BUTANOL EXTRACTS FROM MYELIN FRAGMENTS: SOME CHARACTERISTICS OF TRYPTAMINE BINDING

Akihisa MIYAKAWA, Su-Ja KIM, Tsutomu KAMEYAMA* and Ryoichi ISHITANI**

Group of Biochemical Pharmacology, Josai University, Saitama 350-02, Japan
*Department of Chemical Pharmacology, Faculty of Pharmaceutical Sciences, Meijo University, Nagoya 468, Japan
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Abstract—Myelin fragments from rat brain stems were treated with butanol-water mixtures and binding experiments of 14C-tryptamine to these extracts (i.e. proteolipids) were performed by Sephadex LH20 column chromatography. The elution peak of 14C-tryptamine appeared in a boundary between 6:1 and 4:1 chloroform-methanol, together with 19.2 and 7.3% of the total recoveries of protein and lipid phosphorus, respectively. Various compounds were studied to examine their inhibitory effects on the tryptamine binding to these extracts. The results indicated that only tryptamine and 5-methoxytryptamine inhibited the tryptamine binding, but indole analogues and other neurotransmitters had no effect. The kinetic analysis revealed that the tryptamine binding components present in the myelin butanol extracts are composed of saturable and non-saturable components, and the saturable binding components had an apparent Kd of 1.14×10^-7 M. As a preliminary study, myelin butanol extracts were separated into a lipid and protein fractions with ice-cold ether treatment, and then the 14C-tryptamine binding capacities of both fractions were examined. The results indicated that only the lipid fraction possessed a tryptamine binding capacity and a chromatographic profile similar to the original butanol extracts. Moreover, the heat-treated preparation of myelin extracts also retained the tryptamine binding capacity. All these observations suggest that the myelin butanol extracts have a specific binding capacity for tryptamine, and its binding components may be lipid in nature.

Several investigators (1–5) have reported that in central nervous tissue, the hydrophobic proteins of Folch-Lees (6), i.e., proteolipids, could possibly be considered as the receptor components for neurotransmitters. From experiments on 5-hydroxytryptamine (5-HT) binding to rat brain stems butanol extracts, Fiszer and De Robertis (7) and Godwin and Sneddon (8) suggested that the 5-HT binding components were special proteolipids. However, we found that the crude particulate fractions that they used contained a considerable number of myelin fragments; moreover, myelin butanol extracts possessed a specific binding capacity for 14C-5-HT (9, 10). Based on these evidences, we indicated that what has been reported to be a possible proteolipid 5-HT receptor originated not from the synaptic membranes but from the myelin fragments.

Furthermore, displacement studies revealed that among indoleamine analogues, only
tryptamine inhibited the 5-HT binding to myelin butanol extracts (11). On the other hand, Boulton et al. (12) have reported that tryptamine is associated with a myelin fraction as well as with a synaptosomal fraction. All these observations led us to study the binding properties of myelin butanol extracts for $^{14}$C-tryptamine.

MATERIALS AND METHODS

Preparation of myelin butanol extracts: Male Wistar rats (150–200 g) were decapitated and the brains removed. All procedures were carried out at 4°C unless otherwise stated. The brain stems including the hypothalamus, midbrain and medulla oblongata+pons were homogenized in 0.32 M sucrose (5%) with a Teflon-glass homogenizer. The myelin fragments were isolated from the homogenate by the method of Norton and Poduslo (13). They were then pelleted by centrifugation, resuspended in 50% sucrose (2 ml/g brain stem), and then extracted with 10 vol. of water-saturated butanol for 2 hr at room temperature. The mixtures were centrifuged at 1,000 x g for 20 min, and the butanol phase was retained. This was concentrated under N$_2$ at 38°C to about 1/3 of its original volume and stored at 4°C until use. Just before use, these extracts were treated with water (14%, v/v) to dissolve the precipitated materials (total extracts=TE). The purity of the myelin fraction was confirmed by electron microscopy.

Binding experiments: An aliquot of TE (1.5 ml) was incubated at room temperature for 20 min with several concentrations of $^{14}$C-tryptamine. After incubation, the mixtures were loaded onto a Sephadex LH$_{20}$ column (1.3×20 cm). Discontinuous elution was carried out with solvents of increasing polarity: 50 ml of chloroform, 25 ml each of 15:1, 10:1 and 6:1 mixtures of chloroform-methanol (CM) and then 60 ml of a 4:1 mixture of CM. After chromatography, an aliquot of each of the fractions (0.4 ml) was added to counting vials containing 10 ml of emulsion phosphor (toluene containing 0.4% PPO and 0.01% dimethyl-POPOP: Triton X-100=2:1), and radioactivity was measured in a liquid scintillation counter. In addition, $^{14}$C-tryptamine ($5 \times 10^{-7}$ and $5 \times 10^{-4}$ M) was incubated with TE for various periods of time, i.e., 10, 20 and 30 min at room temperature. The amount of bound tryptamine was the same value each time.

Protein and lipid phosphorus assay: Protein contents of samples were determined by the method of Lees and Paxman (14) using crystalline bovine serum albumin as a standard. Lipid phosphorus was assayed according to the method of Chen et al. (15).

Chemicals: Sephadex LH$_{20}$ was obtained from Pharmacia. 5-HT oxalate, 5-hydroxytryptophan, 5-hydroxy-3-indole acetic acid, acetylcholine iodide, tryptamine hydrochloride, 5-methoxytryptamine hydrochloride, dopamine hydrochloride, noradrenaline hydrochloride and $\gamma$-aminobutyric acid were from the Sigma Chemical Co. Tryptamine-[2-$^{14}$C] bisuccinate (51.5 mCi/mmol) and Triton X-100 were from New England Nuclear. N-Benzoyl-tryptamine and N,N-dimethyltryptamine were the generous gifts of Dr. Y. Kikugawa (from the university at which this experiment was carried out). Chloroform and methanol were redistilled before use.

RESULTS

The elution profile of protein, lipid phosphorus and $^{14}$C-tryptamine from the Sephadex LH$_{20}$ column is shown in Fig. 1. Total recoveries of protein and lipid phosphorus from the column were 4.9 and 100%, respectively. The major portion of the protein peaks which accounted for 35.3% of the total recovery were eluted with chloroform, together with 64.2% of the lipid phosphorus. The second peaks of both components were
eluted with 10:1 CM. On the other hand, the elution peak of $^{14}$C-tryptamine appeared in a boundary between 6:1 and 4:1 CM; and the protein and lipid phosphorus in this area were 19.2 and 7.3% of the total recovery, respectively. To clarify whether or not the eluted radioactivity was of the bound type, the ligand was run without butanol extracts. In this case, no radioactivity was detected in the same elution area. Thus, the elution peak of radioactivity represents a bound type. At $5 \times 10^{-7}$ M of $^{14}$C-tryptamine, the amount of bound ligand was 72.2±6.4% of input total radioactivity (mean±S.E.M. of 3 experiments).

Various agents were examined to determine their inhibitory effects on the binding of $^{14}$C-tryptamine to myelin butanol extracts. Displacement experiments indicated that only tryptamine and 5-methoxytryptamine inhibited the tryptamine binding; but other compounds, indoleamine analogues and other neurotransmitters, had no effect (Table 1). Since previous displacement experiments had already revealed that tryptamine inhibited 5-HT binding to myelin extracts (11), it was interesting to find that 5-HT did not show any inhibitory effect on the tryptamine binding.

The binding of $^{14}$C-tryptamine to myelin extracts was measured as a function of ligand concentration, and the binding mode shown in Fig. 2 was obtained. When the binding pattern appeared as shown in Fig. 2 (curve A), it was reasonable to assume that this type of binding curve was a composite of the saturated and non-saturated groups. In this case, a commonly used procedure can be adopted to resolve the adsorption isotherm into its two presumed components (16). The observed linear slope in the high concentration range is used for the slope of a straight line which goes through the origin (curve B). This is taken to represent the non-saturating component, and the difference between curves A and B represents the binding to the presumed group of high affinity sites which saturate. Thus, the binding curve of Fig. 2 was resolved into saturable and non-saturable binding components, and the saturable binding components were represented as a double reciprocal plot. A double reciprocal plot of $^{14}$C-tryptamine
Table 1. Inhibition of 14C-tryptamine binding by various compounds

| Compound                   | % Inhibition |
|----------------------------|--------------|
| Tryptamine                 | 59.4±12.3    |
| 5-Hydroxytryptophan        | None         |
| 5-Hydroxy-3-indole acetic acid | None       |
| Dimethyltryptamine         | None         |
| Benzyloxytryptamine        | None         |
| 5-Methoxytryptamine        | 78.9±4.0     |
| 5-Hydroxytryptamine        | None         |
| Acetylcholine              | None         |
| Dopamine                   | None         |
| Noradrenaline              | None         |
| γ-Aminobutyric acid        | None         |
| Tyramine                   | None         |

TE was preincubated with various compounds (all: 5x10⁻⁴ M) for 30 min, and then 5 x 10⁻⁷ M of 14C-tryptamine was added. After incubation, the sample was chromatographed through a Sephadex LH₂₀ column as described in Fig. 1, and the total bound radioactivity was measured (mean±S.E.M. of 4 experiments).

Fig. 2. Binding curves and double reciprocal plot of 14C-tryptamine to TE. The binding mode of 14C-tryptamine shown (A) was resolved into saturable and non-saturable bindings (B) as described in the text, and the saturable binding components are plotted as a double reciprocal. The points are the mean of 2 experiments.

binding showed a straight line having an apparent Kd of 1.14 x 10⁻⁷ M. At present, the nature of tryptamine binding components is obscure, and thus, the amount of bound ligand was expressed as a mol/incubation mixture.

As a first step toward the examination of the nature of tryptamine binding components present in the myelin butanol extracts, these extracts were roughly separated into a lipid and protein fraction by the ice-cold ether treatment method of Mokrasch (17), and then the 14C-tryptamine binding capacities of both fractions were assayed by Sephadex LH₂₀ column chromatography. The lipid fraction had a tryptamine binding capacity (71.8±5.3% of input total radioactivity, mean ±S.E.M. of 3 experiments), but the other fraction did not. Moreover, as shown in Fig. 3, the two preparations (myelin butanol extracts and lipid fraction) also showed similarities with respect to their chromatographic properties. In addition, the heat-treated butanol extracts (80°C, 30 min) also retained the native binding capacity for 14C-tryptamine.

DISCUSSION

There is evidence to support the role of tryptamine in neurotransmission in the central...
TRiptamine binding to myelin proteolipids

Fig. 3. Sephadex LH20 chromatogram of the lipid fraction. The lipid fraction obtained from TE was dissolved in a butanol-water mixture (14%, v/v), and an aliquot of sample (0.75 ml, 2.1 µmoles as lipid phosphorus) was incubated with 5×10^{-7} M of [14C]-tryptamine. After incubation, the mixtures were loaded onto a Sephadex LH20 column (0.9 x 20 cm). Stepwise elution was carried out with the following solvents: 25 ml of chloroform; 12.5 ml each of 15:1, 10:1 and 6:1 CM; and then 30 ml of 4:1 CM.

nervous system. For example, Martin et al. (18) have reported that tryptamine may be a transmitter in certain descending spinal pathways. On the other hand, Ungar and Callaghan (19) have reported that synaptic membrane particles labelled with [14C]-tryptamine were extracted with CM (2:1) and described its proteolipid-like nature. The present studies reveal that the butanol extracts from myelin fragments (myelin proteolipids) possess a specific binding capacity for [14C]-tryptamine, and its binding components may be lipids.

Displacement studies indicated that among various compounds, i.e., indoleamine analogues and other neurotransmitters, only tryptamine and 5-methoxytryptamine inhibited the tryptamine binding. This observation suggests that the tryptamine binding components present in the myelin butanol extracts show a relatively high selectivity. In previous experiments (11), we found that tryptamine, dopamine (DA) and acetylcholine (ACh) inhibited the 5-HT binding to myelin extracts. In the case of tryptamine binding, however, 5-HT, DA and ACh did not show any inhibitory effect. From these discrepancies, it is reasonable to presume that the tryptamine binding components may differ from those of 5-HT.

Calculation of the saturable ligand binding was generally made by subtraction of the non-saturable component which persisted in the presence of a large excess of unlabelled ligand from the total binding that occurred in the absence of non-radioactive ligand. However, as shown in Table 1, the elution pattern of [14C]-tryptamine in the displacement experiments of unlabelled ligand shifted each time; and thus, the amount of the saturable bound tryptamine had a large range of S.E. (i.e. 59.4±12.3%). On the other hand, 5-methoxytryptamine showed a higher inhibitory effect than that of tryptamine. In the solubilized DA receptors from rat striatum, Gorissen et al. (20) have pointed out that the displaceable [3H]-spiperone binding mode by (+)-butaclamol shows a shallow curve, while the other one by spiperone is steep in nature. We have demonstrated the presence of high affinity [3H]-imipramine binding sites by a theoretical kinetic analysis (16) in addition to the reports based on the displacement method by unlabelled imipramine (21–23). From these evidences, the tryptamine binding mode was examined by a theoretical kinetic treatment. As shown in Fig. 2, the tryptamine binding components of myelin extracts appeared to be composed of saturable and non-saturable components, and the saturable binding components had an apparent Kd of 1.14×10^{-7} M.

The cold-ether treatment method of Mokrasch (17) selectively disrupts the association between protein and lipid moieties of proteolipids. When the myelin butanol
extracts were treated with 4 vol. of ice-cold ether, 6.1 and 82.1% of protein and lipid phosphorus were recovered in the supernatant portion (i.e. lipid fraction), respectively (11). Binding experiments clearly revealed that only the lipid fraction displayed a tryptamine binding capacity and had chromatographic properties similar to that of the myelin butanol extracts. Moreover, the heat-treated preparation also retained the original tryptamine binding capacity. All these observations strongly suggest that the tryptamine binding components present in the myelin butanol extracts may be lipid in nature. Recently, several investigations (24) have suggested that certain special lipids (i.e. acidic lipids) can act directly as binding sites themselves. For the 5-HT binding components of myelin proteolipids, we concluded that they were three acidic lipids, i.e., sulphatides, phosphatidylserine and phosphatidylinositol (25, 26). More detailed isolation experiments of the tryptamine binding components from the lipid fraction are now in progress.

Finally, we have no explanation for the physiological function of these tryptamine binding components, but these components may be implicated in some physiological function except for the receptors in the central nervous system. In fact, we found that the myelin butanol extracts from Quaking mice showed a relatively lower binding affinity for tryptamine than that of the littermate controls (Karasawa and Ishitani, submitted). 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 (27).

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