marked difference in the nature of ion channels in the same species is difficult to rationalize. It is, however, possible that the discrepancy may arise from the difference in the developmental stages of the animals used. Different ionic requirements for the regenerative responses have been reported during the stage of development in the embryonic excitable cell membrane[20]. In our experiments, immature larvae smaller than 10 mm in their total length were used exclusively.

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Butanol extracts from myelin fragments. III. SDS-urea gel electrophoretic studies

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Summary. The butanol extracts from myelin fragments showing 5-HT binding capacity were studied by SDS-urea gel electrophoresis. 5 main bands were observed and their molecular weights were determined by the method of Ferguson relationship, and revealed that these extracts contained proteolipid protein, DM-20 and basic proteins. Furthermore, the reconstituted fraction with crude basic proteins and lipids showed the saturable binding capacity for C^{14} · 5-HT.

Some investigators[2,3] reported the butanol extracts of central nervous tissue having 5-HT binding affinity, but their materials were the total particulate fractions. In previous experiments[4], we demonstrated that the particulate fraction of Godwin and Sneddon[3] contained a considerable number of myelin fragments and, moreover, butanol extracts from myelin fragments showed binding capacity for C^{14} · 5-HT. Pasquini and Soto[5] have reported that the butanol extracts of bovine brain grey and white matter contain a complex mixture of phospholipids, galactolipids, cholesterol and proteolipid protein of Folch-Lees[6]. They also indicated the results of acrylamide gel electrophoresis, but detailed observations were not made. The objective of the present work is to analyze the binding components present in the butanol extracts of myelin by SDS-urea polyacrylamide gel electrophoresis.

Materials and methods. The details of preparation of butanol extracts from the myelin fragments of rat brain stems were as reported previously[7]. Briefly, the brain stems were homogenized in 0.32 M sucrose (10%). The myelin fragments were isolated from the homogenate by the method of Whittaker et al.[8] and extracted with butanol-water mixtures. The butanol phase thus obtained was concentrated under N_2 to about one-third of its original volume (TE). TE was treated with water (14%, v/v) to dissolve the insoluble materials (water-treated TE), and C^{14} · 5-HT binding capacity was checked by Sephadex LH_{20} column chromatography as described previously[4,7]. SDS-urea polyacrylamide gel electrophoresis was carried out according to the method of Chan and Lees[9]. The water-treated TE was dried under N_2 and emulsified in a medium containing 300 mg of SDS, 450 mg of dithiothreitol, and 800 mg of sucrose per 10 ml of water (mg protein/ml). To obtain complete solubilization, the mixtures were heated at 60 °C for 10 min, then 8 M urea was added and heated 10 min more at same temperature, and 50 µl of samples including 0.005% Bromophenol blue were loaded onto SDS-urea gels. Electrophoresis was performed for 18 h at a constant current of 1 mA/tube, and then gels were stained in 0.25% Coomassie blue. Crude basic protein fractions (0.1 N HCl extracts) were prepared from myelin fragments by the method of Martenson et al.[10] and lipid mixtures were obtained from the water-treated TE by the method of Mokrasch[11].

Results and discussion. Typical gel profile of the butanol extracts from myelin fragments showing C^{14} · 5-HT binding capacity is shown in figure 1. 5 main bands (A-E) were identified, and sometimes a fast anode component (lipid) was lightly stained. Low mobility components (i.e., high mol.wt proteins = HMW) were also observed. From the studies of synaptic membrane proteins, Banker and Cotman[12] pointed out the risk of depending on a single gel concentration for mol.wt determination without prior validation of the procedures by the method of Ferguson relationship. Theoretical treatment of the Ferguson relationship and its application to SDS-urea system have been thoroughly reported[9,13]. On a plot of log R_F (relative mobility) vs. T (% of total acrylamide concentration = 7, 8.5, 10, 11.5 and 13%), i.e., Ferguson plot, lines of 5 protein bands fall into 3 Y_0 (y intercept). Lines of A and B have the same Y_0 (1.65); C has a Y_0 of 1.45. Lines D and E have a Y_0 of 1.03. These results indicate that 5 bands are separating by size and charge differences, and thus their mol.wt can be determined only from the K_R (slope of line) vs. mol.wt relationship as shown in figure 2. Based on this method, we obtained the mol.wt of A, B, C, D, and E is 12,180±1650, 18,330±2520, 19,830±2260, 22,410±1110 and 31,460±1700, respectively (mean±SEM of 6 experiments). The relative ratio of C to E was approx. 1. Chan and Lees[9] obtained 30,600, 24,300 and 18,900 for proteolipid protein (PLP), DM-20 and basic protein (BP) from bovine white matter

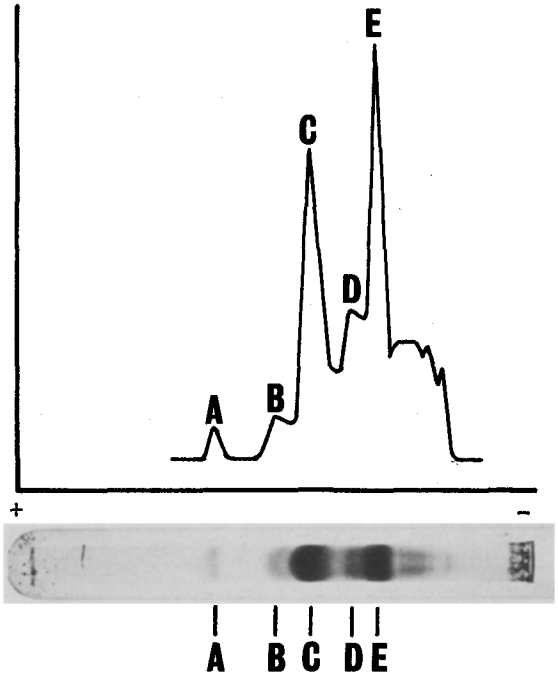

Fig. 1. SDS-urea electrophoresis on 8.5% acrylamide of the butanol extracts from myelin fragments and its densitometric scan. Methods of gel electrophoresis are described in the text; 50 µg protein of sample was applied. The densitometric scan was recorded in Gelman Densitometer-R scanner. The different protein bands are labelled No. A-E.

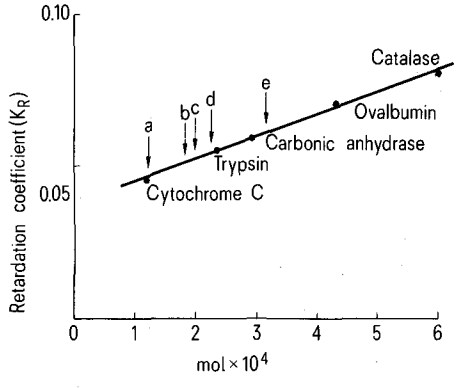

Fig. 2. Molecular weights of the protein bands observed upon electrophoresis in the SDS-urea system. When standard proteins were employed, a linear function for the plots of the retardation coefficient (K_R) of each protein and their mol.wt permitted the estimation of the mol.wt of each of the protein bands (a-e). Notations of the protein bands are shown in figure 1.

myelin. Barbarese et al.[14] reported that mouse myelin contained 4 BPs (21,500, 18,500, 17,000 and 14,000). Agrawal et al.[15] indicated that DM-20 was migrated between PLP and BP. All these observations suggest that the separated protein bands A, B and C are BPs, and D and E is DM-20 and PLP, respectively. Since the components eluted in chloroform-methanol 4:1 by Sephadex LH_{20} column chromatography have the binding capacity for C^{14}· 5-HT, the fractions eluted in this solvent system were pooled, and analyzed by SDS-urea gel electrophoresis as in the case of butanol extracts. The results indicated that this fraction also contained 3 main protein bands, i.e., band B (BP), C (BP) and E (PLP).

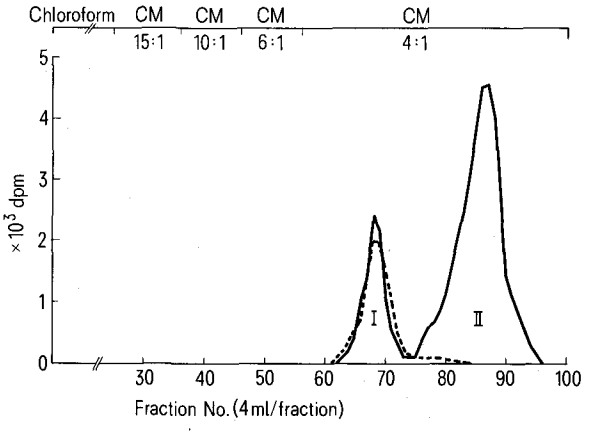

Fig. 3. Sephadex LH_{20} chromatogram of radioactivity of the reconstituted fraction with crude basic proteins and lipids. The sample was incubated with 5×10^{-7} M of C^{14}· 5-HT in the presence of 1000-fold unlabelled 5-HT (dashed line) or absence (solid line). Elution was carried out with the following solvents: 100 ml of chloroform, 50 ml each of chloroform-methanol (CM) 15:1, 10:1 and 6:1, and then 160 ml of CM 4:1.

In a preliminary study[16], we found that the butanol extracts from mitochondrial fraction did not show the C^{14}· 5-HT binding capacity and these extracts contained PLP and HMW, but not BPs. From these observations, it can be assumed that BPs are likely candidates for 5-HT binding materials. To confirm this possibility, the recombination experiments of BPs and lipid mixtures were performed. As shown in figure 3, the recombinate of crude basic proteins and lipids showed the saturable binding capacity for C^{14}· 5-HT (peak II). In the control experiment without butanol extracts, peak I also appeared and thus this peak should be a nonbound 5-HT. In addition, neither BPs nor lipids showed the C^{14}· 5-HT binding capacity.

Finally, the present results suggest that the identified BPs in the butanol extracts from myelin may be implicated in the binding of 5-HT under the participation of lipids. Carnegie[17] has also pointed out the possibility of BP as receptor site for 5-HT. The detailed biochemical and morphological examinations on the recombinate of BPs and lipids are now in progress.

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