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BRIEF COMMUNICATION

Metaphit Fails to Antagonize PCP-Induced Passive Avoidance Deficit

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DANYSZ, W. *Metaphit fails to antagonize PCP-induced passive avoidance deficit.* PHARMACOL BIOCHEM BEHAV **38**(1) 231–233, 1991.—Pretreatment with metaphit (1-[1-(3-isothiocyanotophenyl)cyclohexyl]piperidine), a putative irreversible antagonist of phencyclidine (PCP) receptors, did not antagonize PCP-induced passive avoidance deficit in rats, and did not decrease [^{3}H]MK-801 (5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate) binding to PCP recognition sites coupled to NMDA receptors. The effectiveness of the metaphit treatment was evidenced by the occurrence of audiogenic seizures. These results suggest that previously reported antagonism in vivo actions of PCP by metaphit, is mediated by sites not involved in PCP-induced passive avoidance deficit, and not related to the NMDA receptor complex in brain structures studied (striatum, hippocampus, and cortex).

Metaphit PCP MK-801 Learning Passive avoidance Binding

PHENCYCLIDINE (PCP), a major drug of abuse, produces psychotropic effects that resemble some of the symptoms of schizophrenia. The psychomimetic actions of PCP may result in part from its noncompetitive blockade of ionic channel coupled to glutamatergic receptors of the N-methyl-D-aspartate (NMDA) type (1,22). [This site is called PCP site, in contrast to the sigma site (previously termed sigma-opioid), which is unrelated to NMDA receptor complex, but is sensitive to haloperidol (13).] Endogenous ligands for this site may be involved in schizophrenia (14). Hence, extensive efforts have been made to search for a PCP antagonist, as putative antidotum in PCP intoxication and as a possible treatment of schizophrenia.

Recently, 1-[1-(3-isothiocyanotophenyl)cyclohexyl]piperidine (Metaphit) has been shown to decrease irreversibly the number of PCP binding sites in vitro (15) and to antagonize some behavioral effects of PCP in rats (6). However, it is not clear whether antagonism of PCP by metaphit results from interactions with the NMDA receptor complex or with other sites possibly involved in the psychomimetic effects of PCP, e.g., the dopamine carrier (2). Hence, the present paper was aimed at testing whether metaphit antagonizes PCP-induced passive avoidance deficit, an effect that seems to be related primarily to antagonist activity at the NMDA receptor complex. The binding of [^{3}H](+)5-methyl-10,11-dihydro-5H-dibenzo-cyclohepten-5,10-imine maleate (MK-801), the ligand for PCP site coupled to NMDA receptors, was used to examine possible changes in receptor number and/or affinity.

METHOD

Subjects and Treatment

Male Wistar rats (180–240 g) were used in the experiments. Animals anesthetized with pentobarbital (40 mg/kg) were implanted with polyethylene cannulas aimed at the lateral ventricle (L = 1.0, H = 4.5 from the surface of the skull and AP at bregma). After surgery animals were housed in single cages. After 3–4 days rats were injected ICV with 1 μmol of metaphit (in 5 μl) or with saline. The rats were used 24 hours later either for behavioral testing or for binding experiments. Brain samples from 6 rats were pooled to obtain the required protein concentration.

Binding Assay

Synaptic membranes were prepared as described previously (8). After centrifugation membranes were frozen for 24 hours. On the next day membranes were thawed at room temperature in 30 volumes of 5 mM Tris-HCl buffer (pH = 7.4 at 22°C), which was subsequently used for the binding assay. Then the membranes were homogenized (Polytron) and incubated for 20 min in 37°C. Then membranes were centrifuged for 20 min at 48,000 × g. The pellet was resuspended in the buffer by homogenization. The washing procedure was repeated 5 times. The final pellet was resuspended in a buffer to obtain a final protein concentration between 0.4 and 1.2 mg/ml. Incubations were started by adding [^{3}H]MK-801 (29.4 Ci/mmol, 1 nM) with various concentrations

TABLE 1

EFFECT OF IN VIVO PRETREATMENT WITH METAPHIT (1 μmol IN 5 μl, LATERAL VENTRICLE) ON [^{3}H]MK-801 BINDING IN MEMBRANES PREPARED FROM STRIATUM, HIPPOCAMPUS AND CORTEX 24 HOURS LATER

	Striatum		Hippocampus		Cortex	
	K_d	B_{max}	K_d	B_{max}	K_d	B_{max}
Control	14.1 ± 1.0	1.8 ± 0.52	12.8 ± 0.49	3.5 ± 0.12	10.4 ± 1.53	2.1 ± 0.15
Metaphit	14.7 ± 3.1	2.1 ± 0.42	13.1 ± 1.7	3.3 ± 0.43	9.3 ± 2.27	2.1 ± 0.40

K_d values are expressed in nM and B_{max} in pmol/mg protein. Results are mean ± SE of three Scatchard plots (10 points each) derived from 3 separate experiments.

of cold MK-801, and continued at room temperature for 3 h in a total volume of 0.5 ml. One μM glycine and glutamate were included in the incubation medium to accelerate the association rate. Incubations were terminated by filtration through Whatman GF/C glass fiber filters followed by 3 washes with 5 ml of buffer.

Passive Avoidance Test

The passive avoidance apparatus consisted of two identical chambers, one of which was lighted. The rat was placed in the lighted compartment 15 minutes after PCP (2 mg/kg) or saline administration. Then the door separating both chambers was lifted. As soon as a rat entered the dark compartment the door was closed and foot shock was applied (2.5 s, 0.3 mA); thereafter, the rat was returned to its home cage. Twenty-four hours later the rat was placed again in the lighted compartment and the latency to enter the dark compartment was measured, and served as a measure of retention of the avoidance response. Cut off time was 180 s.

Chemicals

MK-801 was obtained from RBI, [^{3}H]MK-801 from New England Nuclear, phencyclidine (PCP) and metaphit were generously supplied by Prof. Alan Kozikowski from the University of Pittsburgh. All other chemicals were obtained from Sigma.

RESULTS

Most of rats treated with metaphit exhibited audiogenic clonic convulsions and/or escape reaction (wild running) in response to any noise in the room, e.g., knocking at the cage. This reaction appeared as soon as 4 hours after metaphit injection. Within 12 hours of the treatment with metaphit about 20% mortality was observed. The death was proceeded by clonic-tonic convulsions.

Treatment with metaphit failed to affect either the B_{max} or the K_d value of [^{3}H]MK-801 binding in the hippocampus, cortex and striatum (Table 1). The binding revealed single site saturable characteristics.

Injection of PCP before the passive avoidance training session produced remarkable performance deficits, which were not affected by pretreatment with metaphit (Fig. 1).

DISCUSSION

Metaphit has been initially shown to decrease irreversibly the number of PCP binding sites in vitro (15). Similar observations has been made after in vivo treatment with metaphit but the decrease was smaller (25%), (7). However, in all cases either [^{3}H]PCP or its derivative [^{3}H]TCP (1-[1-(2-thienyl)cyclohexyl] piperidine) have been used. Both ligands do not bind exclusively to PCP sites coupled to NMDA receptors but may interact with sigma sites (13, 16, 21), the dopamine carrier (4), or other entities (5). In the present study metaphit failed to affect the binding of [^{3}H]MK-801, the most selective ligand for PCP receptors. Metaphit decreases the binding of [^{3}H]1,3-di-2-tolylguanidine (DTG) (3) to sigma receptors as well as the binding of cocaine or other ligands to the dopamine carrier (23). Thus, the discrepancy between present data and previous reports may result from the selectivity differences among the ligands used.

In agreement with previous reports (10), in the present study some rats showed audiogenic seizures, with appearance identical to behavioral effects of ventricularly administered NMDA (8). It may be then assumed that in those rats NMDA receptor complex was overactivated.

It has been demonstrated that metaphit antagonizes some of the actions of PCP, such as ataxia and stereotypy (7), electrophysiological responses of cerebellar Purkinje cells (20), and an increased 2-deoxyglucose utilization in the rat brain (19).

It is suggested that metaphit probably does not act as a pure antagonist, but behaves as a partial agonist at PCP sites. Indeed, this compound may produce or potentiate effects of PCP such as electrophysiological responses in the spinal cord preparation (9), inhibition of acetylcholine release in slices (18), and catalepsy (11).

Antagonism of certain behavioral effects of PCP by metaphit may result from the interaction with sites not related to the NMDA

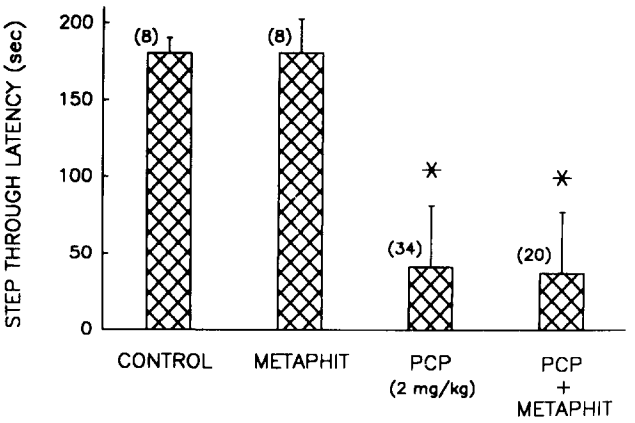

FIG. 1. Effect of pretreatment with metaphit (1 μmol in 5 μl, lateral ventricle) on amnesic action of PCP (2 mg/kg IP, 15 min before training) in passive avoidance test. Results are expressed as medians (± semi-interquartile range) step through latency (s) during retention testing 24 hours after the training. Numbers in parentheses indicate number of animals per group. *$p>0.05$ in respect to control group (Mann-Whitney U-test).

receptor complex. It has been reported that metaphit inhibits the locomotor effects of cocaine, but not those of PCP or amphetamine (17). Metaphit antagonizes head-twitching and the binding to 5-HT-2 receptors, and decreases back pedalling, which is characteristic for 5-HT-1 receptor stimulation (12).

It has been proposed that the amnesic actions of PCP may result from its antagonist activity at the NMDA receptor complex (8). In the present study metaphit did not antagonize PCP-induced amnesia and did not affect the binding of [^{3}H]MK-801 to the PCP site. This evidence indicates that in vivo treatment with metaphit may not antagonize actions of PCP related to the blockade of the channels coupled to NMDA receptors, and does not change the number of PCP binding sites in structures such as striatum, hippocampus, and cortex. Since metaphit has been shown to antagonize PCP-induced ataxia and stereotypy (7), but did not affect PCP mediated passive avoidance deficit, it may be suggested that these behavioral actions of PCP are not related.

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