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23. We thank I. Jamry for technical help and S. Mandel of the Division of Biostatistics for statistical analysis. This work was supported by USPHS grants AM 19537, AM 12129, TO-AM 07284, HL 31102, and HL 14147 from the NIH, 5T32 GM07200, and in part by the Washington University–Monsanto Biomedical Research Agreement.

19 May 1986; accepted 17 September 1986

A Selective Imidazobenzodiazepine Antagonist of Ethanol in the Rat

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Ethanol, at pharmacologically relevant concentrations of 20 to 100 m*M*, stimulates γ-aminobutyric (GABA) receptor–mediated uptake of ^{36}Cl-labeled chlorine into isolated brain vesicles. One drug that acts at GABA-benzodiazepine receptors, the imidazobenzodiazepine Ro15-4513, has been found to be a potent antagonist of ethanol-stimulated $^{36}Cl^-$ uptake into brain vesicles, but it fails to antagonize either pentobarbital- or muscimol-stimulated $^{36}Cl^-$ uptake. Pretreatment of rats with Ro15-4513 blocks the anticonflict activity of low doses of ethanol (but not pentobarbital) as well as the behavioral intoxication observed with higher doses of ethanol. The effects of Ro15-4513 in antagonizing ethanol-stimulated $^{36}Cl^-$ uptake and behavior are completely blocked by benzodiazepine receptor antagonists. However, other benzodiazepine receptor inverse agonists fail to antagonize the actions of ethanol in vitro or in vivo, suggesting a novel interaction of Ro15-4513 with the GABA receptor–coupled chloride ion channel complex. The identification of a selective benzodiazepine antagonist of ethanol-stimulated $^{36}Cl^-$ uptake in vitro that blocks the anxiolytic and intoxicating actions of ethanol suggests that many of the neuropharmacologic actions of ethanol may be mediated via central GABA receptors.

ETHANOL IS ONE OF MAN'S OLDEST and most ubiquitous psychoactive drugs. The acute administration of relatively low doses of ethanol results in anticonflict and anxiolytic activity in laboratory animals (*1*) and man (*2*), and higher doses produce sedation, intoxication, coma, and death (*3*). Although ethanol produces a variety of diverse central nervous system (CNS) effects (*4*), it is still unclear which, if any, of these actions are related to its behavioral effects. Moreover, it is generally accepted that the pharmacological actions of ethanol and related short-chain alcohols result from relatively nonspecific interactions with biomembranes (*5*) because many of the behavioral effects of short-chain alcohols are highly correlated with both their lipid solubilities (*6*) and membrane fluidizing and disordering properties (*7*). However, these nonspecific membrane effects of alcohol may result in specific alterations in membrane function, including changes in the conformation of membrane-bound receptors such as those coupled to adenylate cyclase (*8*) and ion channels (*9*). We have reported that, at pharmacologically relevant concentrations (20 to 100 m*M*), ethanol and related short-chain alcohols stimulate γ-aminobutyric acid (GABA) receptor–mediated uptake of ^{36}Cl-labeled chlorine into isolated brain vesicles, and that this effect was correlated with both their membrane-buffer partition coefficients and intoxication potencies in rats (*10*). The action of short-chain alcohols in stimulating $^{36}Cl^-$ uptake in vitro appears to be mediated via the GABA-coupled Cl^- ion channel because the effects are blocked by the specific $GABA_A$ receptor antagonist bicuculline and the Cl^- channel antagonist picrotoxin (*10*).

In view of the many common neuropharmacological actions of benzodiazepines, barbiturates, and ethanol (*1, 3*) and, because both benzodiazepines and barbiturates have been shown to augment GABAergic neurotransmission (*11*), we have postulated that ethanol's action at the level of the GABA-coupled Cl^- ion channel may underlie many of its behavioral properties (*10*). In the course of studying the interactions of benzodiazepines, barbiturates, and ethanol on GABA receptor–mediated $^{36}Cl^-$ uptake in vitro, we found that the imidazobenzodiazepine Ro15-4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5α][1,4] benzodiazepine-3-carboxylate) (*12*) potently and selectively antagonized the ability of ethanol (but not pentobarbital or muscimol) to stimulate $^{36}Cl^-$ uptake in vitro. Moreover, parenteral administration of Ro15-4513 to rats resulted in a blockade of the anticonflict and intoxicating actions of ethanol. Both the in vitro and in vivo effects of Ro15-4513 in antagonizing the actions of ethanol were prevented by the central benzodiazepine receptor antagonists Ro15-1788 and CGS-8216, suggesting a novel interaction of Ro15-4513 with the benzodiazepine receptor complex. Thus, many of the behavioral and biochemical actions of ethanol may be mediated by central GABA-benzodiazepine receptors.

In our initial experiments, we examined a

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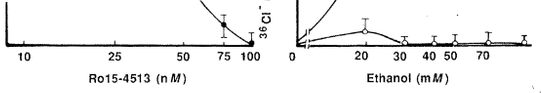

250 g) as described (*20*). $^{36}Cl^-$ uptake into these isolated brain vesicles was also carried out as described (*10, 13*). Various concentrations of Ro15-4513 (10 to 100 n*M*) were added to the synaptoneurosomes 5 minutes before the addition of ethanol (50 m*M*) and $^{36}Cl^-$. The data represent the mean ± SEM of quadruplicate determinations from a typical experiment carried out three times with similar results. Percent stimulation refers to the amount of $^{36}Cl^-$ uptake in the presence of ethanol with various concentrations of Ro15-4513 expressed as a percent of that observed with ethanol (50 m*M*) alone. (B) The effect of various concentrations of ethanol (20 to 100 m*M*) alone (●) or in the presence of Ro15-4513 (100 n*M*) (○) on the uptake of $^{36}Cl^-$ into cerebral cortical synaptoneurosomes (*20*). Uptake of $^{36}Cl^-$ was determined as described in (*13*) and above. Data represent the mean ± SEM of quadruplicate determinations from a typical experiment carried out three times with similar results. At all concentrations of ethanol tested, Ro15-4513 significantly decreased ethanol-stimulated $^{36}Cl^-$ uptake ($P < 0.01$, analysis of variance (ANOVA) followed by Newman-Keuls test).

series of benzodiazepine receptor antagonists and inverse agonists for their abilities to affect ethanol-stimulated $^{36}Cl^-$ uptake into brain vesicles (*10, 13, 14*). $^{36}Cl^-$ uptake into isolated brain vesicles (synaptoneurosomes) was measured as described [Fig. 1 and (*10, 13*)]. At pharmacologically relevant concentrations (10 to 100 m*M*), ethanol stimulated $^{36}Cl^-$ uptake into brain vesicles in a biphasic manner (*10*). Low concentrations of ethanol (≤10 m*M*) potentiated both muscimol- and pentobarbital-stimulated $^{36}Cl^-$ uptake, whereas higher (≥20 m*M*) concentrations directly stimulated $^{36}Cl^-$ uptake (*10*). Of the benzodiazepine receptor ligands tested, only the imidazobenzodiazepine Ro15-4513 antagonized ethanol-stimulated $^{36}Cl^-$ uptake in vitro (Table 1). Ro15-4513 is a very potent antagonist of ethanol-stimulated $^{36}Cl^-$ uptake (Fig. 1A); concentrations as low as 25 n*M* significantly inhibited $^{36}Cl^-$ uptake induced by a maximally effective concentration of ethanol (50 m*M*). At a concentration of 100 n*M*, Ro15-4513 completely antagonized ethanol-stimulated $^{36}Cl^-$ uptake in vitro (Fig. 1B). Because the GABA antagonists bicuculline and picrotoxin block both barbiturate- and GABA-stimulated $^{36}Cl^-$ uptake in vitro (*10, 13*), we determined whether Ro15-4513 would also antagonize pentobarbital- or muscimol-stimulated $^{36}Cl^-$ uptake. Ro15-4513 failed to affect either pentobarbital- or muscimol-stimulated $^{36}Cl^-$ uptake at concentrations as high as 1 μ*M* (Fig. 2). The specificity of Ro15-4513 in blocking the actions of ethanol at the GABA-coupled Cl^- ion channel is further indicated by experiments examining the effects of low (subthreshold) concentrations of ethanol or pentobarbital. Ro15-1788 (300 n*M*) was added simultaneously with the $^{36}Cl^-$ and ethanol. Preliminary experiments on the time course and dose response curve of the antagonism of Ro15-4513 by Ro15-1788 demonstrated that 300 n*M* Ro15-1788 produced a maximal antagonism of the inhibition of ethanol-stimulated $^{36}Cl^-$ uptake by Ro15-4513 with no preincubation. Data represent the mean ± SEM of quadruplicate determinations from a typical experiment repeated three times with similar results. Ro15-4513 significantly antagonized ethanol-stimulated $^{36}Cl^-$ uptake (**$P < 0.01$, ANOVA followed by Newman-Keuls test), an effect that was blocked by the benzodiazepine receptor antagonists Ro15-1788 (Ro15-1788 plus Ro15-4513 and ethanol groups were not significantly different) and CGS-8216 [CGS-8216 (1 μ*M*) plus Ro15-4513 and ethanol groups were not significantly different]. In contrast, Ro15-4513 failed to alter either muscimol- or pentobarbital-stimulated $^{36}Cl^-$ uptake.

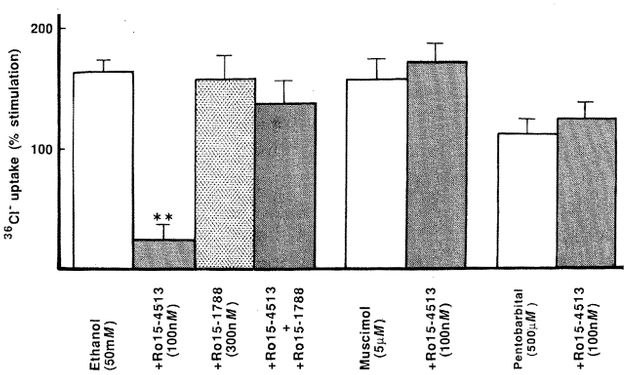

Fig. 2. The effect of the imidazobenzodiazepine Ro15-4513 (100 n*M*) on ethanol- (50 m*M*), muscimol- (5 μ*M*), and pentobarbital (500 μ*M*)-stimulated $^{36}Cl^-$ uptake into rat cortical synaptoneurosomes (*20*). Uptake of $^{36}Cl^-$ was determined as described in (*13*) and Fig. 1A. Ro15-4513 (100 n*M*) was added 5 minutes prior to the addition of 0.5 μCi of $^{36}Cl^-$ and ethanol, muscimol, or pentobarbital. Ro15-1788 (300 n*M*)

ligand of the benzodiazepine receptor (*12*), it was important to determine whether its action in blocking ethanol-stimulated $^{36}Cl^-$ uptake was in fact mediated by central benzodiazepine receptors. Preincubation of brain vesicles with a structurally related imidazobenzodiazepine, Ro15-1788 (ethyl-8-fluoro-5,6 dihydro-5-methyl-6-oxo-4H-imidazo[1,5α][1,4] benzodiazepine-3-carboxylate), a specific benzodiazepine receptor antagonist (*15*), had no effect on ethanol-stimulated $^{36}Cl^-$ uptake but completely blocked the actions of Ro15-4513 (Figs. 2 and 3). The effects of Ro15-4513 in antagonizing the action of both high (Fig. 2) and low (Fig. 3) concentrations of ethanol were reversed after preincubation with Ro15-1788. The pyrazoloquinolinone benzodiazepine receptor antagonist CGS-8216 (*15*) also reversed the effects of Ro15-4513. If the effect of Ro15-4513 in antagonizing ethanol-stimulated $^{36}Cl^-$ uptake is related to its weak inverse agonist properties (*12, 16*) then other inverse agonists of central benzodiazepine receptors should also be effective ethanol antagonists. However, none of the other partial or full benzodiazepine receptor inverse agonists tested had any effect on ethanol-stimulated $^{36}Cl^-$ uptake (Table 1).

From these data it is apparent that submicromolar concentrations of Ro15-4513 selectively block ethanol-stimulated $^{36}Cl^-$ uptake in vitro. To determine whether Ro15-4513 also modifies the behavioral actions of ethanol, we examined Ro15-4513 in two behavioral paradigms. At relatively low doses ethanol administration has been previously shown to produce an anticonflict action in a variety of species (*1*). In one such test in which the drinking behavior of thirsty rats is inhibited by punishment (*17*), the parenteral administration of ethanol (1 g per kilogram of body weight) resulted in a significant increase in punished responding (Fig. 4A). Pretreatment of rats with Ro15-4513 (3 mg per kilogram of body weight) completely blocked the anticonflict actions of ethanol in this paradigm. As observed in vitro, the effect of Ro15-4513 was reversed by pretreatment with Ro15-1788 (Fig. 4A). In contrast, at a dose of pentobarbital (4 mg per kilogram of body weight) that resulted in an increase in punished responding of similar magnitude to ethanol or at a dose (10 mg per kilogram of body weight) that resulted in a near maximal increase in punished responding, Ro15-4513 had no effect on pentobarbital-induced increases in pun-

ished responding (Fig. 4A). Ro15-4513 alone had no significant effect on punished or nonpunished responding in these same experiments.

The ability of ethanol to induce a state of behavioral intoxication is one of its most important behavioral actions. Using the behavioral rating scale of Majchrowicz (*18*), we determined a dose of ethanol that produced an intoxication score of approximately 3 (ED_3), defined as the presence of general sedation, accentuated staggered gait, and impaired righting reflex. Rats were pretreated with Ro15-4513 (2.5 to 10 mg per kilogram of body weight) or vehicle, then with ethanol (2 g per kilogram of body weight), and finally they were rated by an investigator who was unaware of the animal's treatment status. Pretreatment with Ro15-4513 markedly reduced the level of intoxication induced by ethanol (Fig. 4B). Again, the antagonism of ethanol-induced intoxication by Ro15-4513 was reversed by pretreatment with Ro15-1788 and CGS-8216. The actions of Ro15-4513 in antagonizing the behavioral effects of ethanol were not due to a drug-induced reduction in blood ethanol concentration because no change in peak blood ethanol concentration was observed in Ro15-4513–treated rats (*19*). Finally, like the in vitro actions of Ro15-4513 in blocking $^{36}Cl^-$ uptake, the behavioral actions of this drug were not duplicated by the benzodiazepine receptor inverse agonist FG-7142, further suggesting a novel interaction of Ro15-4513 with the benzodiazepine receptor complex. Moreover, in contrast to other benzodiazepine receptor inverse agonists, Ro15-4513 alone at doses as high as 10 mg per kilogram of body weight produced no overt behavioral actions.

Our data demonstrate that a novel imidazobenzodiazepine, Ro15-4513, which selectively antagonizes ethanol-stimulated $^{36}Cl^-$ uptake via the GABA-coupled Cl^- ion channel, also blocks the anticonflict and intoxicating properties of ethanol in vivo. This is consistent with a recent report that Ro15-4513 antagonizes the sedation and motor impairment induced by ethanol in rodents (*14*). Thus, the behavioral effects of low to moderate doses of ethanol appear to be in part mediated by central GABA receptors. It is as yet unknown, however, whether Ro15-4513 will antagonize the behavioral and CNS effects (for example, respiratory depression and coma) observed after higher doses of ethanol; the latter could be mediat-

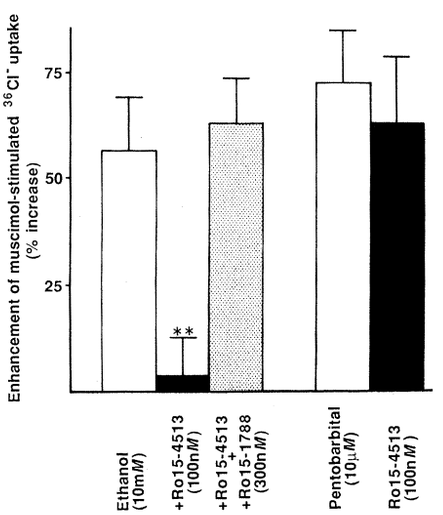

Fig. 3. The effect of Ro15-4513 (100 n*M*) on the ethanol- (10 m*M*) and pentobarbital- (10 μ*M*) induced potentiation of muscimol (5 μ*M*)-stimulated $^{36}Cl^-$ uptake into rat cortical synaptoneurosomes (*20*). $^{36}Cl^-$ uptake was determined as described in (*13*) and Fig. 1A. At the concentrations indicated, neither ethanol or pentobarbital stimulated $^{36}Cl^-$ uptake alone. Data are expressed as the percent enhancement of muscimol-stimulated $^{36}Cl^-$ uptake. In this experiment muscimol (5 μ*M*) alone stimulated $^{36}Cl^-$ uptake by 90 ± 10%. Data represent the mean ± SEM of quadruplicate determinations from a typical experiment carried out three times with similar results. Ro15-4513 significantly attenuated ethanol ($P < 0.01$, ANOVA followed by Newman-Keuls test), but not pentobarbital-induced potentiation of muscimol-stimulated $^{36}Cl^-$ uptake, and this effect was reversed by Ro15-1788 (300 n*M*) ($P < 0.01$, ANOVA followed by Newman-Keuls test).

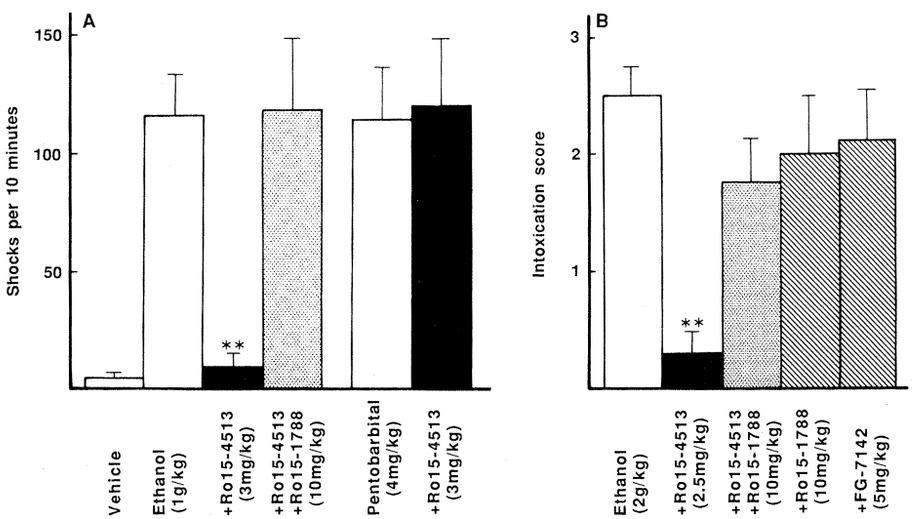

Fig. 4 (A). The anticonflict effects of ethanol were assessed by the method of Vogel *et al.* (*17*). Data are expressed as the mean ± SEM of the number of shocks during 10-minute sessions and are from a representative experiment. All groups contain ten rats unless otherwise indicated. Ro15-4513 had no significant effect on punished responding (4.7 ± 1.3 shocks per session for Ro15-4513 and 6.9 ± 1.5 shocks per session for saline control; not significantly different; $n = 6$) or nonpunished responding (119 ± 43.2 shocks per session for Ro15-4513 and 178 ± 18.3 shocks per session for saline control; not significantly different; $n = 6$). Ro15-1788 (10 mg per kilogram of body weight) alone did not potentiate the effects of ethanol (1 g per kilogram body weight) on punished responding. Pentobarbital (10 mg per kilogram of body weight) produced a near maximal increase in punished responding (445.6 ± 20.1 shocks per session), an effect that was not different from that observed following pretreatment with Ro15-4513 (3 mg per kilogram body weight) (424 ± 36.5 shocks per session; not significantly different). **Significantly different from ethanol alone ($P < 0.001$ ANOVA followed by Newman-Keuls test). The blockade of the ethanol-induced increase in punished responding by Ro15-4513 was reversed by pretreatment with Ro15-1788 [Ro15-1788 plus Ro15-4513 and Ro15-4513 alone are significantly different ($P < 0.001$); Ro15-4513 plus Ro15-1788 and ethanol are not significantly different; ANOVA followed by Newman-Keuls test]. (B) The effect of Ro15-4513 on ethanol-induced intoxication in the rat. Ro15-4513 (2.5 to 5.0 mg per kilogram of body weight) was administered 5 minutes prior to ethanol. Ro15-1788 (10 mg per kilogram of body weight, intraperitoneally) was administered 1 minute prior to Ro15-4513. Behavioral intoxication was scored by an observer who was unaware of the treatment conditions with the rating scale of Majchrowicz (*6, 18*). Ethanol was administered as a 20% volume to volume solution in saline. Ro15-4513 and Ro15-1788 were given as a suspension in 4% Tween-80 in saline. A dose of ethanol (2 g per kilogram of body weight) was chosen that produced an intoxication score of between 2 and 3 (*18*). Values represent the mean ± SEM of individual intoxication scores from a representative experiment ($n = 10$) for each group. Ro15-4513 (2.5 to 5.0 mg per kilogram of body weight) significantly ($P < 0.01$, ANOVA followed by Newman-Keuls test) blocked the intoxication induced by ethanol (2 g per kilogram of body weight). This was not due to a decrease in blood ethanol concentration since there was no difference in blood ethanol levels between the two groups (*19*). Ro15-1788 significantly attenuated the ability of Ro15-4513 to reverse ethanol-induced intoxication [Ro15-4513 (2.5 mg per kilogram of body weight) plus ethanol (2 g per kilogram of body weight), 0.25 ± 0.16; Ro15-1788 (10 mg per kilogram of body weight) plus Ro15-4513 (2.5 mg per kilogram of body weight) plus ethanol (2 g per kilogram of body weight), 1.75 ± 0.36 ($P < 0.01$, ANOVA followed by Newman-Keuls test)]. Ro15-1788 (10 mg per kilogram of body weight) alone or the inverse agonist FG-7142 (5 or 10 mg per kilogram of body weight) had no significant effect on ethanol-induced intoxication.

ed by other neurotransmitter receptors or voltage-dependent channels known to be affected by higher concentrations of ethanol (*8, 9*). Finally, although it is possible that the in vitro actions of Ro15-4513 are unrelated to its behavioral actions, the reversal of both by the selective benzodiazepine receptor antagonists Ro15-1788 and CGS-8216 suggests a common receptor-mediated mechanism.

The exact mechanism underlying the specificity of Ro15-4513 in blocking the neurochemical and behavioral actions of ethanol is not clear but presumably involves some unique interaction with the benzodiazepine recognition site associated with the $GABA_A$ receptor complex. Because the ability of ethanol and other short-chain alcohols to stimulate GABA receptor–mediated $^{36}Cl^-$ uptake is highly correlated with their membrane-buffer partition coefficients (*10*), we have suggested that ethanol's effects on Cl^- ion flux are mediated via an alteration in the lipid-protein microenvironment of the GABA receptor complex, rather than by binding directly to the receptor complex.

Table 1. The effect of benzodiazepine receptor antagonists and inverse agonists on ethanol-stimulated $^{36}Cl^-$ uptake in vitro. Brain vesicles (synaptoneurosomes) were prepared (*13, 14*) and incubated in assay buffer at 30°C for 20 minutes. Five minutes before the addition of 0.5 μCi of $^{36}Cl^-$ and ethanol (50 m*M*), different benzodiazepine receptor antagonists or inverse agonists were added. For all experiments Ro15-4513, Ro15-1788, FG-7142 (*N*-methyl-β-carboline-3-carboxamide), β-CCE (β-carboline-3-carboxylate), and DMCM (6,7-dimethyl-4-ethyl-β-carboline-3-carboxylate) were dissolved in ethanol and subsequently diluted with assay buffer. The final concentration of ethanol in the diluent for these drugs never exceeded 0.7 m*M*. Parallel sets of tubes were run as controls and contained the same amount of ethanol. CGS-8216 (2-phenylpyrazolo[4,3-*c*] quinolin-3(5H)-one) was dissolved in 0.1*N* HCl and diluted in assay buffer, and the final *p*H adjusted to 7.4. An aliquot of vehicle, prepared in the same manner, was also added to a series of tubes assayed in parallel. Uptake of $^{36}Cl^-$ was terminated 5 seconds later by the addition of 5 ml of assay buffer. Each value represents the mean ± SEM of quadruplicate determinations from three separate experiments. Only the imidazobenzodiazepine Ro15-4513 significantly decreased ethanol-stimulated $^{36}Cl^-$ uptake ($P < 0.01$, ANOVA followed by Newman-Keuls test).

Drug	Concentration	Ethanol-stimulated $^{36}Cl^-$ uptake (% of ethanol response)
Ethanol	50 m*M*	100 ± 12
+ DMCM	1 μ*M*	116 ± 19
+ β-CCE	1 μ*M*	97 ± 16
+ FG-7142	1 μ*M*	103 ± 12
+ CGS-8216	1 μ*M*	109 ± 29
+ Ro15-1788	1 μ*M*	105 ± 26
+ Ro15-4513	0.1 μ*M*	6 ± 12

Since the GABA-benzodiazepine receptor is believed to be a transmembrane receptor–coupled ionophore (*11*), it is conceivable that ethanol and Ro15-4513 may interact at a common hydrophobic domain of this receptor complex. Regardless of its exact mechanism of action, Ro15-4513 promises to be a valuable tool for studying the behaviorally relevant CNS actions of ethanol and for unraveling the mechanisms underlying the rewarding and dependency-promoting properties of ethanol. Moreover, based on our observations, it may be possible to develop a clinically effective ethanol antagonist.

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muscles); 5, complete loss of righting reflex; 6, coma; 7, death. Animals were observed for 6 to 10 minutes after the administration of ethanol.

19. Blood ethanol was measured in parallel experiments in trunk blood [P. K. Wilkinson, J. G. Wagner, A. J. Sedman, *Anal. Chem.* **47**, 1506 (1975)] and was: Ro15-4513 pretreated, 257 ± 31 mg/dl; vehicle, 280 ± 14 mg/dl (n = 12); not significantly different by Student's t test.
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21. We thank W. E. Haefely (F. Hoffmann–La Roche and Co., Basel, Switzerland) for the gift of Ro15-4513, E. Lane (NIAAA, NIH, Bethesda, MD) for determining blood ethanol concentrations, and T. Welsh and J. Infante for typing the manuscript.

18 March 1986; accepted 1 October 1986

Age of the Earliest African Anthropoids

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The earliest fossil record of African anthropoid primates (monkeys and apes) comes from the Jebel Qatrani Formation in the Fayum depression of Egypt. Reevaluation of both geologic and faunal evidence indicates that this formation was deposited in the early part of the Oligocene Epoch, more than 31 million years ago, earlier than previous estimates. The great antiquity of the fossil higher primates from Egypt accords well with their primitive morphology compared with later Old World higher primates. Thus, the anthropoid primates and hystricomorph rodents from Fayum are also considerably older than the earliest higher primates and rodents from South America.

THE EARLIEST ANTHROPOID PRIMATES of Africa come from extensive early Tertiary deposits in the Fayum depression of Egypt, approximately 100 km southwest of Cairo. From their initial discovery in the beginning of the century, the fossil primate species from this area have played a critical role in our understanding of anthropoid origins and the evolution of monkeys, apes, and humans (*1–3*). Moreover, the Fayum provides the best record of Paleogene mammals from all of Africa and is critical for understanding the evolution of many mammalian groups on that continent including marsupials, pangolins, elephant shrews, bats, insectivorans, creodonts, hyracoids, elephants, anthracotheres, embrithopods, and hystricomorph rodents. Knowledge of the age of the Fayum deposits is critical for calibrating early aspects of higher primate evolution and for understanding the biogeography and evolution of many other mammals (*4, 5*).

The preponderance of apparently endemic African elements in the Fayum mammalian fauna, however, precludes precise faunal correlation between the Fayum and other paleontological localities that could place this fauna in a worldwide chronological framework. Comparison of the Fayum mammals with Eocene and Oligocene mammals from Eurasia showed only three common genera (*Peratherium*, *Pterodon*, and *Apterodon*) and five common families. All faunal comparisons indicated that the Egyptian fossils were most comparable to late Eocene and early Oligocene taxa from Europe, but sufficiently distinct that a more precise correlation was impossible (*6*).

All the Fayum primates and most of the terrestrial mammals have come from the fluvial Jebel Qatrani Formation (Fig. 1), which conformably overlies the nearshore marine and fluvial Qasr el Sagha Formation. The Jebel Qatrani Formation is comprised of 340 m of variegated alluvial rocks and fine to coarse sandstones, conglomerates, sandy mudstones, carbonaceous mudstones, and limestones, all of which show evidence of profound mechanical and geochemical alteration due to ancient soil (paleosol) formation. Vertebrate fossils have been recovered from dozens of localities throughout the formation (Fig. 1).

The Jebel Qatrani Formation is a complex alluvial unit characterized by distinct large- and small-scale lateral and vertical facies changes (*7*). In general, deposition was by meandering streams. Local, small-scale changes in lithology reflect shifting from one local channel environment to another and transitions from channel to floodplain deposits. The Jebel Qatrani lithotope was low and had little relief. The occasional occurrence of sirenians and brackish water mollusks, sharks, and rays suggests that storms, tidal incursions, or both, increased the salinity of the streams for several kilometers inland. The flood basins of the Jebel Qatrani streams were apparently heavily vegetated in many areas, as evidenced by numerous fossil root casts and areas with abundant fossil trees. There were areally large, but shallow and probably ephemeral, nonsaline ponds; soils were generally damp with probably seasonal rainfall.

The fossil megafloras show affinities with present-day tropical Indomalaysian floras. They suggest a "tropical forest existing in a wet, perhaps monsoonal climate" (*5*). Like the paleoflora, the soils indicate seasonal wetness with good drainage in some areas and swampy conditions in others.

Inasmuch as neither the sedimentary rocks nor the mammalian fauna of the Jebel Qatrani Formation provide precise evidence regarding the age of the formation, the best evidence for the age of this formation and the primates found there comes from the respective ages of the immediately overlying and underlying rocks—the Widan el Faras Basalt above and the partly marine and partly fluvial Qasr el Sagha Formation below.

In the Fayum depression, the upper Qatrani escarpment is capped by 2 to 25 m of the Widan el Faras Basalt, a dark, densely aphanitic, iron-rich extrusive basalt that is exposed for over 50 km. In outcrops where the basalt is thinnest, it appears to be a single flow; however, weathered and scorched contacts within the basalt as well as lenses of gravelly sand containing basaltic clasts attest to the presence of at least two and probably three separate flows over much of the area of exposure. The flows overlie the Jebel Qatrani Formation with a pronounced erosional unconformity, evidenced locally by broad scours of up to 25 m in depth. The thickness, and even the presence of individual flows, is controlled by the topographic irregularities at the top of the Jebel Qatrani Formation. In places where the basalt flows are absent, the Jebel Qatrani Formation is

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