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Phencyclidine-Like Effect of Cyclazocine on Pentylentetrazol-Induced Seizures in Laboratory Animals

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SAGRATELLA, S., T. NIGLIO, E. ORTOLANI, A. SCOTTI DE CAROLIS AND A. PÉZZOLA. *Phencyclidine-like effect of cyclazocine on pentylentetrazol-induced seizures in laboratory animals.* PHARMACOL BIOCHEM BEHAV 22(4) 515–519, 1985.—The present work deals with an EEG and behavioural study of the effect of cyclazocine against the convulsions due to pentylentetrazol (PTZ) in mice, rats and rabbits. In rats, cyclazocine, at the high doses (15–25 mg/kg) prevents the tonic motor convulsions and EEG epileptiform "grand mal" seizure induced by PTZ. In rabbits and mice, cyclazocine inhibits the tonic motor convulsions without modifying either the spike-frequency or the duration of the PTZ-induced EEG seizures. Naloxone, even at high doses, was not able to block the anticonvulsive effects of cyclazocine on PTZ-induced convulsions in the rat. The effects of cyclazocine were compared to those of phencyclidine. These results confirm the multiple behavioural effects of cyclazocine and support the idea that both cyclazocine and phencyclidine, may act on the PCP/sigma receptor identified in binding studies.

Cyclazocine PTZ-seizures Naloxone PCP/sigma receptor

RECENT studies have shown some similarities between the phamacological properties of dissociative anesthetics (phencyclidine, ketamine) and those of psychotomimetic benzomorphans, cyclazocine, N-allynormetazocine indicated as sigma opiate receptor agonists [13]. Phencyclidine (PCP), cyclazocine and N-allynormetazocine (SKF 10,047) induce similar hallucinogenic effects in man and similar abnormal behaviour in dogs [10] and they are all effective in prolonging the latency of flurothyl-induced seizures in the rat [7,8]. Cyclazocine and PCP also block the long-term potentiation of synaptic transmission in rat hippocampal slide [15].

Furthermore the above mentioned psychotomimetic benzomorphans (cyclazocine and SKF 10,047) but not other opioids such as morphine and endogenous peptides can compete at low concentrations for [^{3}H] PCP binding [9]; while Itzhak *et al.* [10] demonstrated that PCP displaces [^{3}H] N-allylnormetazocine binding to rat membrane. It has been, thus, suggested that cyclazocine SKF 10,047 and PCP share a common site of action [19,20].

Previous investigations carried out in this laboratory [14] have shown that PCP antagonizes the convulsions induced by pentylentetrazol (PTZ) in rats and rabbits. A study was, therefore, undertaken on the effects of cyclazocine against the motor seizures and EEG convulsions due to PTZ in mice, rats and rabbits, in order to verify whether cyclazocine and phencyclidine exert a similar antagonistic effect towards the PTZ-induced convulsions.

METHOD

Electroencephalographic recordings were obtained from 26 Wistar male rats and 17 Swiss mice of either sex bearing chronically implanted cortical electrodes. In rats and in mice, under pentobarbital anesthesia (35–40 mg/kg, IP) four electrodes were permanently fixed with dental cement to the skull over the bilateral sensorimotor and optic cerebral cortices. EEG and behavioral observations started 5–6 days after implantation. Fifteen male rabbits were prepared for the acute EEG experiments under local anesthesia with 2% xylocaine. Six screw electrodes were fixed over the sensorimotor and optic cerebral cortices, two concentric deep electrodes were placed into the dorsal hippocampus and into the thalamus (n. ventralis anterior) according to a technique described previously [12]. Rabbits were placed on a restraining table, while rats and mice were put into a round Plexiglas container (diameter 35 cm). All animals were connected through long wires to an EEG Galileo apparatus (Model E 10b polygraph).

Drugs were dissolved with saline and injected intravenously (IV) in rabbits and intraperitoneally (IP) in rats and mice. Drug doses refer to the weight of the base.

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Data have been statistically analized with nonparametric tests (Mann-Whitney U-test; exact Fisher test).

RESULTS

Effects of PTZ

Rabbit. PTZ (20 mg/kg) was administered IV to six animals. This dose induced within 30–40 seconds a "grand mal" ictal seizure consisting of continuous high voltage (0.3–0.6 mV) and high frequency (20 c/sec) spikes lasting about 60 sec. Concomitantly the animals showed a tonic extension of the head and of the hindlimbs. This tonic phase was followed by a clonic phase with repeated violent movements of the head and limbs characterized on the EEG by a progressive slowing of the frequency of the spikes. Then a characteristic isoelectric pattern occurred, which later was followed by trains of spike-and-wave complexes. The electrical silence and the spike-waves were associated with the postictal behavioral depression and lack of response to stimuli.

Rats. PTZ was administered IP at the dose of 50 mg/kg to six rats. Two minutes after drug administration all animals exhibited a "grand-mal" ictal seizure made up of continuous high voltage spikes (20–25 c/sec) lasting 30–40 seconds, concomitantly the rats showed tonic extension of the hindlimbs and head, followed by clonic movements. In the postictal period lasting 6–7 minutes, the EEG was flattened with intermingled slow waves (2–3 c/sec) and spikes, which were correlated to jerks of the head and forelimbs. Later, spike-and-waves appeared and persisted for about one hour (50–60 min).

Mice. PTZ was administered at the dose of 75 mg/kg to 4 mice. Thirty-forty seconds after drug administration all the animals showed a "grand mal" ictal seizure made up of continuous high voltage spikes (20–25 c/sec) lasting 20–30 seconds, concomitantly mice showed tonic extension of the hindlimbs followed by clonic movements. In the postictal period lasting 5–6 minutes the EEG was flattened with isolated spikes, which were correlated to jerks of the head and forelimbs. Later spike-and-wave complexes appeared and persisted for 30–60 minutes.

Effects of Cyclazocine on the EEG and Behaviour of Rabbits, Rats and Mice

The influence of various doses of cyclazocine was studied in a separate series of experiments carried out in 5 rabbits, 6 rats and 6 mice.

Cyclazocine was administered in rabbits at the doses varying from 0.05 to 5 mg/kg. One–two minutes after administration of cyclazocine at the lower doses (up to 1 mg/kg), rabbits presented head nodding and slight ataxia. Doses from 1 to 3 mg/kg induced ataxia and catatonia, doses of 4 and 5 mg/kg provoked also reduction of the muscle tonus. Doses lower than 0.05 mg/kg did not affect gross behaviour.

Rats treated with 10, 15 and 25 mg/kg of cyclazocine IP showed, within ten minutes after administration, increase of locomotor activity, stereotyped behavior such as circling movements and head swinging followed by totter gait and ataxia. In addition to these effects, a reduction of muscle tonus was observed in most of animals at the highest dose of cyclazocine.

Mice treated at the doses varying from 10 to 25 mg/kg IP showed within few minutes after administration ataxia and catatonia.

In all animals the behavioral manifestations were accompanied at the higher doses by EEG changes consisting of low voltage fast activity at 20–30 c/sec, with scattered 3–4 c/sec waves in the sensorimotor and optic areas. The EEG response to external stimulation was absent. Cyclazocine also induced in rabbits the disruption of the hippocampal theta waves which were replaced by fast, low voltage activity. In rat after the administration of the highest dose (25 mg/kg) of cyclazocine scattered spikes appeared in the tracing and were intermingled with 3–4 c/sec waves in the sensorimotor and optic areas.

Influence of Cyclazocine on the Motor and EEG Effects Induced by PTZ

The influence of cyclazocine pretreatment on the convulsions due to PTZ was studied in 10 rabbits, 20 rats and 13 mice. In rabbits, PTZ (20 mg/kg,IV) was administered 15–20 minutes after treatment with cyclazocine at the doses varying from 0.05 to 5.0 mg/kg, IV.

Cyclazocine was able to prevent the appearance of tonic convulsions in all rabbits treated with PTZ, but the typical EEG tracing which is observed during the tonic phase of convulsions (and consisting of high voltage, high frequency spikes) nevertheless was observed. An apparent dissociation between the EEG and behavioural effects therefore occurred.

The animals showed jerking movements of head and limbs accompanied by the typical EEG "grand mal" pattern without any significant reduction in latency of seizure onset and in spike-frequency (20 c/sec) and in duration of the EEG ictal "grand mal" seizure (Table 1).

In rats, pretreatment with cyclazocine (10–25 mg/kg) was able to significantly prevent not only the tonic motor convulsions, but also the EEG "grand mal" pattern elicited by PTZ (50 mg/kg) in 65% of the animals (see Table 1). In these animals twitching of the ears and jerking movements of the head were accompanied by continuous trains of spike-and-wave complexes (3–5 c/sec) intermingled with isolated high voltage spikes. This pattern lasted for 80–90 min. The remaining 35% of animals, presented the EEG "grand mal" without any reduction of the intensity and duration of the tonic and clonic phases of convulsions (see Table 1).

In mice, PTZ (75 mg/kg, IP) was administered 15–20 minutes after treatment of cyclazocine at the doses ranging from 10 to 25 mg/kg, IP. Cyclazocine, at the doses of 15–25 mg/kg, was able to completely block the tonic motor convulsions due to PTZ in 10/10 animals. These animals showed the EEG "grand mal" pattern without any reduction of the duration and of the spike-frequency (20–25 c/sec) of the ictal seizure (Table 1). These seizures were associated only with tremors and movements of the head. The lower dose (10 mg/kg) of cyclazocine was less effective in preventing PTZ-convulsions: only 2 out of 3 animals treated with PTZ did not show the motor tonic phase of the attack. Cyclazocine in mice was also able to increase significantly the latency to the first convulsion induced by PTZ in a dose related way (Table 1).

In order to further investigate the nature of the antagonistic effect of cyclazocine towards the convulsions due to PTZ, additional experiments with naloxone were carried out in 4 rats. Naloxone (10 mg/kg) injected 5 min after PTZ had no effect on the anticonvulsant action of cyclazocine against PTZ. Naloxone up to 10 mg/kg injected 10 min before PTZ had no effect on antagonistic influence of cyclazocine towards PTZ convulsions.

TABLE 1

Animal Species	Cyclazocine (or Saline) mg/kg	PTZ mg/kg	Latency in sec of Seizures Onset*	Duration in sec of E.E.G. "Grand Mal"*	Animals With E.E.G. "Grand Mal"	Animals With Tonic Convulsions	Animals With Clonic Convulsions
Rabbit treated IV	(SAL)	20	27 (±12)	68.4 (± 6.6)	6/6	6/6	6/6
	2.5	20	32 (± 7.99)	52 (± 8.44)	5/5	0/5§	5/5
Rat treated IP	(SAL)	50	110 (± 6.45)	29.17 (±11.9)	6/6	6/6	6/6
	10	50	116 (±34.8)	45 (± 8.67)	3/4	3/4	4/4
	15	50	—	—	2/5¶	2/5¶	5/5
	20	50	—	—	0/5§	0/5§	5/5
	25	50	—	—	2/6§	2/6§	6/6
Mouse treated IP	(SAL)	75	30.5 (±13)	22 (±11)	4/4	4/4	4/4
	10	75	63 (± 2.89)	15.33 (± 6.57)	3/3	1/3¶	3/3
	15	75	110.6 (±26.8)†	16 (± 5.04)	3/3	0/3§	3/3
	25	75	113.86 (±16.75)‡	23.14 (± 5.3)	7/7	0/7§	7/7

*Values are means ± S.E. Mean values are evaluated only for the doses that are able to induce E.E.G. "Grand Mal" seizures in at least 50% of treated animals.
†Differs from PTZ control; $p<0.028$; U Test.
‡Differs from PTZ control; $p<0.01$; U Test.
§Differs from PTZ control; $p<0.03$; F Test.
¶Differs from PTZ control; $p<0.05$; F Test.

DISCUSSION

PTZ elicits an EEG "grand mal" epileptiform pattern consisting of: (1) ictal seizure period with high frequency spikes; (2) postictal depression period with flattening of the EEG; (3) postictal period with trains of intermittent spike-and-wave complexes.

Our experiments show that the anticonvulsive effect of cyclazocine on PTZ-induced seizures in rats is very similar to the one previously described for PCP [14].

In rats, PCP and cyclazocine, at the same doses eliciting similar behavioral effects (i.e., stereotypies, circling, swinging) prevent the tonic motor convulsions elicited by PTZ. These drugs induced also the disappearance of the EEG "grand mal" pattern, which was substituted by trains of spike-and-wave complexes intermingled with high voltage spikes. In rabbits and in mice, cyclazocine prevents the tonic motor convulsions, but does not modify the PTZ induced EEG "grand mal" pattern in its typical series. An apparent dissociation between EEG and behavioral effects therefore occurs in mice and rabbits.

Other drugs not belonging to the classical anticonvulsant group, such as local anesthetics, spasmolitics, antihistamines, exhibit anticonvulsant effects under certain conditions; in fact they are particularly effective against the tonic component of the convulsions induced by maximal electroshock (MES), but do not influence the EEG epileptiform activity; for these drugs an action at the bulbo-, mesencephalic level has been hypothesized [11]. Thus, it cannot be excluded that the elimination of the tonic component of convulsion, unaccompanied by any modification of EEG "grand mal" pattern in mice and rabbits, under our experimental conditions, might be due to a selective depressive action at the level of areas implied in the genesis of the tonic phase of convulsions.

One of the most conflicting aspects of these results is to reconcile the anticonvulsant action of cyclazocine with the scattered spikes or spike-and-wave complexes elicited by the highest doses of the drug employed in the antagonistic action toward PTZ. It is interesting to report that also other authors [1] showed a strong anticonvulsant effect of cyclazocine in kindled amigdaloid rats in presence of scattered spikes elicited by the drug in the prestimulation EEG. Similar polispikes and slow wave complexes were elicited by PCP in rabbits [14] and by its related drug such as ketamine in cats [5].

Celesia *et al.* [5], describing the anticonvulsant effect of ketamine on penicillin epileptogenic hippocampal foci in cats, established the difficulty "to determine if these electroencephalographic changes elicited by highest doses of this drug were polispike-slow wave complexes rather than a mixture of delta and high voltage beta activity." The same authors reported, on the other hand, that not all spikes are

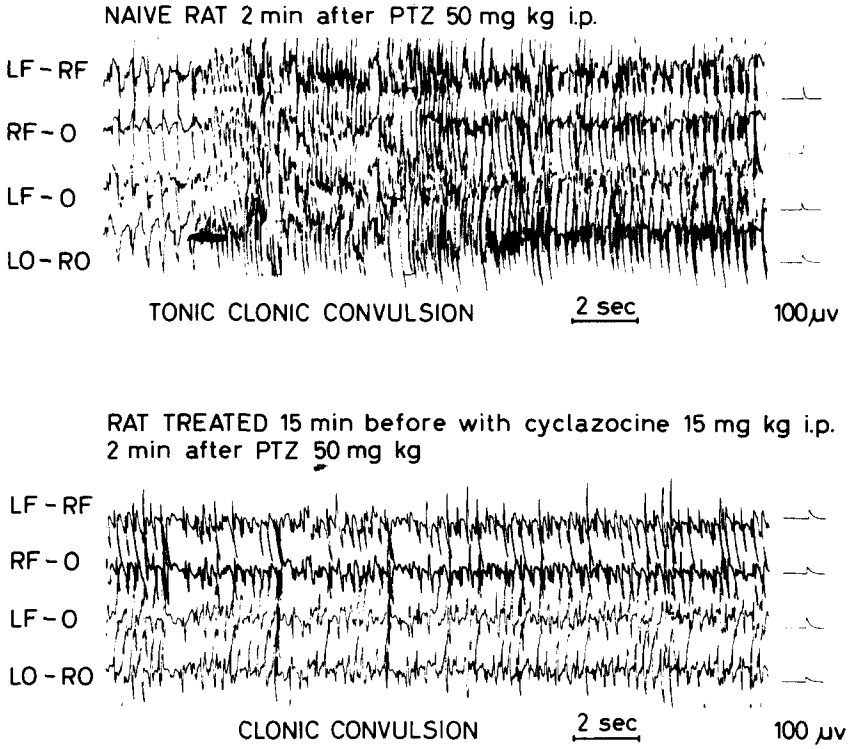

FIG. 1. Antagonistic effects of cyclazocine on the EEG and motor effects induced by PTZ in the rat. Upper record: the tonic-clonic convulsions induced by PTZ. Lower record: two minutes after the administration of PTZ in the rat pretreated with cyclazocine (15 mg/kg, IP). The usual "grand mal" seizures are replaced by continuous trains of spike-wave complexes intermingled with high voltage spikes. The motor manifestations consist only of localized jerking movements. Leads: F=anterior sensorimotor cortex; P=posterior sensorimotor cortex; O=optic cortex.

manifestations of epileptogenetic processes as lambda waves, vertex sharp waves, ponto-geniculate-occipital (PGO) waves which are examples of spikes representing normal physiological events.

On the contrary, Boweyer [3] reported that ketamine induced the reduction of after discharge (AD) on the amigdaloid kindled rats. This reduction was accompanied by an increase of AD spike frequency, and he therefore, hypothesized that, paradoxically, an underlying limbic excitation accelerates the mechanism which terminates the seizures.

It could be alternatively hypothesized that the underlying state of excitation is parallel to the depressive effect of the drug that could be responsible for the inhibitory action on tonic convulsions. This hypothesis on the byphasic action is supported by the reports of some authors: Chen *et al.* [6] described mixed effects of central nervous system excitation and depression after PCP and ketamine in various animal species; Tortella *et al.* [17] reported alternating periods of intermittent behavioral excitation and EEG desynchronization with behavioral stupor and EEG synchronization during the first hour after administration of 2.5 mg/kg of cyclazocine in rats.

Naloxone, even at high doses, did not block the anticonvulsive effects of cyclazocine (20–25 mg/kg) exerted in the rat; thus it appears, that the effect of cyclazocine on PTZ-induced convulsions does not involve a naloxone-sensitive opioid receptor, but might be mediated at the same site of PCP action. On the same line are the findings of others: Cowan *et al.* [7] reported that the anticonvulsant effect of cyclazocine on flurothyl-induced convulsions in rat was not blocked by naloxone (up to 10 mg/kg); Berman and Adler [2] reported that the inhibitory effect on tonic convulsion of MES in rats, was not blocked by naloxone (up to 10 mg/kg); Vaupel [18] described a variety of similar reflex, autonomic, and behavioural effects produced by N-allylmormetazocine (SKF 10,047) and PCP on the spinal dog, which were not prevented by naltrexone pretreatment (1 mg/kg).

(±)Cyclazocine, as well as all the psychotomimetic benzomorphans, are recemic mixture of two stereoisomers; only the (+)-isomer of SKF 10,047 competes for the binding sites labeled from [^{3}H]-PCP and, analogously, rats trained to discriminate PCP from saline, generalized to PCP the response induced by (+)-SKF 10,047 or its racemic mixture but not by the (−) isomer [16,20]. It is, thus, likely that multiple behavioral effect of benzomorphans (cyclazocine and SKF-10,047) are explained by their racemic nature: levo isomers have opioid effects, dextro isomers are responsible for the PCP-like naloxone-resistant effects, presumably exerted at the so-called sigma receptor [4].

On this basis we suggest that PCP-like, naloxone-resistant effect of (±)-cyclazocine in inhibiting PTZ-induced convulsions may really depend on the interaction of the (+)-isomer with the PCP/sigma receptor characterized in binding studies. Studies with the pure stereoisomers of (±)-cyclazocine, will help us in further verifying this possibility.

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