EFFECT OF ANTIDEPRESSANTS ON NEUROTRANSMITTER REUPTAKE BY SYNAPTOSOMES OF CONTROL AND STRESSED RATS

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An important role in the mechanism of action of antidepressants is ascribed to their ability to inhibit neurotransmitter reuptake by synaptosomes [11, 15], for it has been suggested that the monoamine level in the synaptic cleft is lowered in depressions [16]. Investigations of this kind have usually been undertaken on synaptosomes, isolated from the brain of healthy rats, as the biological object. We know, however, that in clinical practice antidepressants exhibit an antidepressive effect only in depressed patients and not in healthy volunteers [8]. The authors showed previously [2] that definite changes in membrane processes, including in neurotransmitter reuptake systems, arise in rats after chronic exposure for 15 days to psychological stress. Depression of behavior also develops in open field and maze tests.

The aim of this investigation was to study the effect of a number of antidepressants on neurotransmitter reuptake by brain synaptosomes after exposure to chronic psychogenic stress, and to compare it with the effect of the drugs on this process when synaptosomes from intact animals are used as the test object.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 180-200 g. Psychogenic stress was produced by the method in [5] for 15 days. The effect of antidepressants of varied chemical structure on neurotransmitter reuptake was studied on the coarse synaptosomal fraction of rat brain [2] by a radioisotope method. A suspension of synaptosomes, 50 µl in volume (on average 2 mg protein to 1 ml) was incubated for 5 min with different concentrations (50, 5, and 0.5 µM) of antidepressants and with standard concentrations of labeled mediators in an incubation medium of the composition described previously [2] at 37°C with constant mixing. The final volume of the sample was 1 ml. The concentration of neurotransmitters in the sample was: $8 \cdot 10^{-8}$ M ^{3}H-serotonin, $2.5 \cdot 10^{-8}$ M ^{3}H-dopamine, and $4.4 \cdot 10^{-6}$ M ^{3}H-GABA. The reaction was stopped by filtration of 100 µl of the incubation mixture on Millipore filters (pore diameter 0.45 µ) followed by washing with 2 ml of incubation medium. The filters were dried at room temperature and radioactivity of the samples was measured in Bray's scintillator on an SL-4000 liquid scintillation counter (Intertechnique, France). Protein was determined by Lowry's method [14].

Reagents. ^{3}H-serotonin-creatinine sulfate (specific activity 12.3 ci/mmole), ^{3}H-dopamine (40 Ci/mmole), and ^{3}H-GABA (16 Ci/mmole) were obtained from Amersham International (England); imipramine was from West Germany, desmethylimipramine from East Germany, mianserin from Sweden, viloxazine and its isomers from the Clinical-Pharmaceutical Research Institute, Bulgaria, and befuraline and maclobamide were resynthesized by the Research Institute of Pharmacology, Academy of Medical Sciences of the USSR, Moscow. All reagents were of the analytical grade.

EXPERIMENTAL RESULTS

The authors showed previously that activity of neurotransmitter reuptake systems changes substantially after chronic stress; the greatest changes in its activity (373% compared with the control — 100%) affected the system of serotonin reuptake (Table 1) [2]. Data on the

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TABLE 1. Effect of Antidepressants on Neurotransmitter Reuptake by Synaptosomes of Control Rats (M ± m)

Experimental conditions	Concentration, μM	Serotonin	Dopamine	GABA
Control	—	100±19,0	100±8,5	100±8,2
Imipramine	50,0	9,3±0,9*	28,6±1,1*	47,4±3,9*
	5,0	39,4±4,0*	55,4±4,9*	82,2±4,8
	0,5	58,4±4,5*	83,9±4,8	—
Desmethylimipramine	50,0	10,9±1,8*	20,3±0,5*	52,2±4,1*
	5,0	43,3±3,9*	48,7±4,2*	84,8±5,8
	0,5	70,6±4,9*	81,2±4,8*	—
Mianserin	50,0	13,5±4,8*	30,8±2,8*	52,1±3,9*
	5,0	49,4±3,9*	73,5±4,1*	84,3±5,6
	0,5	80,2±4,1	91,4±5,8	—
Viloxazine (+)	50,0	31,9±3,2*	43,5±2,8*	82,4±5,5*
	5,0	66,4±4,8	20,2±4,1*	—
	0,5	94,0±6,2	92,1±4,14	—
Viloxazine (−)	50,0	43,1±3,4*	70,0±3,1*	87,8±5,3
	5,0	86,5±5,1	96,4±5,8	—
	0,5	95,1±3,1	—	—
Viloxazine (±)	50,0	38,2±3,1*	61,4±3,7*	95,5±6,0
	5,0	77,3±4,4	87,4±3,9	—
Befuraline	50,0	55,4±3,1*	38,8±3,3*	73,5±5,1*
	5,0	79,5±3,9	73,3±5,1	—
Maclobamide	50,0	94,8±5,1	91,4±5,9	9,02±5,9

Legend. Here and in Table 2, * indicates statistically significant difference from control ($p = 0.05$). M ± m) Concentration of neurotransmitter in synaptosomes as a percent of control, taken as 100: for ^{3}H-serotonin 100% = (11 ± 2.1)•10^{-12} M, for ^{3}H-dopamine (20 ± 1.7)•10^{-12} M, and for GABA (28 ± 2.3)•10^{-9} M. Results of nine experiments are shown.

TABLE 2. Effect of Antidepressants on Neurotransmitter Reuptake by Synaptosomes of Rats Exposed to Chronic Stress (M ± m)

Experimental conditions	Concentration, μM	Serotonin	Dopamine	GABA
Control	—	100,0±19,0	100,0±8,5	100,0±8,2
Stress	—	374,3±37,3	130,0±9,5	43,0±11,0
Imipramine	50,0	30,6±6,39	33,7±6,4*	68,5±3,6*
	5,0	150,7±10,8*	68,1±5,7*	112,8±7,0*
	0,5	206,3±10,1*	108,9±6,6*	—
Desmethylimipramine	50,0	37,3±10,8*	25,0±1,8*	77,5±4,9*
	5,0	168,2±13,1*	65,4±6,6*	116,1±7,7*
	0,5	230,3±12,3*	108,3±6,6*	—
Mianserin	50,0	55,2±9,0*	41,3±2,0*	81,4±5,4*
	5,0	180,2±11,2*	99,8±3,8*	122,1±6,1*
	0,5	291,3±12,3*	114,4±9,1	—
Viloxazine (+)	50,0	100,3±8,2*	58,2±2,7*	120,7±6,1
	5,0	243,6±15,3*	88,7±4,0*	—
	0,5	347,3±19,0	109,6±4,3*	—
Viloxazine (−)	50,0	169,0±7,8*	89,3±3,6*	126,8±5,6
	5,0	331,2±15,7	131,0±3,8	—
	0,5	355,1±26,9	—	—
Viloxazine (±)	50,0	135,4±11,6*	78,7±4,0*	126,1±6,0
	5,0	284,2±12,7*	116,7±4,9	—
	0,5	369,3±15,7	—	—
Befuraline	50,0	211,9±9,3*	44,9±2,5*	107,0±7,0*
	5,0	306,6±22,8*	101,5±5,1*	124,7±8,3
	0,5	351,0±15,3	124,9±6,6	—
Maclobamide	50,0	370,8±17,9	129,4±6,4	125,6±7,0

effect of the drugs on reuptake of the neurotransmitters studied are given in Tables 1 and 2. Activity of the drugs in this test on synaptosomes of the control animals (Table 1) agrees with data in the literature [11, 12, 15]. In a concentration of 5•10^{-5} M all the antidepressants studied except maclobamide significantly inhibited serotonin and dopamine

reuptake by the synaptosomes; tricyclic antidepressants of the imipramine group, moreover, exhibited the strongest activity. Only imipramine, desmethylimipramine, and mianserin, in a concentration of $5 \cdot 10^{-5}$ M, inhibited GABA reuptake.

It will be clear from Table 2 that activity of the drugs increased significantly when synaptosomes from rats exposed to chronic cytogenic stress were investigated. For instance, virtually all the drugs except maclobamide, in a concentration of $5 \cdot 10^{-5}$ M, inhibited serotonin reuptake about 4 times more intensively than in the control experiment. Activity of the antidepressants increased in proportion to the changes taking place in that particular neurotransmitter system. On average the coefficient of increase of activity of the drugs amounted to 3.7 for the serotonin reuptake system, 1.3 for the dopamine reuptake system, and 1.4 for the GABA reuptake system. This suggests that changes in the activity of the drugs in this test were not directly connected with their direct effect on the active centers of the monoamine carriers (in agreement with data obtained by the authors previously for the noncompetitive character of inhibition of serotonin reuptake by imipramine [3]), but it reflects changes introduced by the drugs into the membrane as a whole.

More and more data to show that drugs exert their influence predominantly on membrane lipids are being published in the literature [1, 4]. In turn, the state of the lipid bilayer of membranes substantially modulates activity of Na,K-ATPase [10], adenylate cyclase [9, 13], and cytochrome P-450 [7], and it affects the degree of aggregation of membrane proteins [6] and activity of the neurotransmitter reuptake systems at the synaptosomal level [3]. The most likely target for the antidepressants studied at the membrane level is thus the lipid bilayer of the presynaptic membranes, confirming the suggestions put forward by the authors previously [3].

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