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The Behavioral Effects of NMDA Antagonists in Serotonin Depleted Rats

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PŁAŹNIK, A., M. JESSA, M. NAZAR. *The behavioral effects of NMDA antagonists in serotonin depleted rats.* PHARMACOL BIOCHEM BEHAV **58**(1) 159–166, 1997.—The influence of serotonin (5-HT) depletion (5,7-dihydroxytryptamine, 5,7-DHT, 250.0 μg, ICV), on behavioral effects of non-competitive (MK-801) and competitive (CGP 37849) NMDA antagonists, was examined in rats. 5,7-DHT induced very potent and long lasting decrease in the 5-HT concentration in the brainstem and limbic forebrain. One week after 5,7-DHT administration, dopamine metabolism was found enhanced in the brainstem. The lesion did not change rat baseline motor and exploratory activity, but it significantly disinhibited animals' behavior suppressed by shock, in the Vogel test. Serotonin depletion revealed locomotor stimulating effect of MK-801, administered IP at the doses of 0.05 and 0.2 mg/kg. However, no change in striatal dopamine metabolism was detected in rats injected with the same dose of MK-801 (0.2 mg/kg), and examined one week after serotonergic denervation. Serotonergic lesions antagonized both enhancements of exploratory behavior, and motor suppression produced by the dose of 1.0 and 10.0 mg/kg of CGP 37849, respectively. Thus, 5,7-DHT-induced lesions influenced in a complex way the effects of NMDA antagonists. It is reasoned, that enhancement of motor stimulating effects of MK-801 in neurotoxin pretreated animals, reflects synergistic disinhibition of activity of dopaminergic neurons by MK-801 and serotonin depletion. On the other hand, antagonism of CGP 37849-caused motor depression can be explained by the lowering influence of 5,7-DHT on serotonin content. It is known that the release of serotonin is strongly stimulated by higher doses of CGP 37849, and takes part in the expression of some symptoms of the serotonin-like syndrome, including motor disturbances. © 1997 Elsevier Science Inc.

5,7-DHT MK-801 CGP 37849 Dopamine turnover Locomotor activity Exploration Vogel's test Rats

THERE is an emerging evidence that the interaction between excitatory amino acids (EAA) and serotonin (5-HT) may be important for the control of many brain activities. Raphe nuclei are densely innervated by excitatory amino acids afferents (1,13,34). Both, *N*-methyl-D-aspartate (NMDA) and NMDA receptor antagonists have been found to release 5-HT and to increase its turnover in some brain structures, including the dorsal and median raphe nuclei (2,19,26,36). On the other hand, serotonin depletion with selective neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT), enhanced ^{3}H-glutamate binding to quisqualate-insensitive receptors two weeks after the lesion in all hippocampal layers (23). In functional studies microinjections of NMDA antagonists AP-7 (DL-2-amino-7-phosphonoheptanoic acid) and MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d] cyclohepten-5,10-imine hydrogen maleate) to the median raphe nucleus led to NMDA reversible reinforcement of hippocampal theta activity, reminiscent of a functional lesion of this brain structure (15). Interestingly, similar effect could be observed after median raphe injections of 5-HT_{1A} agonists, selectively inhibiting activity of serotonergic neurons (14). These findings served to construct a model, according to which descending EAA fibers innervating the median raphe are thought to exert a facilitatory influence on GABAergic median raphe interneurons inhibiting, in turn, serotonergic median raphe neurons (15).

Some behavioral effects of non-competitive NMDA receptor antagonist MK-801, are reversed by 5-HT_{1A} receptor antagonist and partial agonist (18). The 5-HT_{1A} receptor agonists reduce also behavioral syndrome caused by intrathecal administration of NMDA (24). Many electrophysiological studies have shown that serotonin exerts a modulatory effect on glutamatergic transmission, depending on brain region studied and

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serotonin receptor subtype involved (8,16,21,25). Thus, there is evidence for both potentiation and depression of glutamate responses by 5-HT in different parts of the brain. Moreover, it appears that the 5-HT system and EAA play important role in a wide range of behaviors including motility and avoidance of fear-evoking stimuli (30,33). However, evidence for 5-HT versus EAA interaction in these behavioral models of brain activity is still unavailable.

The present report examined the effects of non-competitive (MK-801) and competitive (CGP 37849, DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid) NMDA antagonists on motor activity and fear controlled behaviors, in serotonin depleted animals.

METHOD

Animals and Housing

Male Wistar rats (200 ± 20 g) obtained from the licensed breeder were used in the study. After the operation, the animals were housed individually under a 12 h light/dark cycle (lights on at 0600) in a controlled temperature (21 ± 2°C), and humidity, with free access to food and water. Rats used in the Vogel conflict test, shock threshold or baseline drinking experiments, were deprived of water daily for 23 h, during four days preceding the test session. Each experimental and control group consisted of 7–8 animals.

Drugs and Treatments

The following drugs were used in the experiments: MK-801 [(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5, 10-imine hydrogen maleate, Research Biochemicals Inc., USA], CGP 37849 [DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid, CIBA-GEIGY, Switzerland]. For behavioral assays control and lesioned rats were intraperitoneally injected with drugs or vehicle (distilled water). Solutions of drugs were prepared immediately before testing, in a volume of 0.2 ml/100 g. Testing commenced 30 min (MK-801) or 60 min (CGP 37849) after drug treatment.

5,7-DHT Lesions

Animals were pretreated with intraperitoneal injections of desipramine (Sigma, USA) at the dose of 25 mg/kg, sixty min before intracerebroventricular administration of 5,7-dihydroxytryptamine creatinine sulfate (Sigma, USA), to prevent uptake of 5,7-DHT into the noradrenergic terminals. The rats were operated in a stereotaxic apparatus under diethyl ether anesthesia. Lesioned animals received unilateral intra-ventricular infusion of 5,7-DHT (250.0 μg of the free base in 10 μl 0.9% NaCl solution containing 0.2% ascorbic acid) through a Hamilton syringe [coordinates: A-1.2 mm to bregma, V-3.5 mm, L-1.5 mm; according to the atlas of Pellegrino et al. (28)]. Control animals received an equivalent infusion of vehicle. The neurotoxin was administered over a two min period of time. Seven days later, sham-operated and lesioned rats were tested in the open field test. Twenty one days after lesions randomly divided animals were subjected to behavioral testing in the Vogel's conflict procedure. Each 5,7-DHT-lesioned rat received only one drug treatment.

Open Field Test

The open field apparatus used in this experiment consisted of two round arenas (80 cm diameter) with 30 cm high walls, each equipped symmetrically with three photocells. Test was performed in a soundproof chamber under dim light and white noise conditions (75 dB) without previous adaptation. General activity (number of photobeam interruptions) was scored automatically by cumulative recorder for 10 min. Animals were also observed for 10 min from an adjacent chamber by an experimenter via closed-circuit television. The number of entries into the central part of the testing arena was also recorded (this parameter was defined as a movement of an animal from the wall to the central area over a distance of approximately 15 cm). All experiments were performed between 1100 and 1500.

Vogel's Conflict Test

A modified Vogel's drinking procedure was used. Apparatus consisted of 4 plastic boxes (30 × 30 × 60 cm) equipped with grid floor made of stainless steel rods. A water drinking tube was mounted to the wall of a cage, and an electric shock generator was connected with grid floor and the wire embedded in glass drinking tube. During the first two days of four days training, animals were deprived of water 23 h daily. Two subsequent days consisted of a pretest, when animals with sustained 23 h/24 h deprivation cycle were placed for 15 min in the test apparatus with no electric shocks delivered. Subsequently, the rats were allowed to drink water for 45 min in their home cages. After four days of training, drinking levels for all animals were usually stabilized (animals drinking less than 5 ml of water during 15 min long session were not taken into consideration). During the experimental procedure animals were placed into the apparatus and electric shocks were delivered in cycles of five seconds with four second intervals. Shock current was set at 0.4 mA. The amount of water consumption punished by shocks during 15 min of test session was recorded and taken as a measure of conflict behavior.

Baseline Drinking Test

The animals were deprived of water and treated in the same manner as those examined in the Vogel conflict test. The amount of water (ml) consumed spontaneously during a 15 min long session (without electric shock) was measured, after administration of a dose of the drug active in the Vogel test. The time between drug injection and testing was the same as in the Vogel test.

Shock Threshold Test (Flinch-Jump Test)

The rats were deprived of water prior to the test as in the Vogel procedure. The experiment was performed in the Vogel test cages and shocks were delivered to the grid floor in 0.75-s pulses. After 3 min of habituation to the test box, shock titration was continued upward or downward in a stepwise manner (0.05mA, 0.05–0.85 mA range) depending upon responsiveness of the animal. The time between drug administration and testing was the same as in the Vogel test.

Biochemical Analysis

Biochemical analysis of changes in the levels of noradrenaline, dopamine, serotonin, 5-hydroxyindoloacetic acid (5-HIAA) and homovanillic acid (HVA) was performed in separate groups of animals, with a fully automated HPLC system (Shimadzu, Japan), using standard biochemical methods as described previously (33). The animals prepared and treated as described above were not subjected to the behavioral testing. Seven or 21 days after 5,7-DHT lesion, animals were sacrificed and the brains dissected according to Glowin-

ski and Iversen, with some modifications (7), into the limbic forebrain and brainstem, and kept at −80°C for the time of analysis. The brainstem consisted of the mesencephalon and part of the pons, with the caudal borderline at the level of the anterior edge of the fourth ventricle. In another biochemical experiment, seven days after lesion and 30 min after intraperitoneal MK-801 injection rats were decapitated and the striatum was excised for further analysis.

Histological Analysis

All animals, with exception of those used in biochemical analysis, were sacrificed after the final testing day. The brains were removed, stored in 5% formaldehyde solution and verified histologically. The frozen tissue was dissected in the slices and the place of injection examined with a magnifying glass. Only those animals with verified ICV injection sites were included in the study.

Statistical Analysis

The data are shown as mean ± SEM. Behavioral results were analyzed with a one-way or two-way ANOVA, where appropriate, followed by Newman–Keuls test. For neurochemical measurements statistical comparisons between individual control and drug-treated groups were done by Student's *t*-test. In the case of an interactive biochemical experiment with MK-801 and 5,7-DHT-induced lesions, a two-way ANOVA was used. The quotients 5-HIAA/5-HT and HVA/DA used for estimation of 5-HT and DA turnover, were calculated for each rat, and evaluated statistically by means of Student's *t*-test, by individual comparison of each treated group with its own control group. The confidence limit of $p < 0.05$ was considered as statistically significant.

RESULTS

5,7-DHT caused long lasting, selective and a very potent decrease in the forebrain and brainstem concentrations of serotonin and 5-HIAA (Table 1). There was a tendency for these effects to be even more stronger three weeks after the lesion (Table 1). One-way analysis of variance revealed a significant overall treatment effect, 5-HT $F(3, 32) = 30.00$, $p < 0.001$; 5-HIAA $F(3, 32) = 47.32$, $p < 0.001$. In the brainstem, where both dopaminergic and serotonergic nuclei are localized, a significant enhancement of turnover of dopamine and serotonin, measured by metabolite to monoamine ratio, was found one week after neurotoxin administration (Table 1). This effect, as far as serotonin turnover was considered, was no longer present two weeks later (Table 1). In the striatum, but not in the forebrain and the brainstem, there appeared also a significant decrease in the concentration of noradrenaline, one week after the lesion (Table 2). MK-801 at the dose of 0.2 mg/kg, IP, neither changed the concentrations of monoamines, their metabolites, nor 5,7-DHT-induced alterations in the 5-HT and 5-HIAA, in the rat striatum (Table 2). Two-way analysis of variance showed that there was a significant main effect of 5,7-DHT on 5-HT, $F(1, 22) = 108.08$, $p < 0.001$, and 5-HIAA levels, $F(1, 22) = 49.94$, $p < 0.001$. However, no significant effect of MK-801, [5-HT, $F(1, 22) = 0.47$, $p < 0.49$; 5-HIAA, $F(1, 22) = 0.59$, $p < 0.44$], nor MK-801 × 5,7-DHT interaction, [5-HT, $F(1, 22) = 0.54$, $p < 0.46$; 5-HIAA, $F(1, 22) = 2.99$, $p < 0.09$], was found.

5,7-DHT pretreatment did not change spontaneous water intake, $F(1, 28) = 3.55$, $p < 0.069$, and pain threshold, $F(1, 28) = 0.23$, $p < 0.51$ (Table 3). Analysis of variance revealed an overall significant effect of shock on drinking in the Vogel test, $F(1, 28) = 82.08$, $p < 0.001$ (Fig. 1). The clear-cut tendency to disinhibit punished drinking after serotonergic lesion, though not always reaching statistically significant level, was repeatedly found in the present experiment (Figs. 1 and 2). Thus, in some experiments serotonergic denervation caused a significant disinhibition of conflict behavior suppressed by shock in the Vogel test (Fig. 1), whereas motility, exploratory activity, spontaneous drinking, and pain threshold, remained unchanged (Tables 3 and 4). Accordingly, a highly significant and negative correlation was obtained for 5-HT and 5-HIAA levels in the limbic forebrain, and punished drinking in the Vogel test (5-HT, $n = 32$, $r = -0.49$, $p < 0.01$; 5-HIAA, $n = 32$, $r = -0.5$, $p < 0.01$). 5,7-DHT-induced serotonergic lesions revealed the stimulatory influence of MK-801, administered at the dose of 0.05 mg/kg, $F(2, 21) = 8.32$, $p < 0.002$, and 0.2 mg/kg, $F(2, 21) = 8.84$, $p < 0.0017$, IP, on locomotor activity (Table 4). After the dose of 0.2 mg/kg, a parallel increase in the number of central entries in the open field, was recorded, $F(2, 21) = 8.24$, $p < 0.002$, (Table 4). In the Vogel test, MK-801 tested at two dose levels: 0.001 and 0.1 m/kg, IP, did not modify the effects of a shock or serotonin depletion on punished drinking (Fig. 2). Moreover, a two-way analysis of variance indicated that no significant effect of 5,7-DHT × MK-801 (0.1 mg/kg) interaction was present, $F(1, 25) = 0.25$, $p < 0.61$. In this part of the experiment MK-801 was injected at the subthreshold doses, selected on the basis of our previous experiment (30), not active when given alone in both models of anxiety. CGP 37849 injected IP at the dose of 1.0 mg/kg, significantly increased the number of central entries in the open field; this effect was antagonized by 5,7-DHT-induced serotonergic lesions (Table 4). One-way analysis of variance revealed a significant overall treatment effect, $F(2, 21) = 7.69$, $p < 0.003$. Interestingly, CGP 37849 at the highest examined dose of 10.0 mg/kg, IP, potently depressed motility and exploration (Table 4). These animals expressed also flat body posture and abduction of hind limbs. Both recorded behavioral effects were no longer present in the serotonin depleted animals (Table 4). Also in this case one-way analysis of variance showed significant main treatment effect on motility, $F(2, 21) = 4.97$, $p < 0.01$, and exploration, $F(2, 21) = 7.08$, $p < 0.004$. In the Vogel test, from all the examined groups, only 5,7-DHT-pretreated and CGP 37849 administered (0.3 mg/kg, IP) rats showed a significant increase in punished drinking, in comparison with shocked animals (Fig. 3). Two-way analysis of variance revealed that there was a significant main effect of CGP 37849, $F(1, 28) = 10.61$, $p < 0.002$. However, no significant effect of CGP 37849 × 5,7-DHT interaction was found, $F(1, 28) = 0.087$, $p < 0.76$.

DISCUSSION

5,7-dihydroxytryptamine induced a potent and long lasting depletion of 5-HT in the brain. However, selectivity of the effect of this neurotoxin, in spite of a pretreatment of animals with noradrenaline uptake blocker, was not complete. 5,7-DHT caused also a significant decrease in noradrenaline contents in the rat striatum. Concentrations of noradrenaline remained unchanged in the forebrain and in the brainstem. This finding indicates, that the data referring exclusively to changes in the whole brain or forebrain levels of amines after 5,7-DHT are not sufficient, and the influence of the neurotoxin in subcortical structures should be considered as well. The differences in local sensitivity to the 5,7-DHT-neurotoxic action are possible. However, it is unlikely that the decrease

TABLE 1

THE EFFECT OF 5,7-DHT ON THE CONCENTRATIONS OF MONOAMINES, HVA, 5-HIAA, HVA/DA, AND 5-HIAA/5-HT RATIOS, IN THE BRAINSTEM AND LIMBIC FOREBRAIN, 7 AND 21 DAYS AFTER THE LESION

	Brainstem			Limbic Forebrain		
	Sham	5,7-DHT	%	Sham	5,7-DHT	%
7 Days after lesion						
NA	594.3 ± 23.6	599.6 ± 27.2	101	258.1 ± 13.1	223.3 ± 9.8	87
DA	164.0 ± 16.1	168.8 ± 15.9	103	200.5 ± 48.3	201.9 ± 66.7	101
HVA	43.2 ± 5.5	134.9 ± 9.3*	312	147.8 ± 53.7	114.3 ± 18.9	77
HVA/DA	0.28 ± 0.04	0.82 ± 0.04*		1.08 ± 0.4	1.04 ± 0.3	
5-HT	405.3 ± 63.6	62.5 ± 4.1*	15	234.4 ± 16.3	59.4 ± 6.5*	25
5-HIAA	350.8 ± 42.6	121.0 ± 12.8*	34	240.4 ± 17.6	41.4 ± 13.1*	17
5-HIAA/5-HT	0.93 ± 0.08	1.93 ± 0.15*		1.03 ± 0.08	0.75 ± 0.23	
21 Days after lesion						
NA	314.2 ± 30.6	322.5 ± 37.2	102	239.9 ± 33.7	240.8 ± 68.4	100
DA	101.1 ± 17.7	82.9 ± 14.6	82	163.3 ± 25.2	148.5 ± 46.5	91
HVA	ND	ND		ND	ND	
5-HT	487.3 ± 79.7	55.4 ± 12.9*	11	298.1 ± 40.0	49.5 ± 15.6*	17
5-HIAA	469.8 ± 61.4	56.2 ± 13.9*	12	232.3 ± 26.5	31.6 ± 11.1*	14
5-HIAA/5-HT	1.05 ± 0.08	1.25 ± 0.26		0.79 ± 0.04	0.96 ± 0.3	

Data are expressed in ng/g of wet tissue, and shown as mean ± SEM. The number of rats in each experimental group was 7. ND = not detectable; % = refers to appropriate sham lesioned group; * = differs from sham group.

*$p < 0.01$.

in noradrenaline level in the striatum after 5,7-DHT might account for disinhibition of rat behavior in the Vogel test (VT), as a highly significant and negative correlation was obtained for 5-HT and 5-HIAA concentrations in the limbic forebrain and punished drinking. The significant, or close to significance level, drop of noradrenaline contents in some brain areas after 5,7-DHT, administered in a comparable dose-range (200–250 μg), and in spite of an appropriate protection of noradrenergic neurons, has been also observed by other authors (5,31). The temporary changes in 5-HT metabolism, observed one week after the lesion, most probably reflected compensatory enhancement of 5-HT turnover in the remaining intact 5-HT neurons, until new equilibrium of pre- versus post-synaptic activity was established three weeks later.

5,7-DHT-induced lesions disinhibiting animal behavior in the Vogel test, did not change rat motility, neophobic reaction, pain threshold or spontaneous water intake. It has been hypothesized, that the anxiolytic-like effect observed in the conflict test consequent on severe depletion of brain serotonin by means of 5,7-DHT, is indirect and probably involves the $GABA_A$ chloride-ionophore receptor complex (32). On the other hand, enhancement of the locomotor effect of psychostimulants in the 5,7-DHT lesioned animals, has been suggested to stem from disinhibition of central catecholaminergic neurons (6,20). Indeed, the 5-HT lesion was found to enhance motility and locomotor stimulating effect of *d*-amphetamine (3,6,17,20). This phenomenon may be subsequent to compensatory overactivity of catecholaminergic neurons disinhibited by the lesion, and adaptive receptor processes occurring post-synaptically in dopaminergic synapses. Accordingly, serotonin was found to decrease activity of tyrosine hydroxylase in the striatum, a step-limiting factor in the formation of catechola-

TABLE 2

THE EFFECT OF MK-801 (0.2 MG/KG) ADMINISTERED TO 5,7-DHT-LESIONED ANIMALS, ON THE CONCENTRATIONS OF MONOAMINES, HVA, 5-HIAA, HVA/DA, AND 5-HIAA/5-HT RATIOS, IN THE RAT STRIATUM, MEASURED 7 DAYS AFTER THE LESION, AND 30 MINUTES AFTER MK-801 INJECTION

	Sham	(+)-MK-801	%	5,7-DHT	%	(+)-MK-801/5,7-DHT	%
NA	446.8 ± 27.3	349.2 ± 57.0	78	223.7 ± 15.5*	50	294.3 ± 15.6*	66
DA	7682.5 ± 220.7	8043.0 ± 342.6	105	6910.1 ± 394.9	90	7454.7 ± 467.2	97
HVA	821.4 ± 65.1	911.0 ± 47.4	110	862.2 ± 31.1	105	879.2 ± 29.1	107
HVA/DA	0.11 ± 0.07	0.12 ± 0.07		0.1 ± 0.06		0.12 ± 0.05	
5-HT	279.0 ± 26.4	193.2 ± 38.5	70	29.7 ± 8.0*	11	17.2 ± 7.7*	6
5-HIAA	275.5 ± 25.7	194.2 ± 41.9	70	32.1 ± 6.4*	12	30.3 ± 5.7*	11
5-HIAA/5-HT	0.99 ± 0.05	0.99 ± 0.05		1.1 ± 0.06		1.78 ± 1.77	

Data are expressed in ng/g of wet tissue, and shown as mean ± SEM. The number of rats in each experimental group was 7. % = refers to appropriate sham lesioned group; * = differs from sham group.

*$p < 0.01$.

TABLE 3

THE EFFECT OF 5,7-DHT ADMINISTRATION ON SPONTANEOUS, BASELINE DRINKING, AND PAIN THRESHOLD, MEASURED 3 WEEKS AFTER THE LESION, I.E. AT THE TIME OF THE VOGEL TEST

Treaatment	Dose	Spontaneous drinking (ml)	Pain-flinch (mA)
Sham	—	4.9 ± 0.6	0.23 ± 0.01
5,7-DHT	250 μg	5.3 ± 0.4	0.24 ± 0.01

Data are shown as mean ± SEM. The number of rats in each experimental group was 8.

mines (12). It is conceivable, that reduction of serotonin activity by neurotoxin leads to the opposite effect, i.e. to the stimulation of dopamine synthesis.

Some above considerations may be directly referred to the present data. The motor effect of MK-801, non-competitive NMDA receptor antagonist, was potentiated in 5-HT depleted animals. This drug and phencyclidine have a psychostimulant-like profile of action, probably following indirect enhancement of dopaminergic neurons, via blockade of an inhibitory NMDA tone. Indeed, some data indicate augmentation by MK-801 of dopamine metabolism in several brain structures, including the nucleus accumbens septi (NAS) and striatum (19,35). This mechanism, along with 5,7-DHT-induced disinhibition of dopaminergic neurons (see above), might account for stronger stimulation of motility in the group of MK-801 administered and neurotoxin pretreated rats. Accordingly, as the brainstem HVA and dopamine turnover were significantly increased by serotonergic lesions at the time of behavioral testing, such conclusion is proved by the present data. However, the effect of 5,7-DHT on dopamine metabolism was observed in the brainstem area only, it dissipated in time, and did not occur in the striatum. MK-801 did not also significantly change dopamine and 5-HT metabolism in the same brain area. It is possible, that alterations in dopamine turnover in other brain structures (e.g. the nucleus accumbens), might better correlate with the behavioral outcome of the treatment

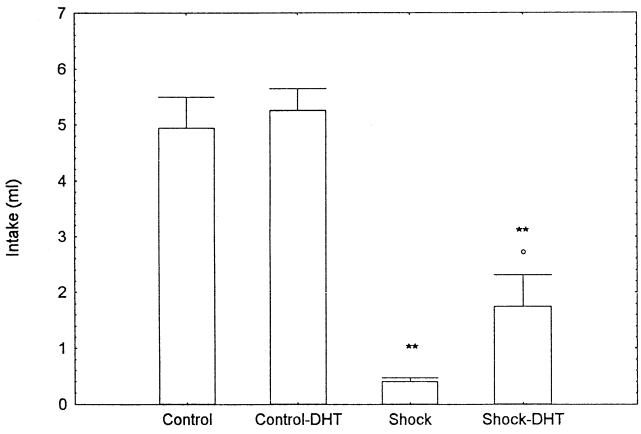

FIG. 1. The effect of 5,7-DHT-induced depletion of serotonin, on rat behavior in the Vogel test, studied 21 days after the lesion. Data are shown as mean ± SEM. Ordinate = the amount of water drunk in ml. The number of rats in each experimental group was 8. * = differs from control, non-shocked rats; ° = differs from shocked rats. ° = $p < 0.05$; ** = $p < 0.01$.

with MK-801. The problem of involvement of dopamine system in the effects of the non-competitive NMDA antagonist is still the matter of controversy. Review of available data indicates surprisingly high number of reports negating such interaction. The doses of MK-801 up to 1.0 mg/kg were found to not affect dopamine metabolism in the rat striatum (9,10). MK-801 suppressed also NMDA-evoked striatal dopamine release (26). A tentative explanation of these disparate findings may refer to differences in the local effects of MK-801 in brain structures. For example, MK-801 (0.3 mg/kg IP) was reported to enhance dopamine metabolism in the nucleus accumbens, medulla oblongata, hypothalamus, but not in the frontal cortex, amygdala, thalamus, hippocampus and pons (19). Importantly, the mucleus accumbens is suggested to play a substantial role in the control of motor activity, and mediation of locomotion stimulating effects of psychostimulants (4).

The blockade of NMDA receptors by MK-801, and depletion of 5-HT, does not seem to interact in changing rat behavior in the VT, as no modification of punished drinking occurred after administration of a subthreshold dose of this drug (0.001 mg/kg), to neurotoxin pretreated animals. Previously, it was found that MK-801 injected in doses as small as 0.005 mg/kg and 0.01 mg/kg, significantly and selectively counteracted the influence of a shock on drinking (30). This effect was no longer present after the dose of 0.05 mg/kg, when locomotor stimulating properties of the drug evolved. It is possible, that the weak influence of MK-801 in the VT could be hindered by the stronger behavioral disinhibition induced by the 5,7-DHT lesion alone. However, the lack of alteration in 5-HT metabolism after MK-801, observed in this and previously published reports, indicates that 5-HT may not be involved in the anti-conflict effect of non-competitive NMDA antagonists (9,10). There are also many other conflicting data concerning the influence of MK-801 on brain serotonergic system. MK-801 was found to block NMDA-induced monoamine release in the rat striatum (26). The drug at a dose of 0.3 mg/kg, did not affect 5-HT turnover in the hippocampus, amygdala, hypothalamus and pons/medulla (19). Moreover, MK-801 given to the median raphe nucleus caused an apparent reduction of the median raphe neurons activity, assessed in the electrophysiological experiment (15). Thus, the involvement of 5-HT system in the effects of NMDA antagonist on fear controlled behavior appears, in contrast to regulation of motor activity, complex and indirect. Finally, it can be concluded that the anti-anxiety-like effect of 5-HT depletion depends more on changes in the activity of $GABA_A$ receptor complex (32), than modification of NMDA receptor function.

Serotonergic lesions affected heterogeneously the effects of competitive NMDA antagonist CGP 37849, in both examined behavioral tests. It is noteworthy, that the doses of NMDA antagonist used in this part of the study, were selected on the basis of our previous experiment (30). On the one hand, serotonin depletion seemed to slightly potentiate the anticonflict action of a dose of 0.3 mg/kg of CGP 37849, in the VT. On the other hand, the lesion attenuated exploratory activity, stimulated by the dose of 1.0 mg/kg, and reversed locomotor suppression, caused by the dose of 10.0 mg/kg of CGP 376849. CGP 37849 was previously found to selectively disinhibit rat behavior in the VT in a dose-dependent way, with the dose of 0.3 mg/kg being marginally effective (30). Importantly, augmentation of punished drinking was not accompanied by alterations in motor activity, pain threshold or spontaneous water intake. It was suggested, that the anxiety-reducing effect is a more general feature of all classes of negative modulators of NMDA receptor complex. It seems that the hyperpolarizing

TABLE 4

THE EFFECT OF MK-801 AND CGP 37849 ON RAT BEHAVIOUR IN THE OPEN FIELD TEST, IN THE 5,7-DHT LESIONED RATS

Treatment	Dose	Locomotor activity	Number of central entries
Sham	—	60.5 ± 11.9	3.1 ± 0.8
5,7-DHT	250 μg	67.1 ± 12.9	5.4 ± 1.2
Sham	—	97.8 ± 6.4	7.6 ± 1.1
(+)-MK-801	0.05 mg/kg	70.5 ± 8.1	5.1 ± 0.9
(+)-MK-801/5,7-DHT		135.3 ± 16.7*‡	8.0 ± 2.9
Sham	—	68.6 ± 5.9	2.9 ± 0.5
(+)-MK-801	0.2 mg/kg	130.1 ± 12.1	2.4 ± 0.9
(+)-MK-801/5,7-DHT		215.9 ± 41.2**†	9.4 ± 2.2**‡
Sham	—	64.9 ± 7.9	4.3 ± 0.9
CGP 37849	1 mg/kg	76.8 ± 8.4	8.6 ± 1.1**
CGP 37849/5,7-DHT		56.0 ± 4.4	3.5 ± 1.0‡
Sham	—	77.4 ± 11.1	4.3 ± 0.9
CGP 37849	10 mg/kg	26.6 ± 5.4*	1.0 ± 0.3**
CGP 37849/5,7-DHT		66.5 ± 16.4†	4.1 ± 0.7‡

Data are shown as mean ± SEM. The number of rats in each experimental group was 8. * = differs from sham, control group; † = differs from drug treated animals; *† = $p < 0.05$; **‡ = $p < 0.01$.

effect of NMDA antagonists on neuronal membranes in the brain limbic structures (e.g., hippocampus), and evolving blockade of neuronal transmission, may be the common and the core mechanism of action of also other groups of anxiolytic compounds [e.g., benzodiazepines, barbiturates, 5-HT_{1A} receptor agonists (29)].

Several studies have shown modulatory action of serotonin on the responses to excitatory amino acids, in the brain. Both suppressive and enhancing effects on the excitation of neurons have been reported (cf. 24). More interestingly, two weeks after 5,7-DHT lesion ^{3}H-glutamate binding was increased in all the hippocampal layers (23), what made the authors to conclude that 5-HT is a direct positive modulator of glutamate receptor subtype. If it is the case, serotonergic lesion-induced hypofunction of hippocampal presynaptic NMDA innervation, might add to the NMDA receptor blockade caused by CGP 37849. Such mechanism should result in a stronger inhibition of neural transmission in the brain structure considered valid for execution of fear-related central processes. This, in turn, could lead to augmentation of the effect of CGP 37849 in the serotonin depleted animals. However, this explanation remains speculative and hypothetical as the influence of 5-HT depletion on the effects of CGP 37849 in the Vogel test appeared only marginally statistically significant.

Changes in the open field effects of CGP 37849, in the serotonin depleted animals, are not easy to interpret. In agreement with our previous report (30), the dose of 1.0 mg/kg of CGP 38849 significantly enhanced rat exploration, an effect regarded as reflecting the anti-neophobic-like reaction of the rats. This phenomenon was selectively suppressed by serotonergic lesions, independently from alterations in rat motor activity. Conceivably, neophobic and conflict behaviors undergo different neural processes. It is pointed out, for example, by the present results showing distinct profiles of behavioral changes in the Vogel and the open field tests, after 5,7-DHT lesions. The sensitivity of both anxiety-modelling reactions to benzodiazepines, serotonergic drugs and NMDA antagonists is also dissimilar (30,33). Thus, having in mind complex cooperation between brain 5-HT and NMDA systems in controlling neuronal processes (see above), the present results may be considered as preliminary, indicating distinct roles of NMDA versus 5-HT interaction, in regulating differently evoked fear-related responses.

The highest administered dose of CGP 37849 (10.0 mg/kg) caused potent motor suppression. The rats exhibited also flat body posture and hind limb abduction. These effects of CGP 37849 were no longer present in 5,7-DHT pretreated animals. In some respects CGP 37849-induced symptoms resembled the 5-HT behavioral syndrome. Moreover, they were quantitatively similar to the behavioral action of the highly selective serotonin releasing agent 5-methoxy-6-methyl-2-aminoindan (22). Accordingly, in biochemical experiments the higher dose of 30.0 mg/kg of CGP 37849 was found to strongly enhance serotonin release and metabolism, in most of the brain regions studied, including the nucleus accumbens and striatum (19). Thus, it is possible that former depletion of brain 5-HT reduces behavioral symptoms produced by higher doses of CGP 37849, most probably related to the release of 5-HT. This could be the mechanism of a disinhibitory effect of 5,7-DHT lesion on CGP 37849-induced locomotor suppression.

Finally, the different behavioral profiles of MK-801 vs. CGP 37849 in serotonin depleted animals should be once more underlined. This may be consequent on the less selective, predominantly motility stimulating, and dopamine oriented profile of action of non-competitive NMDA receptor antagonist. Non-competitive but not competitive NMDA antagonists potently stimulate, at the dose range used in the present experiment, activity of central dopaminergic neurons, enhance glu-

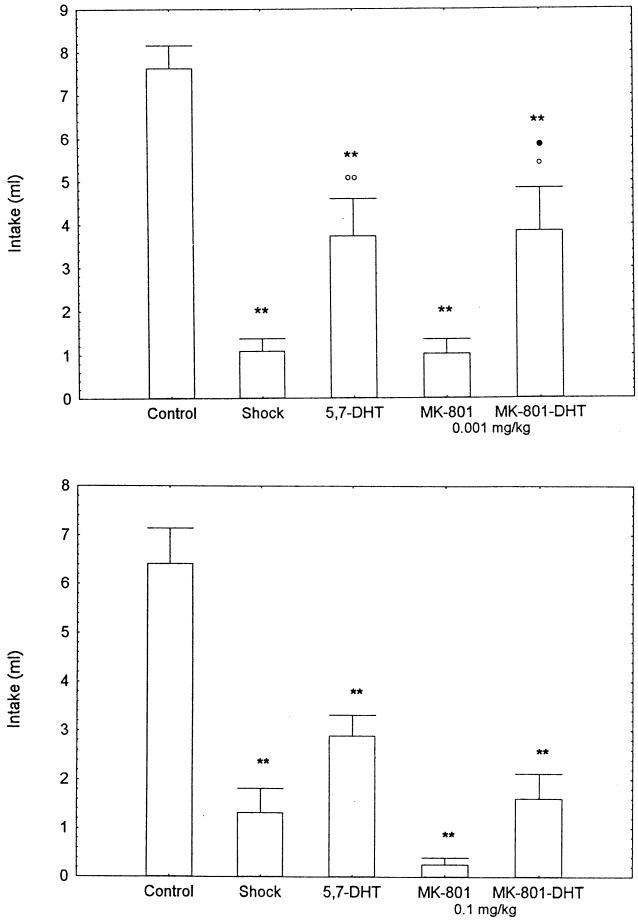

FIG. 2. The effect of MK-801 in the Vogel test, after 5,7-DHT pretreatment. * = differs from drug treated animals. * = $p < 0.05$, ** = $p < 0.01$. For other explanations see Fig. 1.

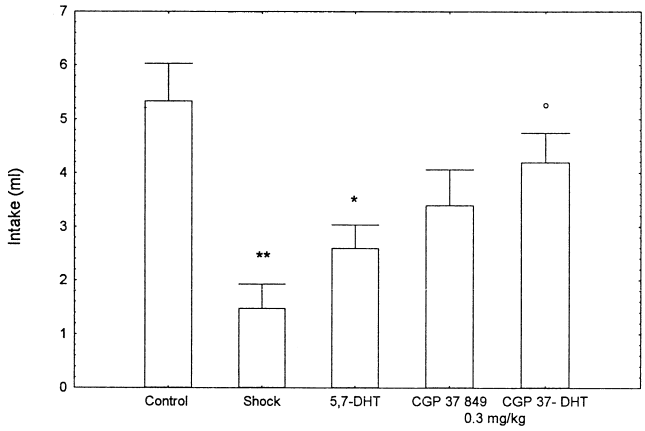

FIG. 3. The effect of CGP 37849 in the Vogel test, after 5,7-DHT pretreatment. * = $p < 0.05$. For other explanations see Fig. 1.

cose utilization in the limbic structures, and attenuate the prepulse-induced inhibition of the acoustic startle response (11,35). Moreover, recently a role of sigma receptors in the psychotomimetic effects of another NMDA receptor channel blocker, phencyclidine, was suggested (27). All in all, these data along with the present results indicate differences, due to either direct or indirect mechanisms, in the central processes called into play by competitive and non-competitive NMDA receptor antagonists.

In conclusion, the present data prove complex interaction between brain 5-HT and NMDA systems. Serotonergic denervation enhanced motor effects of MK-801, and reversed locomotor suppression caused by the higher dose of CGP 37849. These findings indicate that brain 5-HT significantly contributes to the central effects of non-competitive and competitive NMDA receptor antagonists. Such corollary, though preliminary, may be also important for understanding clinical profiles of NMDA antagonists, studied as the possible way of treatment of epilepsy and neurodegenerative diseases. Undoubtedly, the question of 5-HT versus NMDA interaction deserves further studies.

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