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Binding of 5-Hydroxytryptamine to Brain Hydrophobic Proteins. Inhibition by Structural Analogues and Ions

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Binding studies with hydrophobic proteins extracted from cerebral cortex homogenates by mixtures of n-butanol-water and separated by chromatography on LH-20 Sephadex, have been done. 5-HT-(^{14}C) binds to this fraction with high affinity. This binding saturates with 5×10^{-6} M 5-HT, with $K_{1/2}$ value of 1×10^{-7} M. Binding is partially inhibited by a mixture of alkaloids ergocornine, ergocrystine and ergocryptine, as well as by trpytamine. A light inhibition has been observed in presence of tryptophan or lysine, but none in presence of methysergide or hypoxanthine. Binding is strongly inhibited by monovalent ions (Li^+, Na^+ and NH_4^+). The influence of pH in the incubation medium has also been studied; maximal rates of binding were obtained at neutral pH.

Proteolipids are a special group of hydrophobic proteins, first isolated from nervous tissue by FOLCH-PI and LEES (8), which are widely distributed in many mammalian tissues (7). Proteolipids are usually extracted with chloroform-methanol (CM) procedure of FOLCH-PI (9). However other authors by using salt-free tissue preparations, have shown that a mixture of n-butanol-water is also an efficient medium for the extraction of membrane bound proteolipids (4, 10, 11, 13, 15, 16). Studies with CM or butanol extracts of tissue, followed by chromatography on LH-20 Sephadex have suggested a role for hydrophobic proteins in binding of neurotransmitters (1-3, 5, 6, 14).

FISZER and DE ROBERTIS (4) reported the binding of 5-HT to butanol extracts of mammalian brain, and GODWIN and SNEDDON (10, 11) have investigated some of the parameters which control this binding nature. In this work the inhibition of this binding by structural analogues of 5-HT and ions has been investigated, as well as the influence of pH in the incubation medium.

Materials and Methods

Extraction of hydrophobic proteins. Cow cerebral cortex was removed immediately after sacrifice in the slaughterhouse and homogenized with 3 volumes of distilled water. The homogenates were treated with 2 volumes of a mixture of n-butanol-water 1:1 during 30 minutes at room temperature, centrifugated at 4,500 rpm and the butanol phase isolated. The brain butanol extract appeared as a transparent liquid, containing lipids and proteolipids. This constitutes the total lipid extract (TLE).

Chromatography. The butanol phase (TLE) was applied to a column of LH-20 Sephadex (200 × 20 mm) equilibrated over-night with chloroform. The elution was carried out with chloroform and a series of chloroform-methanol mixtures of increasing polarity. The protein content of the fractions was estimated with the Folin phenol reagent according to HESS and LEWIN (12).

Binding studies. The experiments were carried out with 5×10^{-7} M 5-HT-(^{14}C) (50 mCi/mM), incubated with brain butanol phase for 10 minutes at room temperature.

Inhibition experiments. Structural analogues to 5-HT or ions were added to the brain butanol phase before adding 5-HT-(^{14}C). Inhibition was calculated as the percentage binding of 5-HT in presence of different inhibitors.

Results and Discussion

Studies of 5-HT-(^{14}C) binding to butanol brain extracts. Figure 1 shows the elution pattern of the butanol extracts of cerebral cortex. In this experiment 5-HT-(^{14}C) was added to butanol extract (final concentration: 5×10^{-7} M), and ten minutes later the extract was eluted through a Sephadex LH-20 column. Radioactivity appeared with the last protein fraction in the CM 4 : 1 elution. This result was similar to that obtained by FISZER and DE ROBERTIS (4) using cat brain, and GODWIN, with rat brain. This peak contained approximately 80 % of the total radioactivity; and 90 % of free 5-HT-(^{14}C) was retained on the column. This binding of 5-HT was similar to that found in extracts of cow cerebral cortex and hypothalamus.

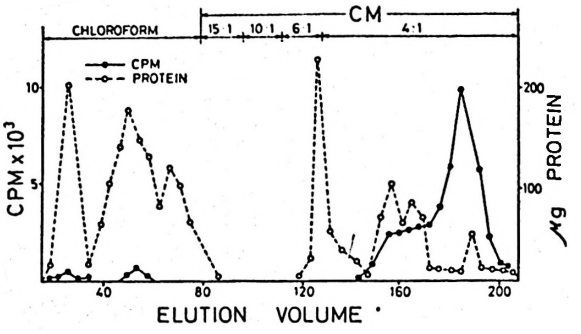

Fig. 1. *Chromatography on LH-20 Sephadex of cow cerebral cortex extracts incubated with 5-HT-(^{14}C).*
Chloroform and chloroform-methanol mixtures were used as eluants. For further explanations, see text. Elution volume units are ml.

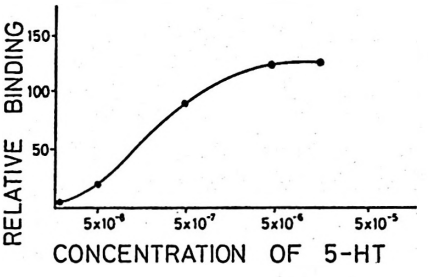

Fig. 2. *Effect of 5-HT concentration on the 5-HT binding to cow cerebral cortex extracts.*
Relative binding was calculated considering the value 100 as the binding obtained at a 5-HT concentration of 5×10^{-7} M.

Under the conditions used, cow cerebral cortex binding becomes saturated in 5×10^{-6} M 5-HT; the value of $K_{1/2}$ is 1×10^{-7} M. Figure 2 shows the effect of 5-HT concentration on the extent of binding. It can be seen that binding is appreciable at very low 5-HT concentrations. This saturability seems to indicate the binding specificity of the protein fraction studied.

Inhibition experiments. Incubation of cerebral cortex extracts were carried out in presence of different substances with molecular structure or with charged groups dispositions similar to those of 5-HT, as well as different monovalent ions. A mixture of alkaloids ergocornine, ergochrystine and ergochryptine (1 : 1 : 1), methysergide, tryptamine, tryptophan, lysine, hypoxanthine, and the ions Li^+, Na^+ and NH^+ were used for this purpose.

Table I shows the inhibitory effect of different substances and ions upon the 5-HT binding to brain extracts. The inhibitory action of tryptamine and ergocornine is especially significant because it proves participation of the indol group at this binding site. However, methysergide, an alkaloid with a methylated indol group is not inhibitory; this effect can be caused by the presence of a methyl group in the indole ring that prevents binding.

Table I. *Inhibition of 5-HT binding to cow cerebral cortex extracts by structural analogues and ions.*
All substances were used at 1×10^{-3} M concentration.

Substance	Percentage
Ergocornine	70
Methysergide	0
Tryptamine	80
Tryptophan	20
Lysine	30
Hypoxanthine	0
Li^+	70
Na^+	32
NH_4^+	56

It has also been found that tryptophan inhibits very weakly the 5-HT binding to brain extracts. This is probably due to the presence of the charged carbonyl group. Lysine inhibits binding more deeply than tryptophan, even though it does not contain the indol group. This can be tentatively explained by the fact that lysine has a charge distribution similar to that of 5-HT.

Hypoxanthine, which does not possess indol groups, or the same charge distribution as that of 5-HT, does not inhibit its binding.

Therefore the specificity of the 5-HT binding is dependent both on the indol group and the distribution of charges.

It is worth noting that the inhibitions produced by ergocornine, tryptamine and lysine are saturable; this seems to be in accordance with the existence of an active center in the binding protein.

The inhibition caused by monovalent ions points to the participation of electrostatic forces in the binding of 5-HT to the protein fraction.

Influence of pH in the incubation medium. Incubations of cerebral cortex extracts have been carried out at different pH, with 5-HT, under standard conditions. For each experiment relative binding was calculated from the radioactivity

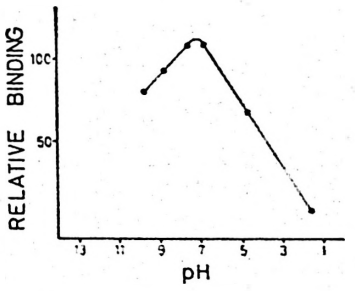

Fig. 3. *Effect of pH on 5-HT binding to cow cerebral cortex extracts.*
Relative binding was obtained considering 100 as the binding found for pH 6.5.

eluted in CM 4:1. The pH desired was obtained by adding HCl or NaOH to butanol extracts (pH = 6.55); the binding obtained at this pH was taken as the control. The extract protein was precipitated when solution pH was higher than 10.

Figure 3 shows the effect of pH on the 5-HT binding to cow brain extracts; this effect is maximal at physiological pH and very small at low pH values. Under neutral conditions the hydroxyl and amino groups of 5-HT are ionized ($pK_1 = 4.9$, and $pK_2 = 9.8$) and their binding is maximal. This is also in accordance with the participation of these groups in the binding of 5-HT to the protein by means of electrostatic forces.

Resumen

Se estudia la fijación de 5-HT a proteínas hidrofóbicas extraídas de homogenizados de corteza cerebral mediante mezclas de n'butanol y agua. Las proteínas, separadas posteriormente por cromatografía en Sephadex LH-20, muestran que una de las fracciones eluidas de la columna presenta una gran afinidad por la 5-HT-C^{14}. Esta unión se satura a una concentración de 5-HT de 5×10^{-8} M, siendo su $k_{1/2}$ de 1×10^{-7} M. La fijación es parcialmente inhibida por una mezcla de los alcaloides ergocornina-ergocristina-ergocriptina, así como por la triptamina; la inhibición es ligera con el triptófano o lisina, y nula utilizando metisergida o hipoxantina. Los iones monovalentes inhiben fuertemente la fijación. El pH del medio de incubación sobre la fijación es óptimo a pH neutro.

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