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δ-9-Tetrahydrocannabinol inhibits the binding of theophylline to mammalian neuronal and non-neuronal membranes

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Abstract—Theophylline (Th) (11.0×10^{-6}–550.0×10^{-6} M) shows dose dependent binding to the subcellular membranes of rat brain and liver. Its binding to neuronal membranes is in the order of synaptosome > microsome or mitochondrion > myelin. However, in the liver, Th binding to microsomes is greater than that to mitochondria. In all the membranes studied δ-9-tetrahydrocannabinol (THC) (1.6×10^{-6}–13.33×10^{-6} M) reduces the binding of Th. Scatchard plot analysis data suggest that this inhibitory effect of THC may arise from an allosteric change in the conformation of the Th binding sites not affecting the binding affinity of Th. Abrogation of the THC-induced inhibition of Th binding to control membranes after solubilization and restoration of the inhibitory effect of THC on Th binding in reconstituted membranes suggest the involvement of membrane lipid in the THC-induced inhibition of Th binding to neuronal and non-neuronal membranes.

δ-9-Tetrahydrocannabinol (THC*), a potent lypophilic psychoactive ingredient, is present in cannabis [1]. Theophylline (Th), a water-soluble methylated xanthine, is found at a high concentration in tea and coffee [2]. Both THC and Th act as potent bronchodilator and central nervous system stimulants [2, 3]. Earlier it has been shown [4–6] that both THC and Th bind to serum albumin and that such binding determines the distribution and elimination of the drugs [7]. Since fatty acids and cholesterol influence these drug–protein interactions [8] and since drug action is primarily manifested through the modulation of membrane properties [9–11], in the present investigation we have studied the effects of THC on the binding of Th to mammalian neuronal and non-neuronal (liver) subcellular membranes in relation to (a) the nature of Th binding and (b) the role of membrane lipid, if any, in such a binding interaction.

Materials and Methods

Male albino rats of Charles Foster strain (body weight 110–120 g) maintained with food and water *ad lib.* were decapitated and the whole brain synaptosomes, myelin, mitochondria and microsomes were prepared by the sucrose density gradients method of Gray and Whittaker [12]. The microsomes of rat liver were prepared by differential centrifugation as described by Schenkman *et al.* [13]. Purity of these membrane preparations was tested by assaying their marker enzymes [14]. Subcellular membrane protein was estimated following the method of Lowry *et al.* [15]. The binding of Th and/or THC to subcellular membranes was estimated by microdialysis under equilibrium conditions [5]. The concentration of Th was determined spectrophotometrically at 277 nm [5]. The binding parameters for the interaction of Th with various subcellular membranes in the absence and presence of THC were evaluated by Scatchard plot analysis [16]. Whole brain synaptosomes, microsomes and liver microsomes were solubilized and then reconstituted following the method of Meissner and Fleischer [17]. Reconstitution was primarily confirmed by assay of membrane-bound acetylcholinesterase activity [14].

* Abbreviations: THC, δ-9-tetrahydrocannabinol; Th, theophylline.

Results

Figure 1 shows that the binding of Th to the membranes of all subcellular fractions of the brain and liver increased with increasing Th concentration (11.0×10^{-6}–550.0×10^{-6} M). At a particular concentration of Th, its binding to brain synaptosomes was maximum followed by brain microsomes, mitochondria and myelin (Fig. 1a). In the liver, the binding of Th to microsomes was greater than to mitochondria (Fig. 1b). Table 1 shows that THC (1.6×10^{-6}, 6.4×10^{-6} and 13.33×10^{-6} M) significantly ($F = 11$, $df = 3$ and 8, $P < 0.01$) inhibited the binding of Th (11.0×10^{-6} and 550.0×10^{-6} M) to rat brain and liver subcellular membranes.

Table 2 appears to show that there were two types of binding, low and high affinity, in all the membranes studied. The number of binding sites and binding affinity were in the order of brain synaptosome = liver microsome > brain microsome = brain mitochondria = liver mitochondria > brain myelin. It was also observed that THC reduced ($F = 11$, $df = 3$ and 8, and $P < 0.01$) the number of both high and low affinity binding sites of Th in all the subcellular membranes without affecting their binding affinity. The maximum inhibition was found at 6.4×10^{-6} M THC in brain synaptosomal and liver microsomal membranes.

Table 3 shows that solubilization of synaptosomal and liver microsomal membranes increased the binding of Th (550.0×10^{-6} M) by 20% and 16%, respectively. Table 3 also shows that THC inhibited the binding of Th to brain synaptosomes and liver microsomes to varying degrees depending on the concentration of THC and the type of membrane.

Discussion

The present study shows that the binding of Th to both neuronal and non-neuronal (hepatic) subcellular membranes is monophasic irrespective of the concentration of Th (Fig. 1). This indicates a single type of binding of Th to biomembranes which is predominantly ionic in nature [4]. Furthermore, the difference in magnitude of Th binding to different subcellular membranes in the brain (Fig. 1) may be explained by the difference in the number of binding sites present in different membranes as observed from the Scatchard plot analysis (Table 2) and not by the lipid–protein ratio of the respective membrane [18]. Unlike in the brain, in the liver the binding of Th to microsomes

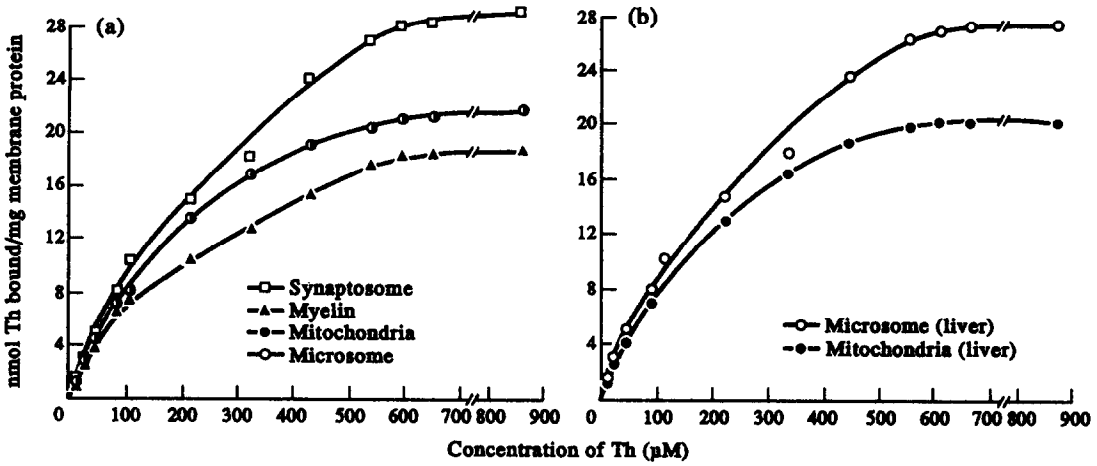

Fig. 1. Binding of Th (nmol/mg membrane protein) to rat (a) brain and (b) liver membranes. Each point represents the mean of four separate determinations.

Table 1. Per cent binding of Th to brain and liver subcellular membranes in the absence and presence of THC

Subcellular fraction	Concentration of Th (μM)	% binding of Th in the presence of different concentrations (μM) of THC			
		0	1.6	6.4	13.33
Brain					
Synaptosomes	11.0	100.0 ± 0.12*	66.6 ± 0.20	51.3 ± 0.10	51.3 ± 0.08
	550.0	1773.3 ± 0.20	1333.3 ± 0.08	1240.0 ± 0.16	1293.3 ± 0.04
Myelin	11.0	100.0 ± 0.10*	70.0 ± 0.08	60.0 ± 0.12	60.0 ± 0.02
	550.0	1666.6 ± 0.20	1483.3 ± 0.16	1425.0 ± 0.10	1483.3 ± 0.10
Microsomes	11.0	100.0 ± 0.08*	94.6 ± 0.04	63.8 ± 0.08	64.3 ± 0.04
	550.0	1666.6 ± 0.20	1483.3 ± 0.16	1425.0 ± 0.10	1483.3 ± 0.10
Mitochondria	11.0	100.0 ± 0.04*	84.6 ± 0.04	63.8 ± 0.12	63.8 ± 0.20
	550.0	1536.8 ± 0.20	1369.2 ± 0.20	1323.0 ± 0.20	1369.2 ± 0.14
Liver					
Microsomes	11.0	100.0 ± 0.10*	73.3 ± 0.08	51.3 ± 0.08	51.3 ± 0.14
	550.0	1774.2 ± 0.11	1346.6 ± 0.24	1226.6 ± 0.06	1260.0 ± 0.10
Mitochondria	11.0	100.0 ± 0.20*	84.6 ± 0.16	59.2 ± 0.20	59.2 ± 0.20
	550.0	1538.4 ± 0.30	1384.6 ± 0.25	1277.0 ± 0.20	1384.6 ± 0.15

Each value represents the mean ± SEM of three separate determinations.

* The binding (nmol/mg membrane protein) of Th (11.0 μM) to control membranes from synaptosomes (1.5), myelin (1.1), microsomes (1.2) and mitochondria (1.3) of the brain, and microsomes (1.5) and mitochondria (1.3) of the liver was considered as 100.

All values are significantly different from control (binding of 11.0 μM Th in the absence of THC), $P < 0.01$.

Statistical analysis was performed using Tukey test of analysis of variance.

is greater than that to mitochondria. It is interesting to note that the total amount of Th binding in liver microsomes (Table 1) is almost identical to that in brain synaptosomes. The greater binding of Th to hepatic microsomes in comparison to hepatic mitochondria may be possible because hepatic microsomes are a site for drug detoxication [19, 20]. Anionic drugs bind to the surface of the membrane while cationic drugs usually bind to the core of the membrane [21]. Intramolecular resonance of Th produces its anionic form [4]. It is also reported that Th binds to the protonated amino group of the lysine residue [4]. Hence, it can be assumed that the membrane having more of such protonated groups on its surface will bind more Th molecules, and in this regard the anionic form of Th may bind more promptly than its non-ionic counterpart [4]. The solubilization of membrane with deoxycholate increases (16–24%) Th binding (Table 1) suggesting that Th binds to membrane protein while the membrane lipid component may inhibit this binding of Th [8], probably by masking some Th binding sites. Thus, solubilization of membrane lipid probably exposes more protonated sites on membrane proteins where Th can bind. With this idea in mind it may be suggested that THC due to its hydrophobicity inhibits the binding of Th to membranes (Table 1). The pentyl side chain of THC may obstruct the attachment of Th to its specific binding site by flopping on the protonated group or THC may bring about a certain allosteric modification of the Th binding site (Table 2) making it difficult for Th

Table 2. Binding parameters of Th (11.0×10^{-6}–550.0×10^{-6} M) to different subcellular membranes in the absence and presence of THC

Subcellular fraction	Concentration THC ($M \times 10^{-6}$)	Number of binding sites		Binding constants	
		High affinity	Low affinity	High affinity ($K_1 \times 10^{-7} M^{-1}$)	Low affinity ($K_2 \times 10^{-7} M^{-1}$)
Brain					
Synaptosomes	0	4.0 ± 0.02	7.5 ± 0.01	0.40 ± 0.06	0.14 ± 0.04
	6.4	2.5 ± 0.01	5.0 ± 0.02	0.30 ± 0.05	0.13 ± 0.04
	13.33	3.4 ± 0.01	5.3 ± 0.02	0.27 ± 0.06	0.13 ± 0.04
Myelin	0	3.0 ± 0.01	5.2 ± 0.02	0.38 ± 0.05	0.13 ± 0.04
	6.4	2.4 ± 0.01	4.0 ± 0.01	0.29 ± 0.04	0.15 ± 0.06
	13.33	2.6 ± 0.01	4.4 ± 0.01	0.28 ± 0.06	0.13 ± 0.03
Microsomes	0	3.3 ± 0.02	7.0 ± 0.03	0.38 ± 0.04	0.13 ± 0.04
	6.4	2.3 ± 0.01	5.0 ± 0.02	0.30 ± 0.04	0.12 ± 0.03
	13.33	2.6 ± 0.01	5.6 ± 0.02	0.30 ± 0.04	0.12 ± 0.02
Mitochondria	0	3.4 ± 0.03	7.0 ± 0.04	0.39 ± 0.05	0.13 ± 0.04
	6.4	2.2 ± 0.02	5.0 ± 0.01	0.35 ± 0.04	0.12 ± 0.05
	13.33	2.6 ± 0.01	5.6 ± 0.01	0.34 ± 0.05	0.13 ± 0.02
Liver					
Microsomes	0	4.0 ± 0.01	7.5 ± 0.02	0.40 ± 0.06	0.12 ± 0.05
	6.4	2.5 ± 0.02	4.8 ± 0.01	0.38 ± 0.05	0.13 ± 0.05
	13.33	3.0 ± 0.02	5.0 ± 0.03	0.40 ± 0.06	0.14 ± 0.04
Mitochondria	0	3.4 ± 0.02	7.0 ± 0.02	0.39 ± 0.05	0.13 ± 0.05
	6.4	2.2 ± 0.01	5.0 ± 0.01	0.38 ± 0.04	0.13 ± 0.02
	13.33	2.7 ± 0.01	5.5 ± 0.01	0.39 ± 0.04	0.13 ± 0.02

Each value represents the mean ± SEM of three separate determinations.
All values under "number of binding sites" were significantly different from control (binding in absence of THC), $P < 0.01$.
Statistical analysis was performed using Tukey test of analysis of variance.

Table 3. Binding of Th (550.0×10^{-6} M) to control, solubilized and reconstituted subcellular membranes in the absence and presence of THC

Subcellular fraction	Concentration of THC	Binding of Th (nmol/mg membrane protein)		
		Control	Solubilized	Reconstituted
Brain				
synaptosomes	0	26.60 ± 0.08	31.90 ± 0.06	26.00 ± 0.04
	6.4	20.75 ± 0.02	25.55 ± 0.06	20.50 ± 0.10
	13.33	18.62 ± 0.04	28.10 ± 0.08	17.75 ± 0.10
Liver				
microsomes	0	27.00 ± 0.05	31.40 ± 0.05	25.80 ± 0.02
	6.4	24.80 ± 0.02	25.23 ± 0.02	21.93 ± 0.06
	13.33	22.80 ± 0.02	25.12 ± 0.02	20.64 ± 0.05

Each value represents the mean ± SEM of three separate determinations.
All values were significantly different from control (binding in absence of THC), $P < 0.01$.
Statistical analysis was performed using Tukey test of analysis of variance.

to bind [22]. The involvement of membrane lipid in the binding of Th in the absence and presence of THC was further supported by the enhancement of Th binding to the solubilized membranes and the withdrawal of this facilitative effect of Th binding to the reconstituted membranes of brain synaptosomes, microsomes and liver microsomes with or without THC (Table 1). Prevention of the THC-induced inhibition of Th binding to control membranes following solubilization and restoration of this inhibition of Th binding in reconstituted membranes suggest that THC in the presence of lipid exerts a greater inhibition of Th binding to neuronal and non-neuronal membranes.

Finally, it may be concluded that (a) the anionic form of Th binds to the exofacial surface proteins of membranes [21] and the binding is predominantly ionic in nature, and (b) THC inhibits Th binding to biological membranes and this inhibitory effect of THC is potentiated in the presence of membrane lipids.

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Department of Biochemistry
University College of Science
University of Calcutta
35 Ballygunge Circular Road
Calcutta 700 019
India

S. K. GHOSH
M. K. PODDAR*

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* Corresponding author.