresponding PS content of each fraction, each sample was concentrated as follows: controls-myelin extracts, 1/6; pups-myelin extracts, 1/12; and mutants-myelin extracts, 1/8 of its original volume. The results indicated that these three myelin extracts displayed similarities with respect to their tryptamine binding capacities (see Table 1).

To investigate the conformational aspects of the tryptamine binding components of myelin proteolipids, competition experiments were performed. As shown in Fig. 1, at 5×10⁻⁴ M tryptamine, (as agonist; 1,000 fold excess) all myelin butanol extracts represented a competition plateau and possessed the same amount of the saturable tryptamine bound (81% of the total binding). However, the apparent IC50 values of controls-, pups- and mutants-myelin butanol extracts were 3×10⁻⁵, 6×10⁻⁵ and 2×10⁻⁴ M, respectively. These observations suggest that the binding affinity for tryptamine differs among these three preparations.

Finally, it is very difficult to evidence the physiological relevancy of these binding components which originate from myelin membranes since the neurophysiological function of myelin is not yet obvious. However, it was demonstrated that the alterations

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**Table 1. Myelin butanol extracts**

<table>
<thead>
<tr>
<th></th>
<th>Controls 26-day-old</th>
<th>Controls 12-day-old</th>
<th>Mutants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid phosphorus  (μmol)*</td>
<td>67.2±4.4</td>
<td>38.0±1.4</td>
<td>44.4±5.1</td>
</tr>
<tr>
<td>Phosphatidylserine (μg)**</td>
<td>24.0±0.8</td>
<td>23.4±0.7</td>
<td>23.8±0.5</td>
</tr>
<tr>
<td>Amount of tryptamine bound (%)***</td>
<td>75.9±1.5</td>
<td>77.8±2.7</td>
<td>75.9±1.1</td>
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Myelin butanol extracts from controls, pups and mutants were concentrated to 1/6, 1/12 and 1/8 of its original volume and then incubated with 5×10⁻⁷ M ¹⁴C-tryptamine. After incubation, the bound radioactivity was measured by Sephadex LH₂₀ column chromatography as described in the text. Mean ±S.E.M. of 3 determinations. *μmol/butanol extracts of g myelin. **μg/incubation mixture. ***% of total input radioactivity.
of tryptamine binding affinity occurred in hypomyelination (9), i.e., pups and dysmyelination (3), i.e., Quaking mutants.

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References


