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BRIEF COMMUNICATION

Failure of the Partial Inverse Benzodiazepine Agonist Ro15-4513 to Block the Lethal Effects of Ethanol in Rats

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POLING, A., H. SCHLINGER AND E. BLAKELY. *Failure of the partial inverse benzodiazepine agonist Ro15-4513 to block the lethal effects of ethanol in rats.* PHARMACOL BIOCHEM BEHAV 31(4) 945–947, 1988.—The partial inverse benzodiazepine agonist Ro15-4513 has been found to antagonize some of the behavioral and physiological effects of low to moderate doses of ethanol. In the present study, pretreating rats with Ro15-4513 (1, 3, 10, and 30 mg/kg) at doses equal to or greater than those used in prior investigations failed to block the lethal effects of intraperitoneal injections of ethanol at a dose of 5.4 g/kg. These results suggest that the lethal actions of ethanol may involve a mechanism that is not blocked by Ro15-4513, which is known to selectively antagonize ethanol-stimulated chloride uptake via the GABA-coupled chloride ion channel.

Ethanol Lethality Ro15-4513 Rats

RECENT in vitro research has demonstrated that the imidazobenzodiazepine Ro15-4513, a partial inverse benzodiazepine agonist, acts at GABA-benzodiazepine receptors to block ethanol-stimulated chloride uptake (10). In vivo studies have also revealed that pretreatment with Ro15-4513 blocks some, but not all, of the behavioral and physiological effects of low to moderate doses of ethanol. Among other actions, Ro15-4513 reportedly blocks a) the ability of ethanol to reduce stress-induced increases in cortical DOPAC (5); b) the sedating effects of ethanol (2, 3, 6, 8, 10); and c) the antipunishment (anticonflict) effects of ethanol (10). It also reduces oral ethanol self-administration (9). These results suggest that many of the behavioral effects of low to moderate doses of ethanol are mediated in part by central GABA receptors.

The possibility that Ro15-4513 is a selective amethystic agent has attracted much attention [e.g., (7)]. There is uncertaintly, however, about the specificity of the effects of Ro15-4513 [e.g., (11)]. The extent to which Ro15-4513 will antagonize the effects of high doses of ethanol is also uncertain. As Suzdak *et al.* (10) noted, the effects of high doses could be mediated by neurotransmitter receptors not affected by the drug, or voltage-dependent channels known to be altered by high concentrations of ethanol. In either case, Ro15-4513 would not block the effects of high ethanol doses. The purpose of the present experiment was to determine whether the lethal effects of a high dose of ethanol could be blocked by pretreatment with Ro15-4513.

METHOD

Subjects and Apparatus

Ninety experimentally-naive young adult male Sprague-Dawley rats (250–360 g body weight), obtained from the Upjohn Company (Kalamazoo, MI), served as subjects. Prior to and during the study they were housed six to a group in stainless steel cages (47 cm long, 31 cm wide, and 20 cm deep) located in a colony room with controlled temperature (74–76°F) and lighting (dark from 7:00 p.m. to 7:00 a.m.). Subjects were always given unlimited access to food (Purina Rat Chow) and water.

Procedure

At the onset of the study, the subjects were randomly divided into five groups, each comprising eighteen rats. All groups received an intraperitoneal (IP) injection of 5.4 g/kg ethanol [the medial lethal dose is 5 g/kg (1)]. Ten min before ethanol administration (95% concentration), an IP injection of 0, 1, 3, 10, or 30 mg/kg Ro15-4513 was given; one group of rats was exposed to each of these doses. The Ro15-4513 was

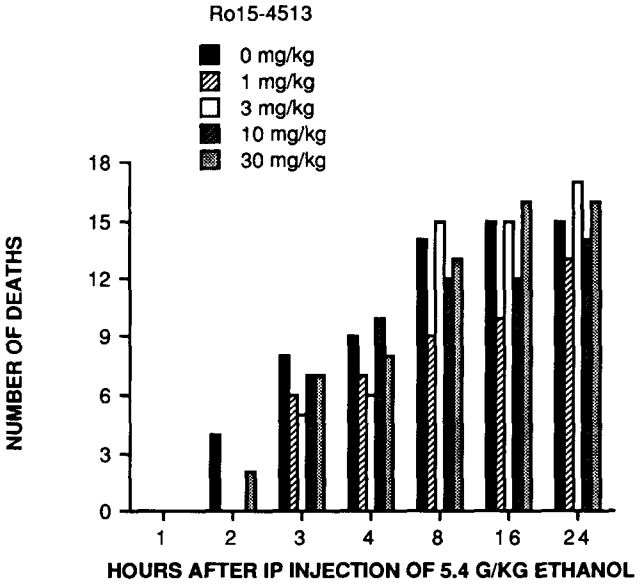

FIG. 1. Number of deaths in groups of 18 rats at various times after IP injection of 5.4 g/kg ethanol as a function of the dose of Ro15-4513 (0, 1, 3, 10, 30 mg/kg) administered 10 min prior to the ethanol. Data are not presented for 1 hour postadministration because no deaths were evident at that time.

prepared just before injection as an ultrasonified suspension in distilled water with Tween 80 added and was injected at a volume of 1 ml/kg. Doses of Ro15-4513 and the time of administration relative to ethanol injection were selected on the basis of values that were effective in prior studies (9,10). Drugs were given to all groups between 1:00 and 3:00 p.m. After drug injection, the rats were housed six to a group in the colony area. The number of subjects that had expired in each group was recorded 1, 2, 3, 4, 8, 16, and 24 hr after ethanol administration.

RESULTS AND DISCUSSION

The number of deaths in each group as a function of time after ethanol injection is shown in Fig. 1. These data indicate that the five groups did not differ appreciably with respect to total number of deaths, or the times at which deaths were recorded. Therefore, pretreatment with Ro15-4513 did not block the lethal effects of ethanol. This assertion in confirmed by statistical test: A chi-square analysis revealed that the observed number of deaths in groups pretreated with Ro15-4513 did not differ significantly ($p>0.05$) from the number expected on the basis of deaths in the groups treated with ethanol alone.

Previous studies have shown that Ro15-4513 is relatively potent [e.g., (3, 4, 9, 10)]; antagonism, when evident, typically occurred at doses of 5 mg/kg or less. Therefore, the doses evaluated in the present study (1, 3, 10, and 30 mg/kg) appear to be reasonable. It is, of course, possible that higher Ro15-4513 doses would antagonize the lethal actions of ethanol, but neither the present data nor previous findings provide support for this possibility.

Nothing has been reported concerning the pharmacokinetics of Ro15-4513, and it is possible that the failure of a single administration of the drug to protect against the lethal effects of ethanol resulted from a short duration of action. That is, so long as Ro15-4513 was active, it interacted with central GABA receptors and thereby prevented lethality. But when this action ended, the ethanol, still present at high levels as a result of the large dose administered, affected those receptors and death ensued. Although this may have occurred, it is not apparent in the time-course data from the present study: The Ro15-4513 groups did not differ systematically from one another or from the control group with respect to the times at which deaths were recorded. Moreover, nonsystemic observations revealed that almost all animals in each group were comatose within 15 min of ethanol administration. This further suggests that the failure of Ro15-4513 to block the lethal effect of ethanol did not simply result from a short duration of action, but rather from an inability of the drug to block some of the neuropharmacological actions of a high (5.4 g/kg) dose of ethanol.

Such an interpretation is rendered problematic by the results of a study (4) reported after the present investigation was submitted for review. In that investigation, Ro15-4513 at 10 mg/kg totally blocked the lethality of ethanol administered orally at doses as high as 15 mg/kg, which killed 7 of 8 rats not pretreated with Ro15-4513. It is not readily apparent why Ro15-4513 blocked the lethal effects of ethanol in this study, but not in the present investigation. An obvious possibility involves the use of different routes of ethanol administration, which may have produced substantial differences in ethanol concentration at the site of action over time. Perhaps the use of IP injections in the present study produced exceptionally high ethanol concentrations, and resulted in death through some mechanism not evident with oral administration.

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