EFFECT OF LONG-TERM TREATMENT WITH ANTIDEPRESSANTS ON BINDING OF [^{3}H]IMIPRAMINE WITH MOUSE BRAIN SYNAPTIC MEMBRANES

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High-affinity stereospecific binding sites of labeled tricyclic anti-depressants, located in the neuronal and glial fractions of brain [3], are probably the primary target for the action of these compounds. Since imipramine inhibits uptake of serotonin by synaptosomes selectively, but not competitively [1, 11], it has been suggested that imipramine binding sites are centers of allosteric regulation of serotonin transport systems [4]. Subchronic administration of imipramine has been shown to lower the concentration of imipramine binding sites in membranes of animal brain homogenate [5, 9] containing both neuronal and glial elements. Meanwhile there are no data in the literature on the effect of long-term administration of various anti-depressants on binding of [^{3}H]imipramine with synaptic membranes. It is likewise not yet clear whether a change in [^{3}H]imipramine binding sites is a common feature of the mechanism of action of tricyclic and the so-called atypical antidepressants.

The object of this investigation was to study the effect of different antidepressants on binding of [^{3}H]imipramine with unpurified mouse brain synaptic membranes.

EXPERIMENTAL METHOD

Male CBWA mice weighing 20-23 g, kept in the animal house under conditions of natural daylight, with free access to food and water, were used. Aqueous solutions of the drugs (or water in the control groups) were administered twice a day perorally in a volume of 0.3 ml for 2 weeks. The doses of the drugs on each occasion were 20 mg/kg for chlorimipramine and zimelidine (generously provided by Professor S. Ross, from Astra, Sweden), and 1 mg/kg for the new morpholine derivative M-1, synthesized at the Institute of Pharmacology, Academy of Medical Sciences of the USSR, in Professor V. A. Zagorevskii's laboratory. The animals were killed 24 h after the last dose of the preparations and the fraction of unpurified brain synaptosomes was isolated as described previously [2]. The residue (P_2) was suspended in 0.05 M Tris-HCl buffer, pH 7.4, and centrifuged for 20 min at 15,000g. This procedure was repeated five times. The residue of washed synaptic membranes was suspended in the same volume of the same buffer, poured out into plastic flasks, and kept in liquid nitrogen for up to 60 days. Before the experiment the suspensions were thawed in a water bath (5-7°C, 90 min), centrifuged, and the residues were suspended in 50 mM Tris-HCl buffer (pH 7.4, 25°C), containing 150 mM NaCl and 10 mM KCl (buffer II), and used for 30 min. Membranes of the homogenate were obtained by the method in [9], by disintegrating the brain in a blender at 14,000 rpm (Type 302, Poland) for 2 × 1 min. The suspensions of membrane homogenate were kept in the same way as the synaptic membranes, but they were not centrifuged after thawing. In each experiment membranes obtained from animals of all four groups of a particular series were used. Binding of [^{3}H]imipramine (Radiochemical Centre, Amersham, England) was carried out by the method in [9] with modifications. The incubation mixture contained, in 0.5 ml of buffer II, 0.3 mg protein of synaptic membranes or 0.6 mg protein of membrane homogenate, and 1.5-8.0 nM [^{3}H]imipramine (six concentrations). Nonspecific binding was measured in the presence of 10 μM imipramine. After incubation for 30 min at 1-2°C 4 ml of cold buffer II was

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TABLE 1. Effect of Subchronic Administration of Antidepressants on Binding of [^{3}H] Imipramine by Washed Mouse Brain Synaptic Membranes (M ± m)

Parameter studied	Compounds injected			
	H_2O (n = 10; 3 series)	zimelidine (n = 7; 2 series)	M-1 (n = 7; 2 series)	chlorimipramine (n = 10; 3 series)
K_D, n M	3,4±0,8	5,7±1,2	2,6±0,5	2,8±0,4
% of control	100	172*	90	107
		(100—305)	(61—146)	(58—240)
B_{max}, pmoles/mg protein	0,93	1,67*	1,27	1,21
	±0,09	±0,23	±0,22	±0,16
% of control	100	150†	107	127†
		(100—251)	(61—161)	(100—159)

Legend. *P ≤ 0.05 by Student's t test, † P ≤ 0.05 by Wilcoxon's T test for paired samples. Each Scatchard plot obtained for 6 points, each point for 3 repetitions. Here and in Table 2, n denotes number of experiments; limits of variations shown in parentheses.

TABLE 2. Comparison of Effect of Subchronic Administration of Chlorimipramine on Binding of [^{3}H]Imipramine by Different Mouse Brain Membrane Preparations (M ± m)

Parameter studied	Washed synaptic membranes		Membranes of homogenate	
	Compound injected			
	H_2O (n=3)	chlorimipramine (n = 3)	H_2O (n=3)	chlorimipramine (n = 3)
K_D, n M	4,3±0,8	5,8±0,9	4,25±0,5	6,6±1,2
% of control	100	114	100	157†
		(77—151)		(110—112)
B_{max}, pmoles/mg protein	0,93±0,26	1,08±0,3	0,51±0,13	0,39±0,16
% of control	100	110	100	73*
		(100—149)		(58—89)

Legend. *P ≤ 0.05 by Wilcoxon's T test relative to corresponding control.

added to each sample and the mixture was quickly filtered through glass fiber GF/C filters (Whatman, England). The filters were rinsed twice with the same volume of buffer, dried in air, and placed into flasks containing 5 ml of Bray's scintillator. After vigorous shaking and extraction for 12 h the radioactivity of the samples was determined with an SL-4000 counter (Kontron, France). Adsorption isotherms were analyzed by Scatchard's method. The protein content in the membrane preparations was determined by Peterson's method [7].

EXPERIMENTAL RESULTS

Subchronic administration of chlorimipramine, zimelidine, and compound M-1 had different effects on the binding characteristics of [H]imipramine with synaptic membranes (Table 1). Examination of the mean values of the dissociation constants (K_D) and the concentration of binding sites (B_{max}) of [^{3}H]imipramine revealed a significant increase in these characteristics only in animals receiving zimelidine. However, since in each experiment the binding characteristics of [H]imipramine were analyzed for all four groups simultaneously, examination of the control and each of the experimental groups at a time is justified. Taking the values of K_D and B_{max} of the control group in each experiment was 100%, it can be concluded that the value of B_{max} in groups of animals receiving chlorimipramine was always higher than or equal to the control level. Analysis of these results by Wilcoxon's paired T test leads to the conclusion that subchronic administration of chlorimipramine, like that of zimelidine, leads to an increase in the concentration of binding sites for [^{3}H]imipramine with synaptic membranes. In the case of simelidine, a decrease in affinity of imipramine-binding sites is observed at the same time, whereas administration of compound M-1 gave heterogeneous results in different series of experiments.

For a more accurate comparison of these results with those obtained previously with brain homogenate membranes [5], an additional series of experiments was undertaken. Animals of

two groups received water and chlorimipramine respectively as described in "Experimental Method." One part of the animals was then used to obtain synaptic membranes, the other to isolate membranes of the homogenate. The characteristics of all four groups were compared in each experiment simultaneously. Subchronic administration of the tricyclic antidepressant under these conditions was shown in fact to cause a decrease in the concentration of binding sites for [^{3}H]imipramine with membranes of the brain homogenate, with at the same time a decrease in affinity (Table 2). No such changes were found in preparations of synaptic membranes (Table 2).

It can be tentatively suggested that this disagreement between the results can be attributed to a fundamental difference in the composition of the membrane preparations studied: Membranes of the homogenate also contained neuronal and glial elements, whereas the method used to isolate synaptic membranes prevented the presence of any significant amount of glial contamination. With this fact in mind it can be postulated that the character of the change in concentration of binding sites for [^{3}H]imipramine in membranes of the glia and in synaptic membranes may be opposite. Another explanation can be based on the presence of imipramine in the preparation of homogenate membranes. The view that membranes isolated by the method in [9] do in fact contain traces of imipramine administered previously is supported by the increase in K_D found in animals of the corresponding group (Table 2) in the present experiments, and also by data of other workers [8]. The presence of such "endogenous" imipramine can considerably prolong the time taken for equilibrium to be reached between the labeled ligand and the membranes. In that case the apparent value of B_{max} may be too low [10].

It can be concluded on the whole from the results of the present experiments that the fact that changes in the state of binding sites of [^{3}H]imipramine with synaptic membranes are in the same direction does not necessarily imply a common element in the action of different groups of antidepressants. Even preparations which actively modify serotoninergic mechanisms act differently during subchronic administration. Chlorimipramine, like an antagonist, increases the concentration of imipramine binding sites, whereas zimelidine, the primary target for whose action is probably the serotonin binding centers of the corresponding carrier [6], by increasing the concentration of [^{3}H]imipramine binding sites, lowers its affinity.

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