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Effect of Filter on Specific [^{3}H]-5-Hydroxytryptamine Binding to Synaptic Membranes*)

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ABSTRACT

In the present study, the authors carried out some basic examinations of specific [^{3}H]-5-Hydroxytryptamine (5-HT, serotonin) receptor binding assay. The most adequate incubation condition for [^{3}H]-5-HT binding assay was at 0°C for 10 to 30 min. Scatchard plot of [^{3}H]-5-HT binding using a Millpore filter and a Glass filter drew biphasic and monophasic, respectivly. It is suggested that filter may affect on specific [^{3}H]-5-HT binding. Therefore, the characteristics of filter should be taken into consideration in [^{3}H]-5-HT binding assay.

INTRODUCTION

In the recent studies on neurotransmitters, a receptor binding assay technique using radioactive ligands has been rapidly advanced. It has been shown that 5-HT might be involved in a number of behaviors including sleep, eating, sexual responses[6] and disease states; depression and schizophrenia[1,3]. The 5-HT receptor binding assay has been often used in psycopharmacological and neurochemical studies. However, the data from these studies were not always coincident. Bennett and Snyder[2] have described a single high-affinity binding site for [^{3}H]-5-HT, while Fillion et al.[4] and Segawa et al.[11] have reported high-affinity and another lower affinity sites.

The present study attempted to examine the most adequate incubation condition for specific [^{3}H]-5-HT binding to synaptic membrane fractions and the effects of two different kinds of filters on Scatchard analysis[9] data of [^{3}H]-5-HT binding assay.

MATERIALS AND METHODS

1) *Preparation of crude synaptic membranes*

Male Wistar rats weighing 200 to 250 g were sacrified by decapitation. The brain was quickly removed and the cerebellum discarded. Crude synaptic membrane fractions were prepared according to the method of Nakata et al.[8]. Briefly, curde mitochondrial P_2 fractions which were prepared by the method of Segawa and Kuruma[10] were dispersed with a Polytron and were centrifugated at 48,000×g for 20 min. The pellets were stored frozen at −30°C. Before the binding assay, all pellets were resuspended in 0.05 M Tris-HCl buffer (pH 7.4) and maintained at room temperature for 40 min to dissociate any 5-HT bound to the membranes. After centrifugation at 48,000×g for 10 min, the pellets were resuspended in Tris-HCl buffer.

2) *Specific [^{3}H]-5-HT binding at various temperatures*

To 0.8 ml of crude synaptic membrane suspension, 0.1 ml of 5.0 nM [^{3}H]-5-HT solution was added. Non-specific binding was determined by addition of 0.1 ml of 5.0 μM unlabeled 5-HT. The tubes with their contents were incubated for 30 min at 0°, 25° and 37°C respectively. The samples were then filtered through a Glass filter (Whatman GF/F) under suction and the incubation tubes rinsed with three times by 5 ml aliquots of Tris-HCl buffer.

*) 山脇成人，前岡邦彦，更井啓介：シナプス膜への特異的セロトニン結合に及ぼすフィルターの影響

The filters were then immersed in 10 ml of Bray's solution, extracted and the radioactivity was measured. Each determination of binding was done in triplicate. Protein was assayed according to the method of Lowry et al.[7].

3) *[^{3}H]-5-HT binding assay using two different filters*

To 0.8 ml of crude synaptic membrane suspension, 0.1 ml of [^{3}H]-5-HT solution (final concentration: 0.1–5.0 nM) and 0.1 ml of 5.0 μM unlabeled 5-HT were added. The samples were incubated at 0°C for 15 min and filtered through a Millipore filter (HAWP 02500, 0.45 μm) or a Glass filter (Whatman GF/F). Then radioactivity was measured.

RESULTS

1) *Time course of specific [^{3}H]-5-HT binding at various temperatures*

As represented in Fig. 1, at 37°C specific [^{3}H]-5-HT binding occured rapidly and attained almost equilibrium within 5 min. At 25° and 0°C specific binding reached maximal values at about 10 min. After reaching maximum, specific binding was apt to decrease within several minutes at 37° and 25°C, while unchanged for 20 min at 0°C

2) *Effects of filter on specific [^{3}H]-5-HT binding*

Saturation curves of specific [^{3}H]-5-HT binding using a Millipore filter and a Glass filter are showed in Fig. 2-a. Scatchard plot of the specific binding using a Millipore filter indicated the exsistence of two types of binding sites: one with high-affinity and the other with low-affinity, while that of using a Glass filter indicated a single high-affinity site (Fig. 2-b). Dissociation constants (Kd) and maximal numbers of binding sites (Bmax) determined by Scatchard analysis are showed in Table 1.

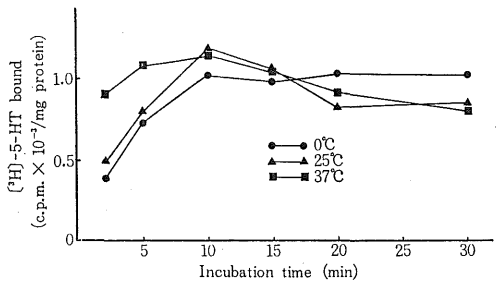

Fig. 1. Time course of specific [^{3}H]-5-HT binding at various temperatures. Each point is the mean of 3 determination.

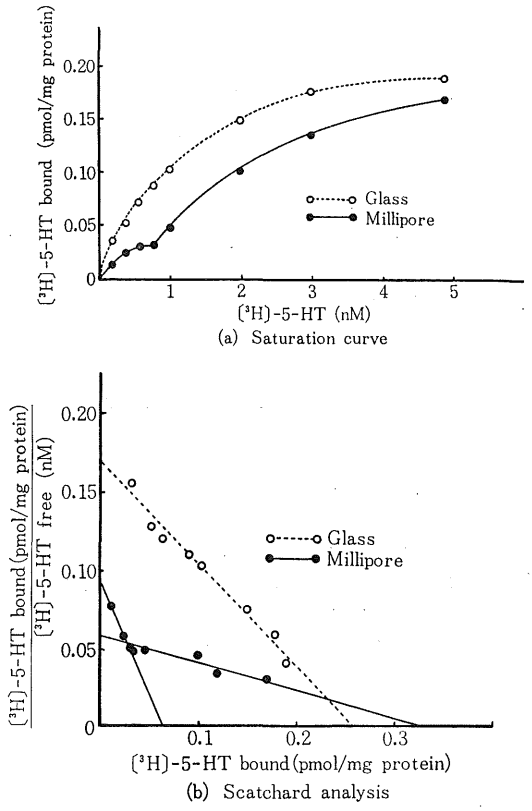

Fig. 2. Saturation curve (a) and Scatchard plot (b) of specific [^{3}H]-5-HT binding to synaptic membranes using a Millipore filter and a Glass filter. Each point is the mean of 12 determinations on 4 animals.

DISCUSSION

Segawa et al.[11] have carried out specific [^{3}H]-5-HT binding assay at 0°C for 10 min, however Bennett and Snyder[2] have described that at 4°C association equilibrium requires at least 120 min. The present study indicates that the most adequate incubation condition is at 0°C for 10 to 30 min and supports the condition of the former.

Scatchard plot of specific [^{3}H]-5-HT binding using a Millipore filter and a Glass filter drew biphasic and monophasic, respectively. This discrepancy suggests that the characteristics of filter may affect on specific [^{3}H]-5-HT binding. Fillion et al.[5] have suggested that the low-affinity site may actually represent receptors on glia cell. From these studies, the low-affinity site such as glia may be filtered through a Glass filter but not through a Millipore filter.

Table 1. Scatchard analysis of specific [^{3}H]-5-HT binding to synaptic membranes using a Millipore filter and a Glass filter

Kind of filter	High affinity		Low affinity	
	Kd (nM)	Bmax (pmol/mg protein)	Kd (nM)	Bmax (pmol/mg protein)
Millipore (0.45 μm)	0.73±0.08	0.066±0.007	6.31±0.26	0.328±0.029
Glass (GF/F)	1.58±0.17	0.267±0.014	—	—

Each value is the mean±S. E. M. of 12 determinations on 4 animals.

In conclusion, The characteristics of filter should be taken into consideration in [^{3}H]-5-HT binding assay.

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