

Is Ethanol Antagonist Ro15-4513 Selective for Ethanol?

Suzdak *et al.* (1) report that the imidazodiazepine Ro15-4513 selectively blocks the anxiolytic and intoxicating properties of ethanol in rats with no adverse behavioral actions on its own. Our observations of the effects of Ro15-4513 on the anxiolytic and behavioral effects of ethanol are not entirely

consistent with those of Suzdak *et al.* Indeed, they support the argument that Ro15-4513 nonselectively antagonizes the behavioral effects of both ethanol and pentobarbital and only at doses that also produce effects that are the opposite of those produced by ethanol and pentobarbital.

In our first set of experiments, we used an operant conflict test modified for incremental shock in which ethanol produces reliable dose-dependent release of punished responding (2). Twenty-four male Wistar rats were trained in the conflict test, randomly divided into four groups, and injected with Ro15-4513 (0, 1.5, 3.0, or 6.0 mg per kilogram of body weight, respectively). Ro15-4513 produced a significant dose-dependent decrease in both punished and nonpunished responding (Fig. 1). The same rats were then randomly reassigned to four groups and injected with saline, ethanol (0.75 g per kilogram of body weight),

Ro15-4513 (6 mg per kilogram of body weight), or a combination of ethanol and Ro15-4513. Ethanol produced a significant increase in responding during the punished component of the test (Fig. 2). Ro15-4513 blocked the anticonflict actions of ethanol, but also produced a significant decrease in nonpunished responding. A lower dose of Ro15-4513 (3 mg per kilogram of body weight) that had no effect on punished responding on its own did not antagonize the anticonflict effects of ethanol (analysis of variance, main effect ethanol only). Ro15-4513 also blocked the anticonflict action of pentobarbital (4 mg per kilogram of body weight) and chlordiazepoxide (5 mg per kilogram of body weight). All compounds were injected intraperitoneally 15 minutes before testing.

In order to characterize further the non-specific effects of Ro15-4513, anterior cortical (AC) and dorsal hippocampal (DHPC) electrodes were implanted in additional rats. After a 10-day recovery period, Ro15-4513 in doses ranging from 0.75 to 6.0 mg per kilogram of body weight was administered by intraperitoneal injection and an electroencephalogram was monitored on paper and

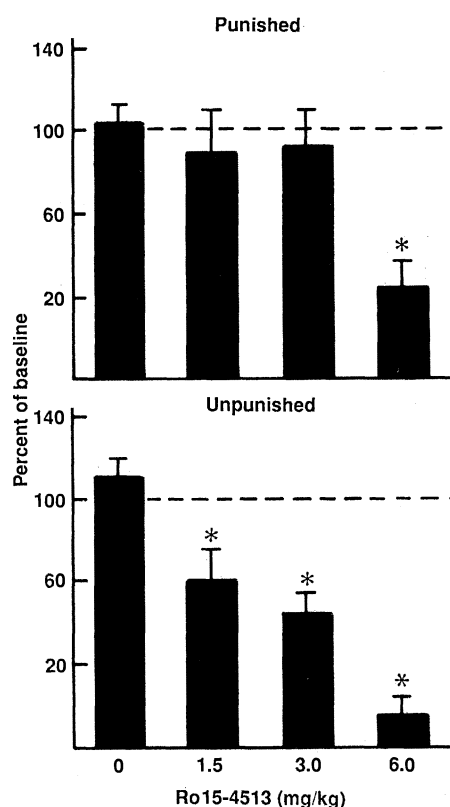
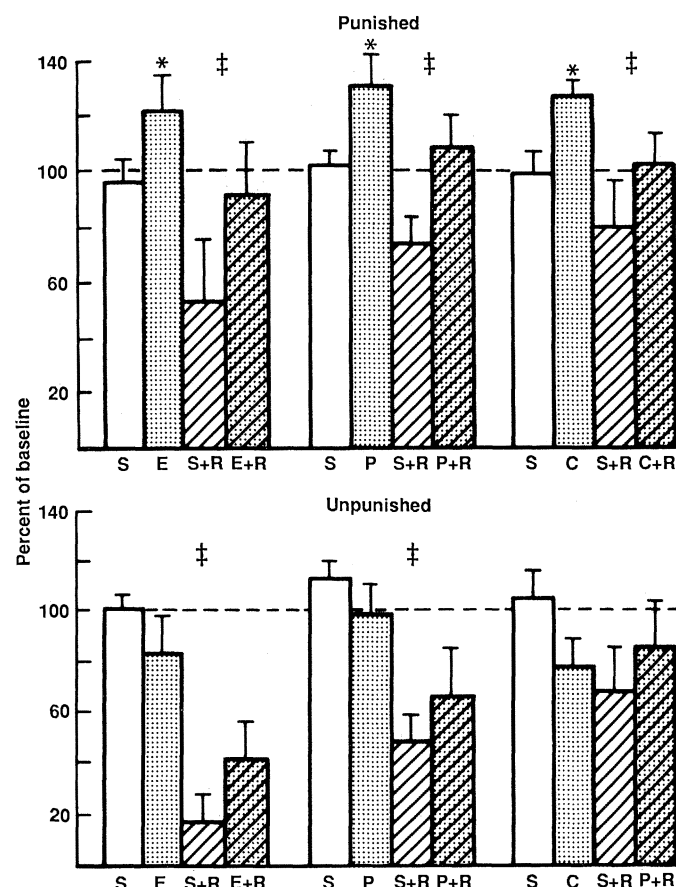


Fig. 1. The effect of Ro15-4513 on punished (conflict) and unpunished (random interval) responding in a conflict test modified for incremental shock. The conflict test consisted of a pure reward component (unpunished) and a conflict component (punished). Responses during the reward component were reinforced on a random-interval, 30-second schedule. Responses during the conflict component were both rewarded by food and punished with foot shock on a continuous reinforcement schedule. Shock (biphasic square wave) was increased by increments of 0.15 mA after each lever press to a maximum of 3.3 mA. Ro15-4513, suspended in emulphor (0.5%), ethanol (0.5%), and saline, produced a generalized suppression of both punished ($F(3, 19) = 7.69$, $P < 0.05$) and nonpunished ($F(3, 19) = 24.0$, $P < 0.05$) responding on the conflict test. This effect was maximal at the highest dose, where punished responding was reduced to 21% of baseline and nonpunished responding to 6.3% of baseline. $n = 5$ rats per group except for the group receiving 1.5 mg per kilogram of body weight, where $n = 7$. *Significantly different from control [analysis of variance (ANOVA) followed by Newman-Keuls test, $P < 0.05$].

Fig. 2. The interaction of Ro15-4513 (R) with ethanol (E) (0.75 g per kilogram of body weight), pentobarbital (P) (4 mg per kilogram of body weight), and chlordiazepoxide (C) (5 mg per kilogram of body weight) on punished (conflict) and unpunished (random interval) responding in the conflict test. Saline (S) was the vehicle. $n = 6$ rats per group except for the chlordiazepoxide vehicle and drug-only groups, where $n = 7$. Results are expressed as percent of baseline responding from previous two non-injection days (mean $\pm$ SEM). For conflict responding, a two-factor ANOVA revealed significant main effects for Ro15-4513, ethanol, pentobarbital, and chlordiazepoxide. Ethanol, pentobarbital, and chlordiazepoxide significantly increased punished responding (* $P < 0.05$, ANOVA E, P, C main effect). In contrast, Ro15-4513 blocked the anticonflict effects of ethanol, pentobarbital, and chlordiazepoxide, but also depressed conflict responding when administered alone ($\ddagger P < 0.05$, ANOVA, Ro15-4513 main effect). For unpunished responding there was a main effect of Ro15-4513 in suppressing responding with ethanol and pentobarbital ($\ddagger P < 0.05$, ANOVA Ro15-4513 main effect) but not with chlordiazepoxide ($F < 1$).



magnetic tape for 1 to 1½ hours. Abnormal encephalographic activity in the form of slow sharp waves was observed in the rats at all doses tested, particularly in the DHPC. High-amplitude encephalographic seizures were also observed in the DHPC 5 to 20 minutes after the administration of Ro15-4513. These ictal episodes were 5 to 10 seconds in length and occurred regularly over a 1-hour period. No overt behavioral signs of seizure activity were noted during the ictal episodes.

Our results demonstrate that administration of Ro15-4513 is effective in reversing the release of punished responding produced by alcohol, pentobarbital, and chlor-diazepoxide. These results are consistent with earlier reports (3). In addition, Ro15-4513 by itself produces a dose-dependent suppression of both punished and nonpunished responding as well as electroencephalographic seizure activity in some limbic sites. While lower doses of Ro15-4513 may be more specific in certain behavioral tests (1), these findings raise questions about the hypothesis that Ro15-4513 is a specific and selective antagonist of ethanol. These results also cast doubt on the potential clinical usefulness of this particular compound, but suggest that Ro15-4513 may be a useful research tool for elucidating the neurochemical substrate of ethanol's effects.

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Response: Britton *et al.* (1) raise two important questions concerning the "anti-ethanol" effects of Ro15-4513. The first is whether Ro15-4513 can antagonize the effects of ethanol at doses that do not produce an opposite, merely subtractive, pharmaco-

logical effect. We have previously noted (2, 3) that at higher doses, or under conditions in which the intrinsic actions of Ro15-4513 are prominent, it would be difficult to ascertain whether Ro15-4513 was "antagonizing" the actions of ethanol or simply producing the opposite behavioral effect. The fact that Britton *et al.* do not demonstrate a selective action of low doses of Ro15-4513 may therefore relate to the prominent rate-decreasing (inverse agonist) actions of Ro15-4513 and to the marginal anticonflict actions of ethanol, pentobarbital, and chlor-diazepoxide observed with their paradigm. By contrast, punished responding was reduced in our study to 5 to 10% of unpunished responding to obtain a robust anticonflict action of ethanol (1000% of control compared with 20 to 30% observed by Britton *et al.*) and to minimize the rate-decreasing actions of Ro15-4513. Moreover, in their experiments, Ro15-4513 (6.0 mg per kilogram of body weight) produced highly variable response decrements ranging from 80 to 90% to less than 20 to 30% for punished and unpunished responding, respectively (Figs. 1 and 2). Such variability could have contributed to their not finding a significant effect of lower doses of Ro15-4513.

We have further examined this question by studying the effects of ethanol and Ro15-4513 on fixed-ratio (FR 30) responding in mice (Fig. 1) (4). Both ethanol (1 to 4 g per kilogram of body weight) and Ro15-4513

(0.3 to 30 mg per kilogram of body weight) decrease responding in this paradigm. However, at low doses of Ro15-4513 (for example, 0.3 mg per kilogram of body weight) we observe antagonism of the rate-decreasing effects of low to intermediate doses of ethanol (Fig. 1A). Moreover, this same dose does not antagonize the decrease in responding produced by pentobarbital (Fig. 1B). Similarly, Engel and Liljequist (5), using Montgomery's elevated maze test in rats, have reported that Ro15-4513 (0.06 mg per kilogram of body weight) antagonizes the anticonflict actions of ethanol at doses that do not alter baseline performance. Samson *et al.* (6) have reported that Ro15-4513 decreases oral ethanol reinforcement in rats at doses (<1 mg per kilogram of body weight) that do not decrease baseline sucrose consumption. Rees and Balster (7) observed that the discriminative stimulus effect of ethanol (but not pentobarbital) is blocked in rats by a low dose of Ro15-4513 (0.1 mg per kilogram of body weight), and the latter did not decrease baseline responding. The doses of Ro15-4513 used in these studies are 20 to 100 times lower than the effective dose reported by Britton *et al.* Finally, Koob and his coworkers (8) have also presented data demonstrating that Ro15-4513 decreases the reaction time deficit produced by ethanol in rats, at a dose (1.5 mg per kilogram of body weight) that does not alter baseline performance. These studies demonstrate that low doses of Ro15-

Fig. 1. (A) Effects of cumulative doses of ethanol (□) (20% volume to volume solution in saline, administered intraperitoneally) and Ro15-4513 (●) on fixed-ratio (FR 30) responding in National Institutes of Health mice (25 g, *n* = 10). Cumulative doses of ethanol were given 5 minutes before responding was assessed for a series of 10 FR 30's or 240 seconds [limited hold (LH), 240 seconds], whichever occurred first. Each of a series of 10 FR 30's (LH, 240 seconds) was separated by a 5-minute time out, with a session comprised of eight series (approximately 60 minutes). Effects were compared with within-session control series (mean of series 2 and series 3 of the session) and expressed as the mean percentage of control over individual mice. The first dose (0.5 g per kilogram of body weight) was given 5 minutes before the series 5 and 6, respectively. The effects of ethanol were assessed twice in each mouse and, as no differences occurred, were averaged to obtain the ethanol-alone curve shown. Ro15-4513 (0.3 mg per kilogram of body weight) was then given (before the fourth series) preceding the determination of a complete ethanol dose-effect function (given before series 5 through 7) on a separate occasion. Under these conditions both drugs decreased responding; many of the large rate-decreasing effects of 1.5 g per kilogram of body weight of ethanol were blocked (86%; *P* < 0.0001 by one-tailed paired *t* test). The higher dose of ethanol (cumulative dose of 3.5 g per kilogram of body weight) was not antagonized by the low dose of Ro15-4513. **(B)** Effects of cumulative doses of sodium pentobarbital (●) (in saline, 1.0 ml/kg, administered intraperitoneally) on fixed-ratio responding in mice, assessed as in (A), in the presence and absence of Ro15-4513 (□) (0.3 mg/kg). Values represent the mean ± SEM of responses.

