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Repeated Treatment With Antidepressant Drugs Does Not Affect the Benzodiazepine Receptors in Preincubated Membrane Preparations From Mouse and Rat Brain

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PRZEGALIŃSKI, E., A. ROKOSZ-PELC, L. BARAN AND J. VETULANI. *Repeated treatment with antidepressant drugs does not affect the benzodiazepine receptors in preincubated membrane preparations from mouse and rat brain.* PHARMACOL BIOCHEM BEHAV **26**(1) 35–36, 1987.—Repeated injections or oral administration of antidepressants: imipramine, amitriptyline and desmethylimipramine (10 mg/kg twice daily for 21 days) did not alter significantly [^{3}H]flunitrazepam binding to frozen-thawed preincubated membranes from the brains of mice or rats.

Antidepressants Imipramine Amitriptyline Desmethylimipramine Benzodiazepine receptors [^{3}H]Flunitrazepam binding sites

CHRONIC treatment with antidepressant drugs or electroconvulsive shock leads to changes in the various populations of cerebral receptors, such as β-, α_1- and α_2-adrenergic, serotonin$_2$, opiate, etc. [1, 4, 6]. Recently, Suranyi-Cadotte *et al.* [5] have reported that chronic administration of four antidepressants: desipramine, zimelidine, bupropion and adinazolam, dramatically reduced the density of [^{3}H]flunitrazepam binding sites in the membranes from the whole rat brain. Based on that result, they suggested that an interaction leading to downregulation of the benzodiazepine receptor should be considered as a possible mechanism of action of an antidepressant treatment. Later, Barbaccia *et al.* [2] reported that an antidepressant, maprotiline, given chronically depresses the density of [^{3}H]flunitrazepam binding sites, but only in unwashed membrane preparations, while no such effect was observed in the membrane preparation which was washed several times, frozen, thawed and preincubated. They suggested that antidepressant treatment may affect an endogenous factor which changes the [^{3}H]flunitrazepam binding.

We investigated presently the effect on [^{3}H]flunitrazepam binding sites of prolonged administration of tricyclic antidepressants in mice and rats, using frozen and thawed, preincubated brain membranes.

METHOD

The experiments were carried out on male Wistar rats (180–200 g) and male Swiss Albino mice (23–25 g) bought from licensed dealers. The animals were kept at room temperature (20–21°C), on a natural day-night cycle, housed in groups of 10–12, having free access to standard food and water.

Imipramine (hydrochloride; Polfa, Starogard Gd.) and amitriptyline (hydrochloride; Polfa, Rzeszów) were given to both mice and rats by a stomach tube. In addition imipramine and desipramine (hydrochloride; CIBA-GEIGY, Basel) were injected intraperitoneally to rats. All drugs were dissolved in 0.9% NaCl solution (saline) and administered in a dose of 10 mg/kg (salt) twice daily (around 9 a.m. and 5 p.m.) for 21 days. The controls received saline IP or PO.

The animals were guillotined 24 hr after the last dosing. Their whole brains were rapidly excised and stored under solid carbon dioxide until used. The membranes were prepared, at 0–4°C, by homogenization of the tissue in 20 vol. of 50 mM Tris-HCl buffer pH 7.6 in a Polytron disintegrator (setting 4, 10 sec). The homogenate was centrifuged at 25,000 g for 30 min, and the pellet was separated and stored for assay at −18°C.

For receptor binding studies the pellets were reconstituted in 50 mM Tris-HCl buffer pH 7.6 to obtain the final concentration of protein (assayed according to Lowry *et al.* [3]) of approximately 3 mg/ml. Suspension of brain membranes (450 μl) was incubated for 40 min at 0°C with 50 μl of various solutions of [^{3}H]flunitrazepam (final conc. 0.05–2 nmol/l) and 50 μl of buffer or displacer solution (clonazepam, final conc. 10 μmol/l). The incubation was terminated by rapid vacuum filtration through Whatman GF/C fiberglass filters, which after washing (twice with 5 ml of ice-cold buf-

TABLE 1

THE EFFECT OF CHRONIC ADMINISTRATION OF ANTIDEPRESSANT DRUGS ON THE DENSITY AND AFFINITY OF [^{3}H]FLUNITRAZEPAM BINDING SITES IN THE MOUSE AND RAT BRAIN

Species	Treatment*	N†	B_{max}‡ fmol/mg prot	K_D‡ nmol/l
Mouse	Saline PO	8	452 ± 25	1.59 ± 0.11
	Imipramine PO	8	439 ± 28	1.64 ± 0.12
	Amitriptyline PO	8	442 ± 24	1.70 ± 0.12
	F(2,21)=		0.07	0.22
Rat	Saline PO/IP	6	732 ± 55	1.26 ± 0.12
	Imipramine PO	5	981 ± 69	1.65 ± 0.14
	Imipramine IP	6	839 ± 53	1.50 ± 0.11
	Amitriptyline PO	6	720 ± 68	1.20 ± 0.14
	Desipramine IP	4	795 ± 83	1.38 ± 0.17
	F(4,22)=		2.63	1.89

*The drugs were administered twice daily in a dose of 10 mg/kg for 3 weeks.
†The number of independent Scatchard plots.
‡Means ± S.E.M.

fer) were counted for radioactivity in 3 ml of Bray solution in a Packard Mod. B 3255 liquid scintillation counter at 38% yield.

For determination of B_{max} and K_D values the Scatchard analysis was employed. A single Scatchard plot was constructed from each individual brain. As the plots were rectilinear, the individual B_{max} and K_D values were calculated with regression analysis. The mean values of B_{max} and K_D values were then calculated and statistical analysis was carried out by one way analysis of variance.

RESULTS

The results are presented in Table 1. The density of [^{3}H]flunitrazepam binding sites in the whole brain of the rat was approximately 60% higher than in mice, while the K_D values in both species did not differ significantly between themselves. The overall analysis of variance did not show any significant effect of antidepressant treatment in either species. However, while the mean B_{max} and K_D values of [^{3}H]flunitrazepam binding sites in mice were very similar in control and antidepressant-treated animals, in the rat imipramine treatment, particularly PO, produced an increase in the B_{max} value. As the F-value in this group (2.63, *df* 4,22) was close to the level of significance (2.82), and the control and oral imipramine group B_{max} values differed significantly if compared after disregarding other groups, it cannot be excluded that the further study may lead to rejection of null hypothesis that imipramine given orally does not affect the [^{3}H]flunitrazepam binding sites in the preincubated membranes from the rat brain.

DISCUSSION

In contrast to the results of Suranyi-Cadotte *et al.* [5], who reported that intraperitoneal administration of various antidepressants to rats produced a fall in the density of [^{3}H]flunitrazepam binding sites by 60–75%, we did not observe any effect of this kind, even in the experiment in which we used the same species, drug and route of administration. If any change was produced this was an increase in the density of [^{3}H]flunitrazepam binding sites, which did not attain, however, the level of statistical significance, and therefore should be confirmed or disproved in further experiments.

The apparent discrepancy between our results and those of Suranyi-Cadotte *et al.* [5] may be probably explained by the difference in preparation of membranes. While we did not wash extensively the membrane preparation, but froze it for storage and preincubated, Suranyi-Cadotte *et al.* [5] washed the preparation, but used it without freezing and preincubation. Barbaccia *et al.* [2] have found that maprotiline given chronically depresses the [^{3}H]flunitrazepam binding in crude unwashed membrane preparation, but does not produce such an effect in the membranes which were washed, frozen-thawed and preincubated. Their results suggested the presence of an endogenous factor interacting with benzodiazepine binding sites, which may be affected by chronic maprotiline treatment and is lost by washing and freeze-thawing procedure. Though washing by itself does not seem to be critical (our preparation was unwashed, while that of Suranyi-Cadotte *et al.* [5] was), the freezing-thawing and preincubation possibly are. In fact, preincubation was introduced into receptor binding studies to destroy endogenous ligands of opioid receptors.

Our results seem to indicate that the characteristics of the basic structure of the membrane [^{3}H]flunitrazepam binding site remains unchanged by antidepressant treatment, but, in conjunction with the results of Suranyi-Cadotte *et al.* [5], may support the opinion of Barbaccia *et al.* [2] that the treatment may affect an endogenous factor influencing the characteristics of benzodiazepine recognition site, and only loosely bound to it.

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