Letters

Seizures in Drug-Treated Animals

In a recent article in the Research News section (5 Dec., p. 1198), Gina Kolata highlights important new results with a novel benzodiazepine derivative, especially its capacity to reverse the intoxicating effects of alcohol. This new substance, an imidazobenzodiazepine identified as Ro15-4513, is reported to (i) "make . . . drunk animals behave as though they were sober," (ii) "block . . . the behavioral effects of alcohol intoxication," (iii) prevent animals from "act[ing] intoxicated in the first place," and (iv) "appear to have no ill effects on the animals." In addition a photograph shows one rat "passed out from alcohol intoxication" and a second rat that "acted sober" after it was given Ro15-4513. Some of the most significant statements of this article concern the safety of Ro15-4513. Repeatedly, the article suggests that Ro15-4513 is without adverse or "ill" effects, "much less risky," and "safe."

In the same issue of *Science*, Suzdak *et al.* (Reports, p. 1243) state that "in contrast to other benzodiazepine receptor inverse agonists, Ro15-4513 alone at doses as high as 10 milligrams per kilogram of body weight produced no overt behavioral actions." Two types of behavioral measurements were performed in this project: (i) the number of licks on a water spout was counted, when every fifth lick produced an 0.4 milliampere electric shock [this measurement is widely used as a preclinical screen for drugs with anxiolytic properties (*1*)]; and (ii) ratings were made by an observer on a seven-point scale for 6 to 10 minutes assessing the magnitude of alcohol intoxication (*2*). Both measurements were conducted in laboratory rats of the Sprague-Dawley strain.

In our experiments with Ro15-4513 in rats and in squirrel monkeys, conducted during the past 4 months, we have found potentially serious side effects that may compromise the usefulness of Ro15-4513 as a drug that "counters alcohol intoxication." The most important detrimental effects of Ro15-4513 were evident in our experiments with adult male squirrel monkeys (*Saimiri sciureus*) housed as members of four social groups (*3*). Administration of Ro15-4513 (1 milligram per kilogram orally) produced severe tremors in seven out of eight monkeys; in two monkeys clear behavioral and physiological signs of convulsive activity, including tonic and clonic phases, were seen. The monkeys were observed to show muscular rigidity and posturing of the limbs, followed by repetitive contractions of somatic musculature. Since none of the information available to us led us to expect any such effects, we were not prepared to record electroencephalographic activity during our behavioral experiments. However, like others, we have not seen any such convulsive effects in our studies with rats.

Intrinsic behavioral effects of Ro15-4513 were evident in our experiments with both squirrel monkeys and rats (*4*). Briefly, disruption of social and aggressive behavior occurred in both species after administration of Ro15-4513 (1 milligram per kilogram by intraperitoneal injection in rats, orally in squirrel monkeys) in the absence of ethanol. The frequency of associative, threat, and attack behavior decreased by 60% to 80% from vehicle control levels. In view of these large changes it appears premature to refer to Ro15-4513 as having "no behavioral actions." However, in confirmation of the observations by Suzdak *et al.* we also recorded a partial restoration of motor activity that was impaired by large alcohol doses (1.5 to 3.0 grams per kilogram orally) in both rats and squirrel monkeys.

Our observations emphasize the necessity to conduct preclinical studies in primates in addition to laboratory rodents. Careful quantitative ethological measurements are required for an adequate assessment of the behavioral specificity and safety of therapeutically promising drugs.

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Response: Miczek and Weerts point out that Ro15-4513 has intrinsic behavioral actions in rats and squirrel monkeys that may limit its use as a potential alcohol antagonist in man. This conclusion has already been made by both research groups who have published data concerning the alcohol-blocking actions of this drug (*1*). Kolata's article deals primarily with the use of Ro15-4513 as a research tool and the pros and cons of developing a clinically effective "derivative or analogue" based on the behavioral and biochemical findings with Ro15-4513.

Since Ro15-4513 is a partial inverse agonist of the benzodiazepine receptor, as emphasized and cited in our report (p. 1244) as well as in the previous publications about this compound (*2–5*), it would be expected (and has already been reported) to have intrinsic pharmacological actions, including "anxiogenic" and proconvulsant effects. For this reason we administered Ro15-4513 in the conflict test at doses that did not significantly decrease the number of either punished or nonpunished responses; at these doses Ro15-4513 blocked the anticonflict effects of ethanol. At higher doses [or under different behavioral parameters (for example, shock intensity or amount of water deprivation)] Ro15-4513, like other inverse agonists, would be expected to decrease punished or nonpunished responses, or both (*6*), and would therefore appear to "antagonize" any drug that increases the number of punished responses, including pentobarbital. We would also like to emphasize that with the use of different behavioral tests, Ro15-4513 has been reported to antagonize the actions of barbiturates (*2, 3*). The observation of Miczek and Weerts that Ro15-4513 disrupts social and aggressive behaviors in more "naturalistic" behavioral paradigms confirms previous reports on other inverse agonists (*7*). Moreover, that Ro15-4513 has no convulsant effects in rats (which confirms our report of no overt or easily observable behavioral actions of Ro15-4513 in this species), but has tremorigenic and convulsive activity in squirrel monkeys, is consistent with previous studies on other inverse agonists reported by several groups (*8*). Schweri *et al.* (*9*) have shown, for example, that β-carboline carboxylate ethyl ester does not produce seizures in rats but is a potent convulsant in squirrel monkeys, a phenomenon that appears to be related to the differential metabolism of the labile ester moiety in rats and monkeys. Pharmacokinetic and pharmacodynamic differences between species have always been a major consideration in drug development.

As we pointed out to Kolata (p. 1198), Ro15-4513 is only a prototype. A clinically effective compound should be "a long-acting derivative" (ideally with a half-life greater than ethanol) devoid of "adverse effects" including those expected of a partial inverse agonist. The ability of Ro15-4513 to reverse or block many (but not all) of alcohol's behavioral effects, however, does not appear to be attributable to its inverse agonist properties, since in both our experiments and those of Bonetti *et al.* (*2*) and of Polc *et al.* (*3*), other partial or full inverse agonists tested either did not attenuate or only slightly attenuated alcohol's behavioral, biochemical, and electrophysiological actions. Thus, on the basis of the available biochemical and behavioral data, it appears that the inverse agonist and alcohol antagonist properties of benzodiazepine receptor ligands are dissociable, which makes the development of an