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 chlordiazepoxide. 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, pharmacological 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 chlordiazepoxide 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 discriminitive 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-

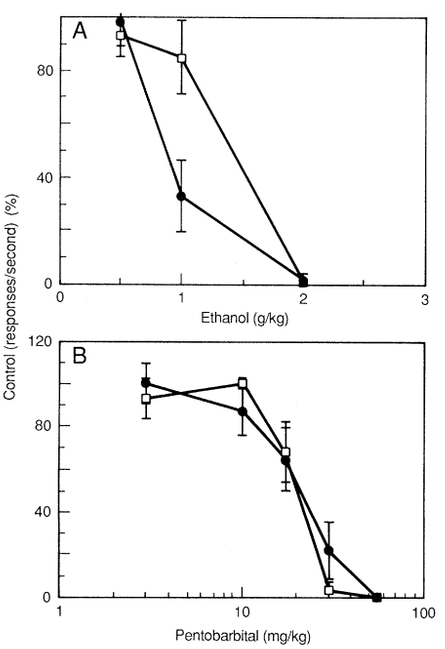

Fig. 1. (**A**) Effects of cumulative doses of ethanol (□) (20% volume to volume solution in saline, administered intraperitoneally) and Ro15-4513 (●) on fixed-ratio (FR 30) responding in National Institutes of Health mice (25 g, $n = 10$). Cumulative doses of ethanol were given 5 minutes before responding 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.

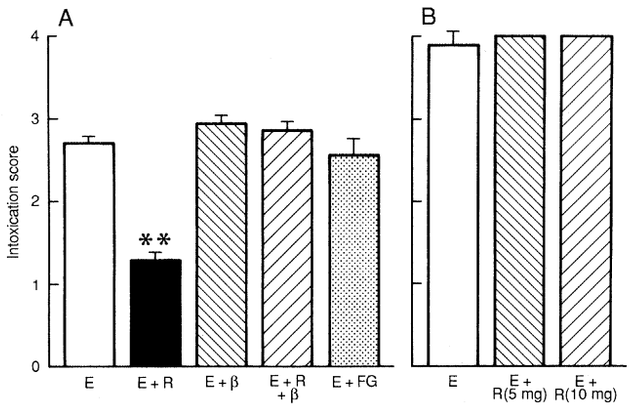

Fig. 2. (**A**) The effect of Ro15-4513 (R) on ethanol-induced intoxication in the rat. Ro15-4513 (2.5 to 10.0 mg per kilogram of body weight), β-CCE (β) (10 mg per kilogram of body weight, or FG-7142 (FG) (30 mg per kilogram of body weight) were administered intraperitoneally 5 minutes before ethanol. Ethanol (E) was administered as a 20% volume to volume solution in saline. Ro15-4513, β-CCE, and FG-7142 were given as a suspension in 4% Tween-80 in saline. Behavioral intoxication was scored by an observer unaware of the treatment conditions (*2*) who used the rating scale of E. Majchrowicz (*11*). A dose of ethanol (2 g per kilogram of body weight, administered intraperitoneally) was chosen that produced an intoxication score of between 2 and 3. Values represent the mean ± SEM of individual intoxication scores ($n \geq 12$) for each group. Ro15-4513 (2.5 mg per kilogram of body weight) significantly [ANOVA, $F(3,115) = 43.4$, $P < 0.01$; Newman-Keuls, $P < 0.01$ ethanol versus Ro15-4513 plus ethanol] blocked the intoxication induced by ethanol (2 g per kilogram of body weight). Neither β-CCE (10 mg per kilogram of body weight) nor FG-7142 (30 mg per kilogram of body weight) attenuated ethanol-induced intoxication. Administration of β-CCE (10 mg per kilogram of body weight) in combination with Ro15-4513 (2.5 mg per kilogram of body weight) blocked the ability of Ro15-4513 to inhibit ethanol-induced intoxication (Newman-Keuls, $P < 0.01$, as compared with Ro15-4513 plus ethanol). (**B**) At a dose of ethanol (E) (4 g per kilogram of body weight, administered intraperitoneally) that produced an intoxication score of 4, Ro15-4513 (R) (5 or 10 mg per kilogram of body weight, administered intraperitoneally) had no statistically significant effect on ethanol-induced intoxication [ANOVA, $F(2,25) = 0.65$].

4513 can antagonize ethanol while not necessarily producing the opposite action, depending on the paradigm employed.

The second question implicit in the comment of Britton *et al.* is whether the "anti-ethanol" actions of Ro15-4513 are shared by other inverse agonists. On the basis of their electroencephalographic (EEG) studies in rats, they have recently suggested that the partial inverse agonist FG-7142 may be even more selective than Ro15-4513 in antagonizing ethanol (*9*). In those behavioral paradigms where γ-aminobutyric acid (GABA) antagonists such as bicuculline or picrotoxin have been shown to block ethanol's actions, we would expect inverse agonists, including Ro15-4513, to also be effective. In our initial report (*2*), however, we tested a series of inverse agonists (including FG-7142) (concentrations, ≤1 μ*M*), and except in the case of Ro15-4513 did not detect antagonism of ethanol-stimulated (or ethanol-potentiated) $^{36}Cl^-$ uptake in synaptoneurosomes. In examining the effects of Ro15-4513 and FG-7142 on ethanol-induced intoxication in rats, we found that only Ro15-4513 antagonized intoxication induced by moderate doses of ethanol (2 g per kilogram of body weight) (*2*). We have also compared the effects of higher doses of FG-7142 (10 and 30 mg per kilogram of body weight), the more potent partial inverse agonist β-CCE (10 mg per kilogram of body weight), and Ro15-4513 (0.1 to 10 mg per kilogram of body weight) administered either before (Fig. 2A) or after ethanol (*10*). As in our initial study, neither FG-7142 nor β-CCE had a significant effect on ethanol-induced intoxication. If, as suggested by Britton *et al.*, Ro15-4513 antagonizes ethanol-induced intoxication solely by means of its inverse agonist properties, then the addition of other partial inverse agonists to a submaximally effective dose of Ro15-4513 should result in an even greater antagonism. As shown in Fig. 2A, β-CCE administered with Ro15-4513 completely blocked the inhibition of intoxication observed with Ro15-4513 alone. These data suggest that Ro15-4513 has "anti-ethanol" effects not shared by other partial inverse agonists. We predict, however, that Ro15-4513 will not be the only partial or full inverse agonist found with "anti-ethanol" properties.

We would also like to reemphasize that not all the behavioral effects of ethanol are antagonized by Ro15-4513; the profound intoxication produced by higher doses of ethanol is not antagonized by Ro15-4513 (Fig. 2B; also see Fig. 1, upper panel).

Finally, we, and others, have repeatedly pointed out that the adverse pharmacokinetic and pharmacodynamic profile of Ro15-4513 would preclude its use in humans (*3*). However, as with any prototype compound, the development of a less toxic and more suitable analog remains a possibility. We do agree with Britton *et al.* that Ro15-4513 has proved to be a useful tool for delineating some of the neurochemical substrates of ethanol's behavioral effects.

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