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A Possible Role of a GABAergic Mechanism in the Convulsant Action of RO5-4864

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RASTOGI, S. K. AND M. K. TICKU. *A possible role of a GABAergic mechanism in the convulsant action of RO5-4864.* PHARMACOL BIOCHEM BEHAV **23**(2) 285–288, 1985.—The purpose of the present investigation was to determine the possible role of GABAergic mechanism in the convulsant action of RO5-4864. Benzodiazepines (BZ) and other agents which facilitate central GABAergic transmission delayed the onset of facial and forelimb clonus, whereas tonic hind limb extension was blocked in a dose-dependent manner. RO5-4864-induced convulsions were blocked by diazepam, clonazepam, pentobarbital, ethanol and amino-oxyacetic acid (AOAA). RO5-4864-induced convulsions were not blocked by the BZ antagonist RO15-1788. Specifically, RO15-1788 caused a decrease in the onset of severity component of tonic seizures, which tended to become generalized and precipitated in a tonic extension of the hindlimbs. Further, subconvulsive doses of a direct GABA receptor antagonist, bicuculline, enhanced the proconvulsant action of RO5-4864, indicating thereby a potential antagonism of the central GABAergic transmission. These observations strongly suggest that RO5-4864 probably elicits convulsions by selective impairment of the GABAergic transmission.

GABAergic transmission Convulsants Receptor interactions Benzodiazepines RO5-4864 Barbiturates Picrotoxin site

A number of experimental studies have shown that benzodiazepines (BZ) and drugs which modulate GABAergic transmission are effective in antagonizing a variety of chemo-convulsants by interacting with one of the sites of the GABA-BZ ionophore complex [3,15]. RO5-4864, a 1,4 benzodiazepine, has been reported to possess several pharmacological effects, including sedative, anxiogenic, convulsant and anticonvulsant (for review, see [11]). Despite controversy regarding the mechanisms by which RO5-4864 produce these effects, it is known that BZs protect against RO5-4864 convulsions [6,18]. There is also evidence indicating that RO5-4864 has a behavioral profile similar to BZ and GABA antagonists [4,5]. Recent electrophysiological studies provided convincing data for a central site of action for RO5-4864 [9,17]. Furthermore, radioligand binding data from our laboratory have demonstrated that RO5-4864 competitively inhibits the binding of [^{35}S]t-butylbicyclophosphorothionate (TBPT) [16], a ligand which binds to a convulsant site present in the GABA receptor complex.

The present investigation was carried out to determine the mechanism of convulsant action of RO5-4864 by using drugs which modify GABA functions. An attempt was also made to assess the proconvulsant component in RO5-4864, by combining it with the chemo-convulsants which bring about seizures by inhibiting GABAergic transmission.

METHOD

Male Sprague-Dawley rats weighing 100–150 g, were used in the present study. They were maintained under identical conditions with free access to food and water. Individual rats, at least five in each group, were tested in a completely randomized schedule.

RO5-4864-Induced Seizures

The onset of initial clonic component of seizures produced by RO5-4864 were characterized by bursts of motor activity, facial twitching, piloerection, rearing and clonic forelimb convulsions. The appearance of myoclonic jerks or tonic hind limb extensions were recorded as the severity component of the seizures. Each animal was observed individually for the onset of clonic component, onset of severity component and total seizure latency (i.e., the duration between the first appearance of clonic or tonic component to a point when the animal died or recovered from convulsions in a 60 min test session). Protection against tonic seizures and extension of the hind limb was considered as a protective response of the drugs. The control group of animals were always tested at the beginning and at the end of each test session.

Initial dose-response studies demonstrated that RO5-4864 (10 mg/kg) produced forelimb convulsions for a shorter duration with no mortality, while higher doses (40 mg/kg) caused both tonic and clonic convulsions and mortality in 80% of rats.

For drug-interaction studies, drugs were injected intraperitoneally (IP) 30 min prior to the challenge dose of RO5-4864 (40 mg/kg). The animals were assumed protected if no convulsion had developed within 60 min after challenge of RO5-4864.

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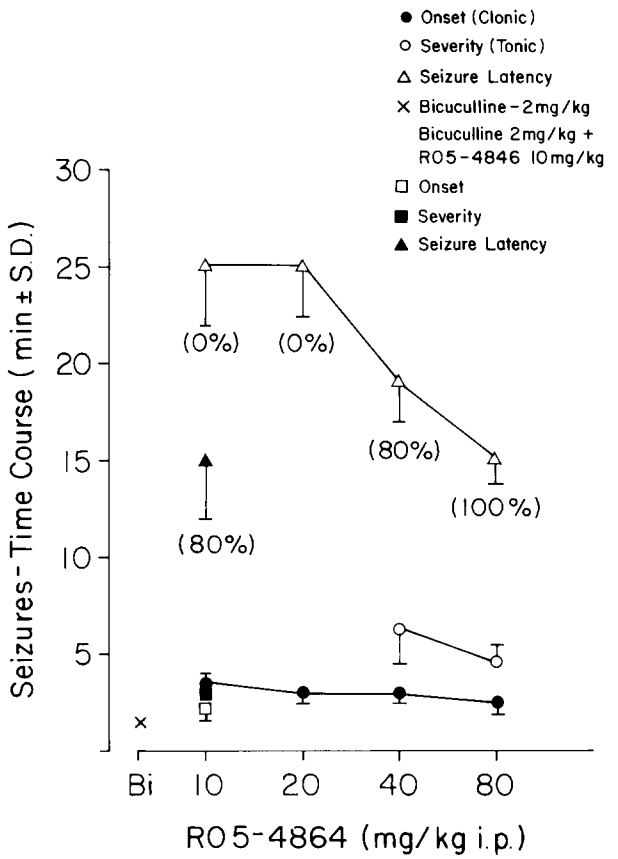

FIG. 1. Dose-response relationship of RO5-4864 alone or in combination with a single subconvulsive dose of bicuculline. Ordinate: Seizure time-course. Abscissa: Dose mg/kg. Each point is the mean±S.D. of five animals in each group. The onset (clonic seizure component ●), severity (tonic seizure component, ○) and total seizure latency △) were recorded in a 60 min test session at each dose level of RO5-4864. Interaction with bicuculline is represented by x=bicuculline alone (2 mg/kg); □=onset; ■=severity; and ▲=total seizure latency following simultaneous administration of RO5-4864 (10 mg/kg) and bicuculline (2 mg/kg). Values in parentheses represent the percentage mortality. (*Note:* Subconvulsive dose of bicuculline produced only facial twitching. However, in combination with RO5-4864 (a dose which produced only clonic seizures), it caused facilitation of each other's effects and resulted in both clonic and tonic seizures. The mortality of this dose-combination is equivalent to a dose of 40 mg/kg of RO5-4864.)

TABLE 1

THE PROTECTIVE EFFECTS OF DRUGS ON RO5-4864-INDUCED CONVULSIONS

Treatment (IP)	Convulsions (min ± S.D.) Onset (Clonic)	Severity (Tonic)	Total Seizure Duration
RO5-4864 + Vehicle			
40(mg/kg)	3.0 ± 0.7	6.5 ± 1.3	16.5 ± 4.2 (80)
Diazepam(mg/kg)			
0.5	6.8 ± 0.8§	*	35.0 ± 4.2 (10)§
2.0	9.8 ± 2.0§	*	30.0 ± 3.6 (0)§
4.0	5.2 ± 0.4†	*	26.0 ± 4.2 (0)†
8.0	*	*	*
Clonazepam (mg/kg)			
0.5	4.8 ± 0.4	*	13.0 ± 2.3 (0)
2.0	4.8 ± 0.4	*	17.0 ± 2.7 (0)
4.0	5.6 ± 0.5‡	*	9.2 ± 0.8 (0)
8.0	*	*	*
Pentobarbital (mg/kg)			
5.0	3.2 ± 0.4	6.8 ± 0.8	47.0 ± 5.7 (0)§
10.0	4.4 ± 1.1	*	39.0 ± 2.2 (0)§
20.0	4.6 ± 1.1	*	45.0 ± 3.6 (0)§
40.0	*	*	*
AOAA(mg/kg)			
10.0	2.6 ± 0.5	4.0 ± 0.7‡	12.0 ± 3.4 (100)
25.0	6.4 ± 3.0	*	24.0 ± 9.0 (40)
50.0	*	*	*
Ethanol(g/kg)			
1.0	3.6 ± 0.5	6.0 ± 1.0	41.0 ± 9.6 (100)§
2.0	4.6 ± 0.5	13.4 ± 1.3‡	54.0 ± 4.2 (10)§
4.0	9.2 ± 1.7§	*	19.0 ± 3.9 (0)
RO15-1788(mg/kg)			
20.0	2.2 ± 0.4	4.4 ± 0.5‡	30.0 ± 7.6 (100)§
40.0	2.4 ± 0.5	3.8 ± 0.4‡	19.0 ± 1.5 (100)
80.0	2.2 ± 0.4	3.6 ± 0.5‡	13.0 ± 1.9 (100)

RO5-4864 was injected 30 min (60 min AOAA) after drug treatment. Data are shown as means ± S.D. of 5 animals (in min). Values in ( ) represent % mortality; *indicates no convulsions; †$p<0.05$; ‡$p<0.01$; §$p<0.001$, compared to RO5-4864 (40 mg/kg) by the Student Newman-Keul's Procedure.

For proconvulsant study, a subconvulsive dose of bicuculline, picrotoxin or strychnine (dose with no convulsive effects) was combined simultaneously with RO5-4864 (10 mg/kg). The rats were observed for the occurrence of clonic and tonic convulsions.

Drug Administration

Drugs were obtained from the following sources: RO5-4864, RO15-1788, diazepam, clonazepam from Hoffman LaRoche, Inc. (Nutley, NJ), sodium pentobarbital, aminoxyacetic acid (AOAA), bicuculline, picrotoxin and strychnine from Sigma (St. Louis, MO).

RO5-4864, RO15-1788 and picrotoxin were dissolved in dimethylsulfoxide (DMSO), whereas diazepam and clonazepam were dissolved in propylene glycol. Ethanol was given as 50% w/v solution made of 95% ethanol and distilled water. Bicuculline was dissolved in 0.1 N HCl and adjusted to pH 5 with 0.1 NaOH. Drugs were injected IP 30 min (except AOAA, 60 min) prior to RO5-4864 challenge. Control animals treated either with saline or vehicle (DMSO or propylene glycol) were always run for a comparison with drug under test.

Data Analysis

Data were calculated for each drug dose or drug combination by one-way analysis of variance. The degree of significance from control was determined by the Student Newman-Keul's procedure.

RESULTS

RO5-4864-Induced Seizures

RO5-4864 produced seizures in a dose-dependent manner in rats. Lower doses (10–20 mg/kg) caused facial twitching, generalized bursts of hyperactivity with face and forelimbs in clonus with no mortality. At higher doses (40 mg/kg), RO5-4864, in addition, produced myoclonic jerks and tonic seizures and extension of hind limbs. Figure 1 shows that this dose also resulted in 80% mortality in rats. Doses higher than 80 mg/kg caused early onset of forelimb convulsions, followed by violent clonic and tonic convulsions of hind limbs and decreased the total seizure latency (i.e., the duration between the first onset of clonic convulsions to a point of recovery from convulsions or death of the animal), and mortality of all the animals.

Effect of Drugs on RO5-4864-Induced Seizures

Table 1 summarizes the effects of various drugs on the different components of RO5-4864-induced seizures in rats. In drug-interaction studies, 40 mg/kg of RO5-4864 (IP) was used as a challenge dose. Table 1 shows that all animals challenged with this dose of the drug had convulsions, irrespective of the vehicle pretreatment. Diazepam (0.5–4 mg/kg) delayed the onset of hyperactivity, facial twitching, piloerection and forelimbs seizures, while clonazepam was less effective in blocking the clonic component. Both diazepam and clonazepam, in equi-effective doses, blocked the myoclonic jerks and tonic seizures of hind limbs of RO5-4864. Seizure latency was significantly increased with diazepam, while clonazepam did not alter the seizure latency. Pentobarbital (10–40 mg/kg), like diazepam, blocked tonic component and enhanced the seizure latency. Administration of RO15-1788, 30 min prior to RO5-4864 challenge, was ineffective in antagonizing RO5-4864-induced convulsions; however, the onset of tonic seizures was significantly reduced. All the animals died with this treatment without affecting the duration of the seizures. These results indicate that RO15-1788 may possess proconvulsant action.

In the presence of AOAA (25 mg/kg), a GABAminergic agent, 60 min prior to RO5-4864, the onset of forelimb convulsions was delayed and the severity of tonic component in two of five animals tested was blocked. Table 1 also shows that at higher dose, AOAA (50 mg/kg) caused protection against either component of the seizure and mortality was significantly reduced. Similar modification in the convulsive pattern following ethanol was also observed (Table 1). At 2 g/kg, ethanol delayed the onset of tonic hindlimbs convulsions and reduced the mortality. However, at higher doses (4 g/kg), prominent facial and forelimb clonus was not reduced, while hindlimbs clonus and myoclonic jerks were blocked.

Effect of Subconvulsive Dose of GABA Antagonists on RO5-4864-Induced Convulsions

Administration of RO5-4864 (10 mg/kg) in combination with bicuculline (a dose which produced mild forelimbs twitching; 2 mg/kg) did not change the onset of clonic convulsions, but caused violent clonic and tonic extension of hind limbs. This drug combination produced a mortality of 80%. As shown in Fig. 1, the response of RO5-4864, at this dose (10 mg/kg) and in combination with bicuculline (2 mg/kg), was equivalent to that produced by 40 mg/kg of RO5-4864. Similarly, the same dose of RO5-4864, when combined with subconvulsive doses of picrotoxin (4 mg/kg) produced a proconvulsant action (data not shown). In contrast, no potentiation of proconvulsant actions of RO5-4864 was observed in combination with subconvulsive doses of strychnine (1 mg/kg) in rats.

DISCUSSION

Several studies have attempted to determine the potential mechanism(s) by which RO5-4864 produces its pharmacological effects. However, no concensus appears to have emerged. Specific binding sites for [^{3}H]RO5-4864 in peripheral tissues and CNS have been demonstrated [10,14]. Moreover, the pharmacological specificity of these sites is different from the central BZ sites, which are coupled to the GABA receptor complex [10,14]. While previous studies ruled out the involvement of GABAergic transmission in the actions of RO5-4864, some recent studies have suggested such a possibility. Thus, RO5-4864 decreases presynaptic inhibition and dorsal root reflexes in cat spinal neurons, an effect opposite to that of BZs [17]. MacNeil *et al.* [9] reported that RO5-4864 inhibited the enhancement of [^{3}H]diazepam binding by GABA. Further, opposite effects of RO5-4864 and BZs were observed in the substantia nigra zona reticulata in rats [18]. The proconvulsant effect of RO5-4864 has also been reported [6] and its convulsant effects are blocked by diazepam, pentobarbital and muscimol [4–6, 17, 18]. Thus, many drugs that facilitate GABAergic transmission block the convulsant effects of RO5-4864. However, contradictory results have been reported with phenytoin and RO15-1788 [18]. The ability of PK11195, a ligand for the peripheral BZ site [8], to block RO5-4864-induced convulsions, does suggest the involvement of this site. However, it may be pointed out that the complete pharmacological profile of PK11195, including its effects on GABAergic transmission, have yet to be elucidated.

Our study demonstrates that RO5-4864 produces a proconvulsant effect with specific GABA antagonists, bicuculline (Fig. 1) and picrotoxin (data not shown). In contrast, proconvulsant effect was not observed in combination with glycine antagonist strychnine. BZs like clonazepam and diazepam were effective blockers of RO5-4864 convulsions. It is not clear why clonazepam, in contrast to diazepam, was a relatively poor antagonist of the initial clonic component of RO5-4864 convulsions. This indicates that some other mechanisms might also be responsible for the complete convulsant profile of RO5-4864. Since clonazepam does not bind to the peripheral BZ binding sites, these results suggest the role of central mechanisms in some, if not all, the components involved in the convulsant action of RO5-4864. This is further substantiated by the findings that several other facilitators of GABAergic transmission, including pentobarbital and ethanol, provided protection against RO5-4864-induced convulsions. General activation of GABA receptors by AOAA, a GABA-T inhibitor (this study), or the administration of a GABA agonist [18], muscimol, protects the animals from RO5-4864-induced seizures. These data indicate that selective stimulation of the GABA receptors of the GABA-BZ receptor complex does play a role in the prevention of RO5-4864 convulsions. TBPT (picrotoxin) sites are also potential sites of action for GABA antagonists and convulsants like picrotoxin, bicyclophosphate esters and tetrazoles [13, 15, 16]. The ability of RO5-4864 to inhibit [^{35}S]TBPT binding competitively to rat brain membranes further suggests the involvement of GABA-BZ-receptor

ionophore complex in the convulsant action of RO5-4864 [16]. The inability of central BZ antagonist RO15-1788 to prevent RO5-4864 convulsions also suggests a different site of action than the central BZ recognition site for RO5-4864-induced convulsions. However, others have reported that RO15-1788 reverses some of the behavioral effects of RO5-4864 [5]. It may be pointed out that RO15-1788 possesses anticonvulsant activity [7] and that the reversal may be due to this phenomenon. Our results with RO15-1788 indicate that it may have some proconvulsant action. A similar proconvulsant effect of RO15-1788 was observed by Corda *et al.* [2] in isoniazid-induced convulsions.

Our behavioral data, together with neurochemical and electrophysiological results from other laboratories [4–6, 9, 12, 13, 15, 16] support the conjecture that a significant aspect of convulsant actions of RO5-4864 is of central origin, and the most likely site appears to be the picrotoxin site of the GABA-BZ receptor complex.

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