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Lipid fluidity markedly modulates the binding of serotonin to mouse brain membranes

(receptor modulation/synaptic transmission/microviscosity/cholesterol/aging)

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ABSTRACT **The binding of [^{3}H]serotonin to mouse brain crude membrane and synaptosomal membrane preparations was investigated as a function of membrane fluidity changes by lipids. The microviscosity ($\bar{\eta}$) of the synaptic membranes was increased by *in vitro* incubation with either cholesteryl hemisuccinate or stearic acid, resulting in an up to 5-fold increase in the specific binding of [^{3}H]serotonin. Serotonin binding increased progressively until it reached a maximum at 1.75 relative $\bar{\eta}$ units; then it declined. Fluidization of membrane lipids, by treatment with lecithin or linoleic acid, caused a small but significant decrease in serotonin binding. These observations are compatible with the concept of vertical displacement of membrane proteins, indicating that in the untreated brain tissue the accessibility (B_{max}) of serotonin receptor binding sites constitutes only a fraction (about 20%) of the potential binding capacity stored in the membrane. Scatchard plots of [^{3}H]serotonin binding, at different $\bar{\eta}$ values, indicate a continuous change in the binding affinity (K_d) of serotonin to its receptor, concomitant with changes in its accessibility. These results may have important implications for physiological processes in the central nervous system, which are associated with modulation of membrane lipids, such as aging. In addition, the regional heterogeneity and plasticity of receptors may be accounted for by differences in membrane lipid fluidity. It was found here that various brain regions differ markedly in their membrane lipid viscosity.**

The overt physiological expression of the interaction between a ligand and its receptor on a cell surface is the product of two distinct steps—the formation of the ligand–receptor complex, and the transmission of the relevant message into the cell interior. The accessibility of surface receptors to ligand binding can be modulated by changes in the membrane lipid fluidity which, in turn, may mediate a shift in the equilibrium position of membrane proteins (1–4). Signal transduction, either through activation of adenylate cyclase by the ligand–receptor complex (5, 6) or by microaggregation of ligand–receptor complexes (7, 8), is associated with lateral movements of components of the membrane which are determined, at least partially, by lipid fluidity (9). This net "passive" modulation of a receptor function by lipid fluidity is therefore a rapid physical process which does not require metabolic energy. It has been suggested (10) that this mechanism may play a key role in the modulation of receptor function in fast cellular processes such as neurotransmission.

The accessibility of a receptor binding site, which is a prerequisite for its function, and the extent and direction of its displacement by changes in the lipid fluidity are determined primarily by the geometry of the receptor in the immediate lipid environment. The β-adrenergic receptor, for example, is affected by lipid fluidity in a different manner in different cells. When lipid microviscosity is decreased, the receptor becomes more exposed in liver cell membranes (11) but less exposed in rabbit reticulocyte membranes (12); in turkey erythrocytes the β-adrenergic receptor is not affected by changes in lipid fluidity (13). This diversity reflects a basic difference in the geometry of the β-adrenergic receptor in membranes of different tissues.

In this study, the passive modulation of the accessibility of the serotonin receptor in brain membranes was investigated. A direct correlation between lipid microviscosity of crude membrane and crude synaptosomal preparations and the binding characteristics and accessibility of the serotonin receptor was found. These findings are of fundamental importance in understanding the mode of action of serotonin as well as other neuroactive substances in the brain.

MATERIALS AND METHODS

[^{3}H]Serotonin (30.2 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) was obtained from New England Nuclear. Unlabeled serotonin, pargyline, cholesteryl hemisuccinate (CholSuc), egg lecithin (grade II), polyvinylpyrrolidone (PVP, M_r 40,000) and Tris free base were purchased from Sigma. Stearic acid (>99%), linoleic acid (>99%), and 1,6-diphenyl-1,3,5-hexatriene were obtained from Koch-Light Laboratories (Colnbrook, England). The following drugs were used: methysergide (Sandoz), metergoline (Farmitalia), cinanserin (Squibb), mianserin (Organon), methiothepin (Hoffmann–LaRoche), fluxethine (Eli Lilly), and haloperidol (Abic, Israel).

Media for Lipid Modulation. A modification of a previous procedure (14) was used, in order to avoid using ethanol. CholSuc was dissolved in glacial acetic acid (40 mg/ml) by heating and stirring. The hot solution was diluted to 100 vol with 50 mM Tris·HCl, pH 7.4/3.5% PVP, with vigorous stirring. The pH was then readjusted to 7.4 with solid Tris base. The resulting translucent suspension of CholSuc in 170 mM Tris acetate buffer was then used for modulating membrane fluidity. Solutions of egg lecithin (80 mg/ml), stearic acid (6 mg/ml), and linoleic acid (6 mg/ml) in glacial acetic acid were used in a similar manner to form the corresponding media.

Crude Membrane Preparation (Crude Homogenate). Male BALB mice (20–25 g; 6–8 weeks old) were decapitated, and their forebrains (whole brain minus cerebellum) were rapidly removed and homogenized in 20 vol of ice-cold 50 mM Tris·HCl (pH 7.4) buffer in an Ultra-Turrax homogenizer (setting 6, 20 sec). The homogenates were then centrifuged at 42,000 × *g* for 10 min at 4°C in a Sorvall RC-5B centrifuge. The pellets were resuspended in 10 vol of the same buffer (100 mg of tissue per ml) and incubated for 2 hr with varying volumes of the lipid suspensions described above. Between 1 and 30 ml of lipid

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Abbreviations: CholSuc, cholesteryl hemisuccinate; PVP, polyvinylpyrrolidone; SPM, synaptic plasma membranes.

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suspension per 1 ml of membrane suspension, at room temperature, with continuous shaking, was used. The samples were then centrifuged at 42,000 × *g* for 10 min, and the pellets were washed twice with 10–20 vol of 50 mM Tris·HCl (pH 7.4) buffer. The final pellet was resuspended in 50 vol of 50 mM Tris·HCl (pH 7.4) buffer containing 0.1 mM pargyline and 0.2 mg of ascorbate per ml and used for [^{3}H]serotonin binding assays (as described below).

Crude Synaptosomal Membrane Preparation. Crude synaptosomal membranes were prepared as described (15). The resulting pellet was suspended in 10 vol (based on original tissue weight) of 50 mM Tris·HCl (pH 7.4) buffer and subjected to lipid treatment, as described above for the crude membrane preparation. Washing and final resuspension with ascorbate and pargyline were as described above.

As controls, each preparation was incubated for 2 hr with either 10 vol of 50 mM Tris·HCl (pH 7.4) buffer or 10 vol of 170 mM Tris acetate (pH 7.4) buffer, containing 3.5% PVP and then washed and resuspended as described above. No significant differences in serotonin binding or in membrane fluidity were found after incubation in these buffers.

Synaptic Plasma Membrane (SPM) Preparation. SPM were prepared as described (16). The final pellet, containing SPM, was weighed, suspended in 10 vol of 50 mM Tris·HCl (pH 7.4) buffer, and subjected to lipid treatment as described above. This preparation was used for membrane fluidity determinations only.

[^{3}H]Serotonin Binding Assay. This assay was performed as described (17, 18), with minor modifications, because endogenous serotonin in the samples used was assumed to be degraded completely after the 2-hr lipid treatment (19). All assays were done in triplicate, together with triplicates containing unlabeled serotonin. Specific binding is defined as the total minus the nonspecific binding—i.e., in the presence of 10 μM unlabeled serotonin.

Measurements of Lipid Fluidity. Membrane lipid microviscosity $\bar{\eta}$ was determined by fluorescence depolarization of a lipid probe, 1,6-diphenyl-1,3,5-hexatriene (for review, see ref. 20). A stock solution of 2 mM diphenylhexatriene in tetrahydrofuran was diluted 1:1000 into vigorously stirred phosphate-buffered saline (pH 7.2). A sample of membranes (containing about 40 μg of protein) was suspended in 2 ml of the diphenylhexatriene dispersion and incubated for 30 min at 25°C. The degree of fluorescence polarization, P, was then recorded at 25°C as described (21). The lipid microviscosity (the reciprocal of fluidity) was estimated by the approximate empirical relation, $\bar{\eta} = 2P/(0.46 - P)$ which, on a relative scale, should be close to linear (20). Preliminary observations showed that CholSuc had a greater effect than cholesterol on lipid fluidity; therefore, CholSuc was selected as the membrane rigidifier (14) to be used in this study.

RESULTS

A plot of the specific binding of [^{3}H]serotonin at 37°C to the crude synaptosomal membrane fraction at different $\bar{\eta}$ values, as a function of ligand concentration is shown in Fig. 1. Specific [^{3}H]serotonin binding increased markedly with increasing microviscosity and binding decreased slightly, although significantly, with an increase in membrane fluidity. The nonspecific binding, constituting 40–50% of the total binding, varied linearly with ligand concentration and also increased as $\bar{\eta}$ increased, although to a much smaller extent than the total binding (data not shown).

Fig. 2 shows the same data as in Fig. 1, replotted according to Scatchard (22). Analogous experiments were carried out with crude membrane preparations, for which Scatchard plots of

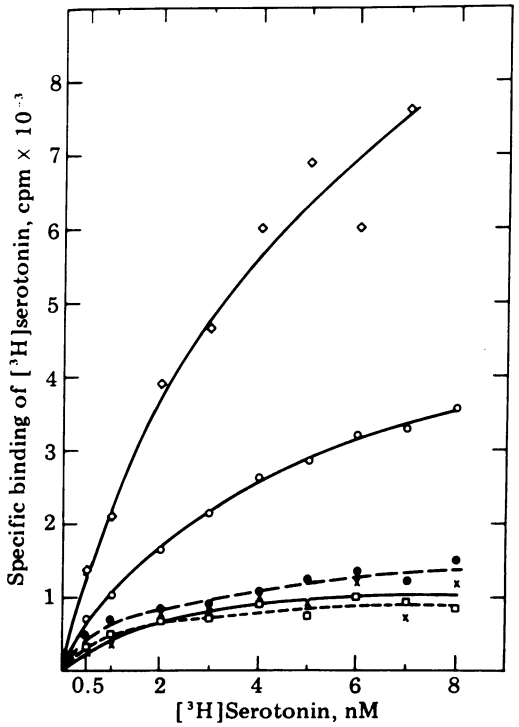

FIG. 1. Specific binding of [^{3}H]serotonin at 37°C as a function of concentration at different $\bar{\eta}$ values. Portions of the same crude synaptosomal membrane suspension (prepared from 10–20 pooled mouse brains) were incubated with varying amounts of CholSuc or lecithin. Control (untreated) samples were incubated with Tris acetate buffer, pH 7.4. Membrane microviscosity and binding were assayed. Each point is the mean of triplicate samples (SEM was <5%). The whole experiment was repeated with similar results. $\bar{\eta}$ values: ◇, 9.90; ○, 7.49; ●, 6.75 (control); □, 5.80; ×, 5.39.

[^{3}H]serotonin binding are presented in Fig. 3. In both figures, the control curves are nonlinear, indicating two types of binding sites. Table 1 summarizes the binding data obtained from Figs. 2 and 3. The K_d values obtained for untreated membranes are in agreement with values reported previously (18, 19). The capacity and K_d of both high- and low-affinity sites increased progressively with increasing $\bar{\eta}$. At high values of $\bar{\eta}$, the difference between the slopes of high- and low-affinity sites seemed to vanish, with a clear trend toward the expression of

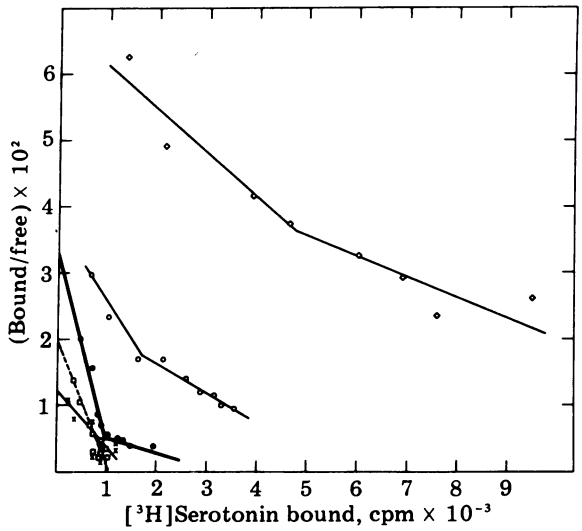

FIG. 2. Data from Fig. 1 replotted according to Scatchard. $\bar{\eta}$ values: ◇, 9.90; ○, 7.49; ●, 6.75 (control); □, 5.80; ×, 5.39.

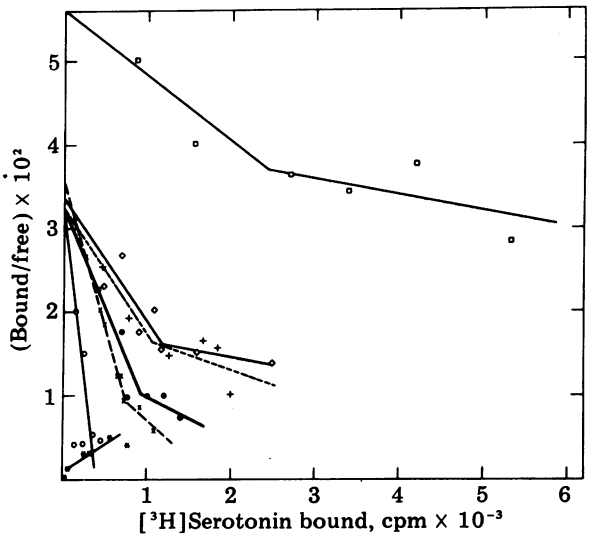

FIG. 3. Scatchard plots of [³H]serotonin binding to crude membrane preparations from mouse brain at different $\bar{\eta}$ values. Experiments were conducted as described in Fig. 1. The data represent the results of at least three experiments. $\bar{\eta}$ values: □, 7.49 (CholSuc treatment); ◇, 6.75 (CholSuc); +, 5.02 (lecithin); ●, 5.26 (control); x, 4.45 (lecithin); o, 4.35 (lecithin); ✱, 3.44 (linoleic acid).

only low-affinity sites. When the membranes were fluidized only slightly (10–20% below control), their binding capacity seemed to increase (as in the more viscous state), in addition to the increase in K_d of both high- and low-affinity sites. However, further fluidization of the membrane caused the expected decrease in binding capacity, and only high-affinity sites could be detected. The slopes (Fig. 3) became steeper with an increase in lipid fluidity until, at very low $\bar{\eta}$ values (about 50% below control), they turned positive.

Competition experiments with various drugs confirmed the substrate specificity of the newly exposed serotonin receptors (Table 2). Various drugs known to compete for serotonin sites displaced bound [³H]serotonin to varying degrees; GABA and atropin did not. As $\bar{\eta}$ increased, the potency of the various drugs in competing for serotonin sites decreased. However, the profile of their potencies was preserved as well as the substrate specificity. GABA and atropine did not compete for the new serotonin sites at any of the $\bar{\eta}$ values tested.

Binding data over a wide range of $\bar{\eta}$ values and ligand concentrations, obtained in a series of independent experiments,

Table 1. Summary of binding data from Figs. 2 and 3

$\bar{\eta}$ at 25°C, poise	K_d, nM		B_{max}, pmol/g tissue	
	High-affinity	Low-affinity	High-affinity	Low-affinity
Crude membrane preparation				
7.49	2.82	11.60	15.91	48.30
6.75	1.55	11.60	5.19	20.10
5.26*	0.95	4.14	3.05	6.55
5.02	1.49	5.27	4.85	12.64
4.45	0.64	2.50	2.26	4.00
4.35	0.29	—	0.90	—
Crude synaptosomal membrane preparation				
9.90	3.44	7.11	23.30	37.20
7.49	1.99	5.10	7.40	12.75
6.75*	0.76	9.87	2.64	7.40
5.80	2.68	—	3.27	—
5.39	1.22	—	2.37	—

* Untreated.

Table 2. Displacement of bound [³H]serotonin from crude homogenate of mouse forebrain at different values of $\bar{\eta}$

Drug	Binding			
	$\bar{\eta}$ = 5.26*		$\bar{\eta}$ = 6.25	
	cpm	%	cpm	%
None	1508		4300	
Serotonin†	940	100	2208	100
Serotonin	1050	81	2746	74
Methysergide	1228	49	3463	40
Metergoline	1165	60	3364	45
Cinanserin	1356	27	3884	20
Mianserin	1230	49	3646	31
Methiothepin	1224	50	3576	35
Fluxethine	1306	21	4097	10
Imipramine	1390	21	4272	1.3
Haloperidol	1214	52	4177	5.9
Atropine	1612	—	5214	—
GABA	1680	—	5179	—

Equal amounts of membranes were incubated with 4 nM [³H]serotonin and the various drugs listed (all drugs were at 1 μM except as marked). Each value is the mean of triplicate samples which varied less than 15%. For each drug used, the percentage displacement occurring with 10 μM unlabeled serotonin is given in parenthesis. The concentration for 50% inhibition (IC_{50}) was determined for serotonin and methysergide by incubation with five different concentrations of the unlabeled drugs; the values were 10 nM and 2 μM, respectively, in the untreated preparation ($\bar{\eta}$ = 5.26) and 0.8 μM and 1 μM when $\bar{\eta}$ = 6.25.

* Untreated.

† At 10 μM.

are summarized in Fig. 4. As $\bar{\eta}$ increased, the binding of [³H]-serotonin increased. The increase seemed to be biphasic, reached a 5-fold maximum at $\bar{\eta}$ values of ≈1.75 relative to control, and then declined sharply. With more fluid membranes, the pattern of binding also was complex and probably biphasic. Slight fluidization of the membranes caused a small but significant increase in binding but upon further increases in membrane fluidity a significant decrease in binding was observed. These data confirm the binding data shown in Table 1.

The $\bar{\eta}$ values reported here were derived from relatively crude preparations. The apparent $\bar{\eta}$ values are average $\bar{\eta}$ values of all the membranes in the system. Mitochondria (2.52 poise) cause a shift toward lower $\bar{\eta}$ values, and the myelin from axons (6.41 poise) cause a shift toward higher $\bar{\eta}$ values. SPM occupy an intermediate position (3.78 poise). The lipid-enrichment procedures used in the cruder preparations are also effective with SPM. When 1 mg of SPM was treated with various lipids, the $\bar{\eta}$ values were as follows: 0.008 mg of CholSuc, 4.35 poise; 0.04 mg of CholSuc, 5.95 poise; 0.016 mg of lecithin, 3.44 poise; 0.08 mg of lecithin, 3.20 poise; 0.12 mg of stearic acid, 4.35 poise; and 0.12 mg of linoleic acid, 2.75 poise.

Table 3 presents $\bar{\eta}$ values of membranes obtained from different brain regions of BALB mice and other rodent species. Although these measurements were made on crude preparations, the data indicate a wide range of lipid microviscosity of the membranes from different brain regions as well as from different species. The data also indicate that the fluidity values, obtained after the various lipid treatments, are within the range obtained for untreated preparations.

In order to exclude the possibility that the effects on binding seen when membrane microviscosity was changed were due to certain specific lipids (such as CholSuc and lecithin), both preparations were treated with stearic acid (which has a rigidifying effect) and linoleic acid (with a lecithin-like fluidizing effect). Both stearic and linoleic acids had effects on membrane

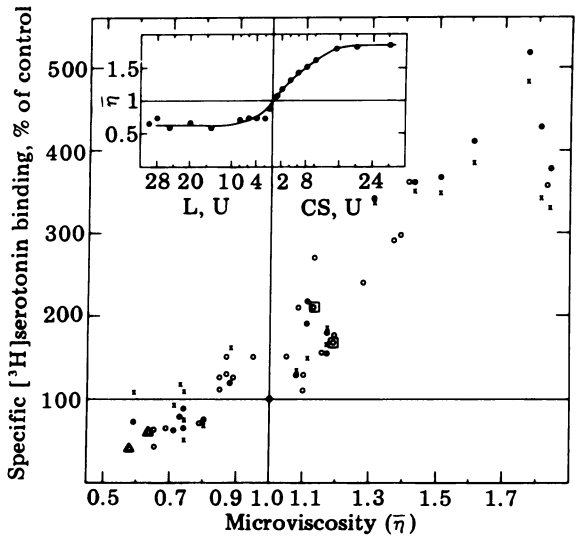

FIG. 4. Specific binding of [³H]serotonin as a function of $\bar{\eta}$ shown as relative to control. Portions from the same mouse brain suspension were incubated with varying amounts of lipids (see below). Control samples were incubated with 170 mM Tris acetate (pH 7.4) buffer. Each set of experimental points contains data from a single experiment. Points to the left of the vertical line were obtained after treatment with lecithin. Points to the right of the vertical line were obtained after treatment with CholSuc. X,●, Crude synaptosomal membrane fractions incubated with 1 nM or 6 nM [³H]serotonin, respectively; O, Crude homogenate incubated with 2 nM [³H]serotonin; □,△, crude homogenate incubated with 2 nM [³H]serotonin after treatment with stearic acid or linoleic acid, respectively. (*Inset*) Representative experiment showing $\bar{\eta}$ (relative to control) of crude synaptosomal membrane fraction as a function of lipid incubation volume. Each unit (U) of CholSuc (CS) or lecithin (L) represents 1 vol of lipid suspension (CholSuc, 0.4 mg/ml; lecithin, 0.8 mg/ml) per volume of membrane suspension (100 mg/ml). Values of $\bar{\eta}$ and [³H]-serotonin binding capacity of the control samples were: crude homogenate, $\bar{\eta}$ = 5.26, specific [³H]serotonin binding = 2.0 pmol/g of tissue; crude synaptosomal membrane fraction, $\bar{\eta}$ = 6.75, binding of [³H]serotonin at 1 nM ligand = 0.72 pmol/g of tissue; binding of [³H]serotonin at 6 nM ligand = 2.98 pmol/g of tissue.

fluidity and [³H]serotonin binding similar to those due to treatment with CholSuc and lecithin, respectively (see Fig. 4). Excess CholSuc was found to coprecipitate with the membranes, thus contributing, at least in part, to the increase in nonspecific binding observed in CholSuc-treated membranes.

The reversibility of the changes in binding described above was confirmed by a two-stage treatment. The sample was first treated with lecithin, and the expected decrease in $\bar{\eta}$ and in binding was observed. Further treatment with CholSuc caused a reversal of these changes. We have also carried out this procedure in the opposite direction (23).

DISCUSSION

The data presented here on [³H]serotonin binding to mouse brain membrane preparations in relation to fluidity can be summarized as follows. As the membrane lipids become more viscous, the specific binding of serotonin increases steadily. The specific binding of [³H]serotonin, as a function of $\bar{\eta}$, has a complex profile (Fig. 4). In the region where $\bar{\eta}$ increases, the binding appears to be biphasic and reaches a maximum at 1.75 relative $\bar{\eta}$ units. Above this level, serotonin binding decreases sharply. This behavior may reflect the progressive, although unequal, exposure of different binding sites for serotonin. In the third phase, at $\bar{\eta} > 1.75$ relative units, the decrease in binding may indicate the irreversible loss of receptors (23).

The results in Table 2 confirm that the new sites satisfy the definition of serotonin receptors because the substrate specificity, as well as the profile of potencies of various drugs in competing for these sites, is preserved. It seems, however, that these new serotonin receptors have different characteristics.

Table 3. Membrane $\bar{\eta}$ values in various brain regions and different species

Species	Preparation	Brain region	$\bar{\eta}$, 25°C poise
BALB	Crude synaptosomal membranes	Cerebral cortex	4.15
		Hypothalamus	4.15
		Thalamus	4.35
		Hippocampus	4.35
		N. caudatus	4.67
		Cerebellum	4.90
		Midbrain	5.39
		Brainstem	6.75
		Whole brain	6.41
	SPM	Whole brain	3.78
	Crude homogenate	Forebrain	5.26
C57 BL/6J (lean)	Crude homogenate	Forebrain	5.14
C57 BL/6J-*ob/ob* (obese)			5.80
(C_3H/eb × C57 BL/6J)F_1 (7 weeks old)			5.26
(C_3H/eb × C57 BL/6J)F_1 (2.5 years old)			5.95
Wistar rats			6.01

Membranes were prepared from 3–10 animals. Values are the mean of at least two experiments, which varied less than 5%.

The Scatchard plots of the specific [³H]serotonin binding (Figs. 2 and 3) indicate a progressive response to membrane microviscosity. Concomitantly with the marked increase in the number of both low- and high-affinity sites, their K_d values increase progressively (indicating lower affinity) with an increase in $\bar{\eta}$. The displacement of the receptor proteins may also affect their rotational and lateral movements as well as cause critical changes in their tertiary and quaternary structure. Apparently, both high- and low-affinity sites can change their binding characteristics markedly in response to the limiting conditions imposed by the surrounding lipid and eventually reach a situation in which the distinction between them is abolished. Furthermore, it could well be that the serotonin binding sites all have the same basic chemical structure which, in different environments, have different binding characteristics. The observation that at high lipid fluidity ($\bar{\eta} < 0.8$ relative units) the detectable serotonin receptors are of high affinity while at low lipid fluidity ($\bar{\eta} > 1.3$) the receptors are predominantly of low affinity (see Table 1) may have an alternative explanation. The low- and high-affinity sites may be located at different levels along the vertical axis of the receptor molecule. As the latter is displaced vertically to the plane of the membrane, the relative contribution of each of these sites is changed as indicated by the Scatchard plot parameters.

One of the most interesting findings presented in this study is that, under normal physiological conditions, the apparent binding capacity of serotonin receptors constitutes only about 20% of the potential binding capacity stored in the membrane (Fig. 4). Obviously, the binding of an agonist to a receptor is just the initial step in a sequence of events. The next step, which is the activation of adenylate cyclase, is probably enhanced by

increasing lipid fluidity (9, 13). The final response of target cells of different membrane fluidity therefore could be the result of two opposing processes—the accessibility of receptors, which decreases with lipid fluidity, and the activation of adenylate cyclase, which increases with lipid fluidity. For maximal response, one therefore may expect an optimal lipid fluidity for each receptor. Other processes involved in synaptic transmission, such as release and reuptake, may also change in response to changes in membrane lipid fluidity.

The increase in the lipid viscosity of synaptic membranes from the brains of aged animals (Table 3) could initially increase receptor accessibility. It has been reported (24) that the number of serotonin binding sites in brains from old men is about 2-fold higher than in brains from young adults, and only low-affinity sites are found. However, the overexposed receptors can overshoot the point of optimal activity and eventually become more vulnerable to degradation. Some of the behavioral aspects of aging, associated with the serotoninergic system (e.g., loss of sleep, appetite, and libido), could be accounted for by this process.

Table 3 shows that membranes prepared from various brain regions differ in their fluidity. This might be a partial explanation of the heterogeneity of receptor sites in the brain. Consistent with our findings that midbrain is composed of more viscous membranes compared to cerebral cortex and hippocampus (with more fluid membranes), Bennet and Snyder (17) have shown that K_d of [^{3}H]serotonin is lower (higher affinity) in cortex and hippocampus as compared to midbrain. We have also observed that the binding of ^{3}H-labeled [D-ala]enkephalin and [^{3}H]naloxone to the opiate receptor is modulated by changes in membrane lipid fluidity (23).

The changes in binding of transmitters, agonists, and antagonists, as well as in synaptic plasticity, may be associated with modulation of membrane architecture caused by changes in lipid fluidity.

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