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EEG Effects of Ro 15-4513 and FG 7142 Alone and in Combination With Ethanol[1]

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EHLERS, C. L., R. I. CHAPLIN AND G. F. KOOB. *EEG effects of Ro 15-4513 and FG 7142 alone and in combination with ethanol.* PHARMACOL BIOCHEM BEHAV **36**(3) 607–611, 1990.—The electrophysiological effects of Ro 15-4513 and FG 7142 alone and in combination with ethanol were investigated. Intraperitoneal administration of Ro 15-4513 (3, 6 mg/kg) was found to significantly increase power in the 6–16 Hz frequency range, an effect which was antagonized by ethanol (1 g/kg). FG 7142 administration (2, 5 mg/kg IP) was found to enhance spectral power in the 6–8 Hz range, an effect which was also blocked by coadministration of ethanol. Both drugs also produced dose-related abnormal EEG activity in the form of episodic bursts of EEG spiking. These ictal episodes, which lasted 5–40 seconds, were associated with an arrest of behavior but no overt behavioral convulsions. Coadministration of ethanol caused an elimination or significant reduction in these episodes. These studies suggest that Ro 15-4513 and FG 7142 have potent electrophysiological actions of their own which are partially antagonized by ethanol.

EEG Ethanol Inverse agonists Benzodiazepines Ro 15-4513 FG 7142

IT has been suggested that the CNS actions of ethanol may result from actions on neuronal membranes (2, 6, 9, 15), which may only secondarily produce dose-dependent effects on specific neurotransmitter processes. However, some studies have provided evidence to indicate that some of the actions of ethanol may result from relatively specific membrane interactions with the benzodiazepine/GABA receptor ionophore complex. For example, acute ethanol administration has been shown to increase the number of low-affinity GABA receptor sites (22). GABA receptor-mediated uptake of $^{36}Cl^-$-labeled chlorine into isolated brain vesicles has also been established (19). The motor impairment and anticonflict effects produced by ethanol have also been demonstrated to be potentiated by CNS injections of GABA agonists (5,12). In addition, the GABA antagonist picrotoxin has been found to reverse ethanol-induced sedation (12), and to block GABA-mediated $^{36}Cl^-$ ion uptake (12).

Recent studies utilizing the benzodiazepine antagonists Ro 15-1788, and the partial inverse agonists Ro 15-4513 and FG 7142 have attempted to further characterize ethanol effects. Administration of Ro 15-4513 has been found to antagonize both the anticonflict and sedative effects of ethanol as well as block the stimulation of $^{36}Cl^-$ uptake in vitro (20). Administration of FG 7142 has also been shown to reverse the anticonflict effects of ethanol (10). Although the selectivity of these effects has been challenged (13,21), these drugs provide valuable tools for the study of behaviorally relevant actions of ethanol.

We have reported in a preliminary study (21) that administration of 6 mg/kg Ro 15-4513 can produce abnormal electrical activity in the form of spike discharges. The present study aims at extending those findings to evaluate a larger dose range, to compare the EEG actions of Ro 15-1413 to FG 7142, and to test whether ethanol administration could antagonize these electrophysiological effects.

METHOD

Animals

The experimental subjects were twenty-nine male, Wistar rats weighing 280–400 grams. The rats were individually housed and maintained in a temperature- and light- (12:12 LD) controlled room. Food and water were given ad lib.

Surgery

At least two weeks prior to the experimental procedures, rats were surgically prepared with recording electrodes. Twenty-one experimentally naive animals were anesthetized (Nembutal, 50 mg/kg IP) and stainless steel bipolar electrodes were aimed at the dorsal hippocampus (AP 3.0, ML 3.0, DV 3.1) and cortical screws were placed in the calvarium over midline sensory-motor and occipital cortices. A second series of eight drug-naive rats were also utilized in this study. These animals had been previously

[1]An abstract of this work was presented at the Seventeenth Annual Society of Neuroscience Meeting, New Orleans, LA.

implanted with electrodes aimed at the locus coeruleus, dorsal hippocampus, nucleus accumbens, as well as cortical screws (3). In all animals electrode attachments were made to a multipin (Amphenol) connector and the entire assembly was anchored to the skull with dental acrylic.

Drug Administration and EEG Recordings

For the Ro 15-4513 study, the first group of twenty-one rats were randomly assigned to 3 groups of 7 and each group received two of six drug conditions spaced two weeks apart. The six conditions were: 6 mg/kg Ro; 3 mg/kg Ro; 1.5 mg/kg Ro; vehicle; 6 mg/kg Ro + 1.0 g/kg ETOH; and vehicle + 1 g/kg ETOH. The vehicle consisted of a solution of 0.5% Emulphor, 0.5% ETOH, 99% saline. A second set of eight rats received two lower doses of Ro 15-4513 (0.75 mg/kg, 0.1 mg/kg) spaced two weeks apart. Two weeks following the end of the Ro 15-4513 study, the twenty-one original rats were randomly assigned to three groups of seven and additionally tested following administration of one of three doses of FG 7142 (5 mg/kg, 2.5 mg/kg, 5 mg/kg + 1 g/kg ETOH). All drug and vehicle solutions were prepared fresh each day. These doses of Ro 15-4513 (0.1–6 mg/kg) and FG 7142 (2.5, 5 mg/kg) were chosen as they were within or below the range of doses which we have previously shown to produce a behavioral effect in the conflict test (10,11).

For EEG recordings on the test day, each rat was adapted to a light-, sound-, and temperature-controlled chamber, in which behavior could be monitored for one hour. All animals were observed during recordings for any behavioral signs of convulsive activity such as running fits, forelimb clonus or tonic-clonic activity. A flexible microdot cable was attached to the animal's electrode connector. Ro 15-4513, FG 7142, and vehicle injections were made intraperitoneally (IP) ten minutes before recordings were begun. For the vehicle + ethanol injections, Ro + ETOH and FG + ETOH studies, the ETOH was injected IP, five minutes prior to the subsequent IP FG, Ro or vehicle injections. EEG (1–70 Hz, high-low pass filtered) was recorded on paper (Grass Model 7D) for one hour and on tape (Vetter model D) for twenty minutes following the injections. Behavior was continuously observed and noted on the polygraphic records.

The first twenty minutes of taped EEG, not containing artifacts, from cortical and dorsal hippocampal leads were selected for computer analysis. The EEG analysis was accomplished on a DEC (LSI 11-73 processor computer) which digitized (128 samples/sec) and calculated the Fourier transform of 4-second continuous epochs from 0 to 127.75 Hz. The power spectra of the 4-second epochs were determined over the 0.25–64 Hz range. The transformed data were then further compressed into seven frequency bands (1–2, 2–4, 4–6, 6–8, 8–16, 16–32, and 32–64 Hz) and mean power density was calculated for each band as previously described (4). Analysis of variance (ANOVA) was utilized to determine if significant differences between groups could be determined.

In addition to computer analysis of the EEG records, paper records were scored for paroxysmal activity and the number of ictal episodes in an 18-minute recording period was counted. A description of the effects of the highest dose of Ro 15-4513 given to these rats (6 mg/kg) on EEG paroxysmal activity has been previously reported (21).

RESULTS

Administration of Ro 15-4513 and FG 7142 were found to produce dose-related changes in the EEG which were significantly different to those produced by vehicle injections. As seen in Fig. 1, these drugs were found to produce bursts of spike activity in hippocampal and cortical leads. These ictal episodes lasted for 5–10 seconds in the case of Ro 15-4513, and up to 40 seconds in the case of FG 7142. EEG spiking was not associated with behavioral signs of convulsions. Each rat's record was scored for the number of these ictal episodes observed, over an 18-minute period, and a mean number of ictal episodes was calculated for the group of rats given each dose of drug. These data are provided in Fig. 2. As this figure shows, even the lowest doses of Ro 15-4513 (0.1, 0.75 mg/kg) were found to produce spiking episodes. The effects of FG 7142 and Ro 15-4513 on the number of paroxysmal episodes seen in the EEG was not observed to be entirely dose dependent. This suggests that once the threshold for paroxysmal activity is reached, the amount of ictal activity depends more strongly on individual variations between animals, rather than dose, as is seen with convulsant drugs (7). Figure 2 also presents the results of the coadministration of ethanol (1 g/kg) and the high doses of Ro 15-4513 (6 mg/kg) and FG 7142 (5 mg/kg). Ethanol was found to completely block the ictal episodes produced by FG 7142, whereas it was only found to reduce ictal episodes produced by Ro 15-4513 by one-half.

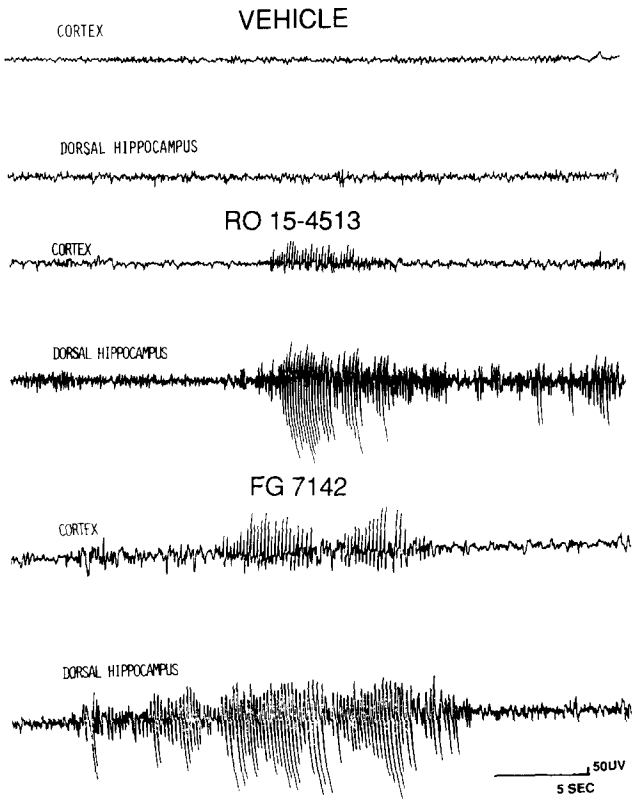

FIG. 1. Effects of vehicle, Ro 15-4513 (6 mg/kg) and FG 7142 (5.0 mg/kg) on the cortical and dorsal hippocampal EEG of the rat. Note the appearance of paroxysmal burst of spikes which occur initially in the hippocampal leads.

Spectral analysis of the records of the first twenty-one rats revealed that Ro 15-4513 and FG 7142 also produced significant, dose-related changes in the frequency characteristics of the EEG, as seen in Fig. 3 for the hippocampal leads. Low doses of Ro 15-4513 (1.5 mg/kg) did not produce highly significant changes in EEG spectra; however, higher doses (3.0, 6.0 mg/kg) did produce significant increases in several EEG bands in both cortical (CTX)

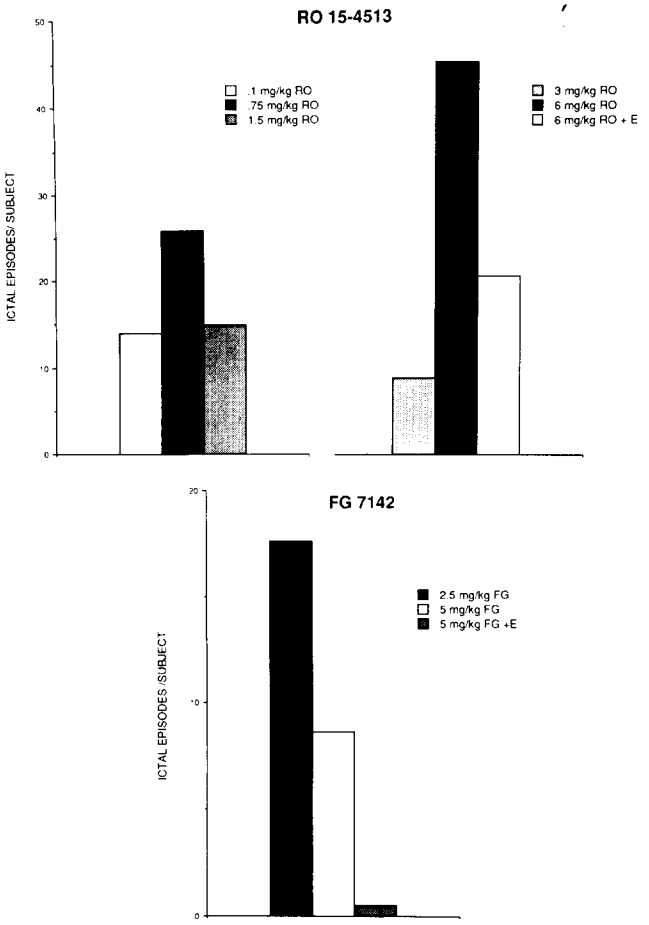

FIG. 2. A quantification of the bursts of EEG paroxysms which occurred in hippocampal leads following administration of various doses of FG 7142, Ro 15-4513 alone and in combination with ethanol. Each bar represents the mean number of ictal events which occurred over the group of rats tested. The vehicle- or ethanol alone-treated rats did not display any EEG paroxysms.

and dorsal hippocampal (DHPC) leads. At the 3.0 mg/kg dose, Ro 15-4513 was found to produce increases in the midrange frequencies (6–8 Hz, $F=7.79$, $p<0.007$; 8–16 Hz, $F=4.95$, $p<0.03$) in DHPC. Higher doses of Ro 15-4513 (6.0 mg/kg) enhanced activity in the lower frequencies in CTX (2–4 Hz, $F=4.91$, $p<0.04$; 4–8 Hz, $F=7.28$, $p<0.01$) and produced further increases in the midrange frequencies in DHPC (6–8 Hz, $F=12.47$, $p<0.001$; 8–16 Hz, $F=8.7$, $p<0.005$). Administration of ethanol alone (1 g/kg) was also found to shift the distribution of spectral power in the EEG. A modest, but highly significant increase in the 4–6 Hz ($F=10.23$, $p<0.003$), 6–8 Hz ($F=12.52$, $p<0.001$), and 8–16 Hz ($F=15.60$, $p<0.001$) frequencies was evident in DHPC leads. Ethanol in combination with Ro 15-4513 was also found to significantly antagonize the increases in the midrange frequencies observed following injections of Ro 15-4513 alone (DHPC, 6–8 Hz, $F=6.04$, $p<0.02$; 8–16 Hz, $F=7.02$, $p<0.01$).

As also seen in Fig. 3, FG 7142 produced significant effects on the EEG which were somewhat different from those observed following Ro 15-4513. The 2.5 mg/kg dose of FG only induced increases in the 6–8 Hz frequencies in both CTX ($F=4.01$, $p<0.05$) and DHPC ($F=8.83$, $p<0.005$). The higher doses of FG (5.0 mg/kg) also produced increases in the 6–8 Hz range ($F=4.07$, $p<0.05$). Coadministration of ethanol with FG 7142 was found to significantly antagonize these increases in the 6–8 Hz range produced by FG 7142 alone ($F=6.28$, $p<0.01$).

DISCUSSION

Some of the behavioral actions of ethanol, particularly its anxiolytic effects, have been suggested to be mediated through the benzodiazepine/GABA receptor ionophore complex (BZ). The data which support this hypothesis are mainly based on pharmacological studies utilizing various antagonists and inverse agonists. For instance, the anti-conflict actions of ethanol have been shown to be antagonized by Ro 15-4513 and FG 7142 (10,12). Ro 15-4513 has also been shown to prevent ethanol-induced decreases in locomotor activity and reverse ethanol-induced impairment in the horizontal wire test (1). However, not all of the actions of ethanol can be reversed by these compounds. Ro 15-4513 has not been found to alter ethanol's lethality (8), nor does it alter ethanol-induced loss of the righting reflex or depression of rotarod performance. Due to the specific pharmacologic profile of Ro 15-4513, it has been suggested that it is a "partial inverse agonist" at the benzodiazepine receptor complex capable of modifying some, but certainly not all, of the actions of ethanol and benzodiazepines (17,18).

One property which inverse and partial inverse agonists at the BZ receptor seem to share is their convulsive or proconvulsive effects. In the present study, all doses of Ro 15-4513 tested (0.1–6 mg/kg) were found to produce EEG paroxysms. These EEG paroxysms consisted of bursts of spikes and sharp waves which averaged 3–40 seconds in length. These ictal episodes were first observed in dorsal hippocampus and later at cortical sites. The nature of the EEG paroxysms produced by Ro 15-4513 were somewhat different than those produced by FG 7142. Ro 15-4513 tended to produce short ictal episodes of 3–10 seconds in duration which contained complex spike and wave forms. Ictal episodes produced by FG 7142 were longer in duration, up to 40 seconds in length and the spike trains appeared more uniform in nature. None of these spiking episodes produced by Ro 15-4513 or FG 7142 were associated with overt behavioral convulsions. Bonetti *et al.* (1) have also recently reported in a small number of rats that Ro 15-4513 can produce EEG paroxysms in cortical areas at doses between 3 and 12 mg/kg IP. In our study we observed EEG paroxysms at doses as low as 0.1 mg/kg IP; however, we were recording from limbic sites where the ictal activity is presumably originating. These data are consistent with previous studies which have demonstrated that this class of drugs can significantly enhance the behavioral seizure inducing effects of classic convulsant agents such as PTZ or picrotoxin (1,14). Overt behavioral seizures have also been reported to occur in monkeys given high doses of Ro 15-4513 (16).

In the present study, administration of ethanol was found to reduce or block the paroxysmal episodes produced by these drugs. A total blockade of any paroxysmal activity was observed when ethanol (1 g/kg IP) was administered five minutes prior to high doses of FG 7142 (5.0 mg/kg). This dose of ethanol was found to only partially block the ictal episodes produced by high doses (6.0 mg/kg) of Ro 15-4513. These findings are consistent with those reported in behavioral studies where FG 7142 and Ro 15-4513 have been found to reduce the anticonflict actions of ethanol (10,11).

The effects of Ro 15-4513 and FG 7142 on the spectral characteristics of the background EEG have not been previously reported. At the 3.0 mg/kg dose, we found significant increases in the 6–18 Hz frequency range in DHPC. Higher doses (6.0 mg/kg) produced significant increases in cortical slow waves (2–8 Hz).

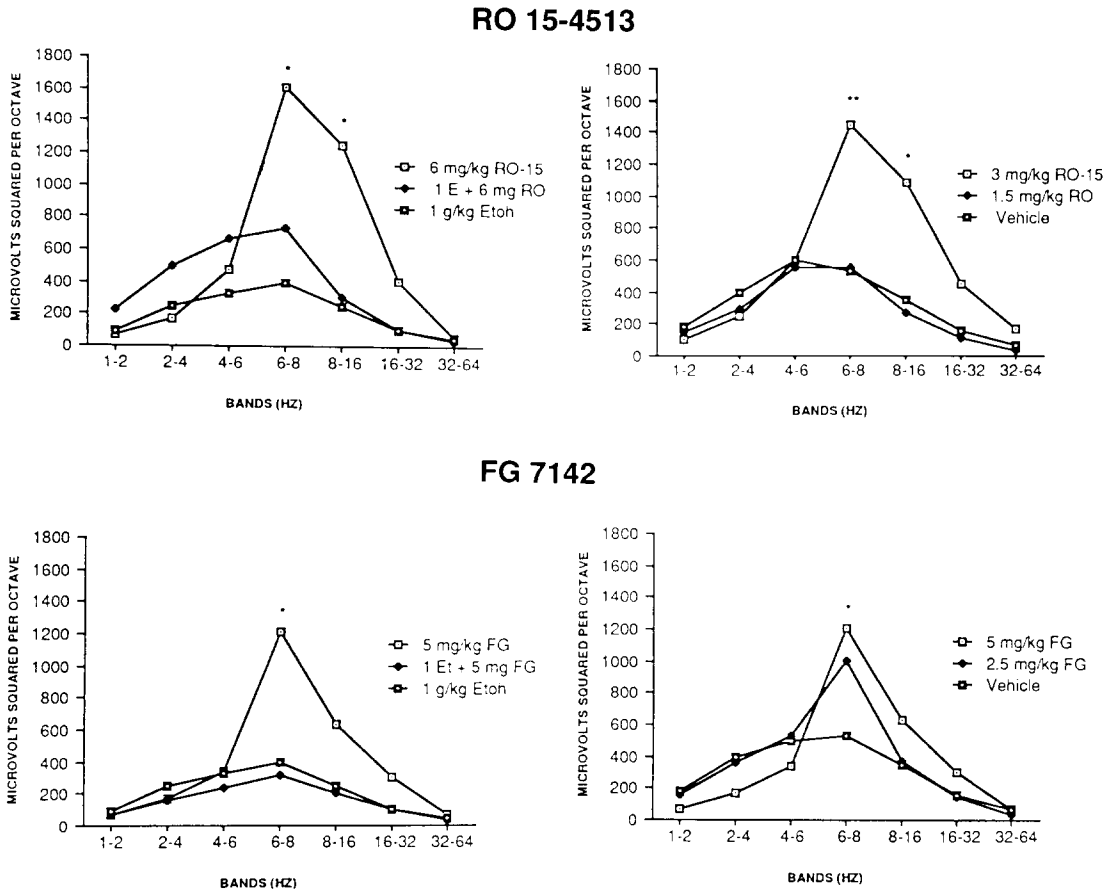

FIG. 3. Changes in the distribution of EEG spectral power in hippocampal leads seen following administration of vehicle, ethanol and Ro 15-4513, FG 7142, alone and in combination with ethanol. For each graph, bandwidth (1–64 Hz) is presented on the abscissa and EEG power (μV^2/octave) is presented on the ordinate.

Administration of ethanol (1 g/kg) was found to significantly reverse these EEG effects. FG 7142 was shown to produce a different distribution of background EEG spectra. An increase in the 6–8 Hz frequencies of the DHPC leads were found at both doses (2.5, 5.0 mg/kg), an effect which was also antagonized by ethanol. The fact that FG 7142 and Ro 15-4513 produce significantly different effects on EEG spectra as well as different types of EEG paroxysms in the same dose-potency range, as measured by EEG spectra and behavioral studies (10,11), suggests that they may have somewhat different mechanisms of action. One possibility is that these drugs may be acting at different combinations of sites in the BZ receptor complex or may also exert actions on neuronal sites separate from the BZ receptor. The fact that ethanol was capable of completely blocking the effects of FG 7142 and only partially block the effects of Ro 15-4513 further suggests that the acute antiepileptic effects of ethanol may be more selective for FG 7142 than Ro 15-4513.

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