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Effects of Ro 15-4513 on Schedule-Induced Ethanol Intake in Rats and Schedule-Maintained Responding in Pigeons

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EFFECTS OF RO 15-4513 ON SCHEDULE-INDUCED ETHANOL INTAKE IN RATS AND SCHEDULE-MAINTAINED RESPONDING IN PIGEONS

by

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EFFECTS OF RO 15-4513 ON SCHEDULE-INDUCED ETHANOL INTAKE IN RATS AND SCHEDULE-MAINTAINED RESPONDING IN PIGEONS

Dawn D. Delaney, Ph.D.

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The partial inverse benzodiazepine receptor agonist, Ro 15-4513, is a recently synthesized compound with the ability to antagonize some of the biochemical and behavioral effects of low to moderate doses of ethanol and related CNS depressants. The present investigations sought to explore further the behavioral actions of Ro 15-4513. In Experiment 1, the effects of acute (0.6-5.4 mg/kg) and chronic (5.4 mg/kg) administrations of Ro 15-4513 on rats' schedule-induced consumption of water and 8% ethanol solution were examined. Acute and chronic administrations of Ro 15-4513 slightly reduced consumption of both liquids. No selective effects on ethanol consumption were observed.

In Experiment 2, the acute and chronic effects of Ro 15-4513 were examined in pigeons responding under a multiple fixed-ratio 25 interresponse-time-greater-than-6-seconds (mult FR 25 IRT > 6-sec) schedule of food delivery. Acute administrations of Ro 15-4513 (1.0-5.4 mg/kg) produced dose-related reductions in response rates under the FR component, but had no effect on rates under the IRT > 6-sec component. Some tolerance to the rate-

suppressing effects of Ro 15-4513 was evident when 5.4 mg/kg was given chronically.

In all, the present findings are consistent with a growing body of literature indicating that (a) Ro 15-4513 does not selectively block all of the behavioral effects of ethanol, and (b) Ro 15-4513 alone is behaviorally active. Contrary to early reports, Ro 15-4513 is not a selective amethystic agent without intrinsic behavioral activity.

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Dawn D. Delaney

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GENERAL INTRODUCTION

Ethyl alcohol (ethanol) is undoubtedly the most widely consumed and abused psychotropic drug known to humankind. Recent estimates from the National Institute on Drug Abuse reveal that nearly 75% of the United States (U.S.) citizens over 12 years of age report consuming ethanol at least once during the past year, and of these, nearly 30% report that they consume ethanol once a week or more (NIDA, 1988). The drinking patterns among American drinkers are far from equal, however. A tenth of the drinking population is estimated to consume half of the alcoholic beverages sold (NIAAA, 1983).

In our society, ethanol has the unique distinction of being the only potent pharmacological agent with which obvious self-induced intoxication is socially acceptable (Jaffe, 1985). Although the popularity of ethanol suggests that it is a relatively safe drug, that is not necessarily the case. An estimated 10 million adults and 3 million adolescents (between the ages of 14 and 17 years) in the U.S. experience problems directly related to their use of ethanol (Drug Abuse Policy Office, 1984). These problems include accidents on the job and in the home, dysfunctional family interactions, traffic accidents, fetal abnormalities, liver and heart disease, and organic brain syndromes (e.g., Korsakoff's psychosis). Measured in terms of accidents, lost employment and productivity, healthcare, and

property loss and crime, the combined costs of problem drinking were estimated for 1980 to exceed $89 billion (NIAAA, 1987). The cost in human misery, however, is beyond calculation.

Although none are as effective as desired, a number of different approaches are currently in use for modifying abusive patterns of ethanol use (Jaffe, 1985). One approach, traditional psychotherapy, places emphasis on the hypothesized emotional problems or personality disorders which are believed to underlie the drug abuse. Self-help groups such as Alcoholics Anonymous (AA) offer another approach to the treatment of ethanol abuse by providing appropriate role models in the form of recovering alcoholics and an environment which supports sobriety and admonishes drug use. Supervisory-deterrent approaches are also available, which typically motivate the individual to abstain from drug use with threats of loss of valuable reinforcers such as a job or freedom from incarcination. One of the popular adjunctive treatments for ethanol abuse in the U.S. is the use of disulfiram (Antabuse), a drug that blocks the second step in ethanol metabolism, the conversion of acetaldehyde to acetyl coenzyme A, by inhibiting acetaldehyde dehydrogenase. The resulting accumulated level of acetaldehyde in the blood produces an aversive condition consisting of flushing, throbbing headache, nausea, vomiting, decreased blood pressure and thirst (Radcliffe, Rush, Sites, & Cruse, 1985). From a learning perspective, ethanol self-administration is punished by this unpleasant reaction,

which occurs within minutes of ethanol ingestion. As will be discussed shortly, another pharmacological agent, Ro 15-4513, has recently been developed which decreases ethanol self-administration. This agent, not yet used in treatment, operates on the principle of operant extinction rather than punishment by blocking several of ethanol's behavioral and stimulus effects.

Pharmacology of Ethanol

Like other general central nervous system (CNS) depressants, ethanol exerts its primary action by depressing all the neurons in the brain. At low doses, ethanol has been found to induce behavioral excitement (i.e., increased locomotor activity) and mild euphoria, effects which contribute to the popular misconception that ethanol is a "stimulant." These stimulating effects are explained, in part, by the depression of inhibitory neurons within the brain (i.e., disinhibition) when low doses of ethanol are ingested. There is little doubt, however, that ethanol is a primary and continuous CNS depressant (Chrusciel, 1982; Goth, 1984; Ritchie, 1985). As the dose of ethanol is increased, excitatory neurons are also depressed, and a predictable sequence of behavioral depression ensues, ranging from relief from anxiety to sedation, release from inhibitions, sleep, general anesthesia, coma and finally death as the result of depression of the respiratory centers in the medulla (Julien, 1985).

The pharmacological profile of ethanol is similar to that of several other general CNS depressants, namely the barbiturates and benzodiazepines. Among the pharmacological properties shared by these agents are sedation, hypnosis, decreased anxiety, muscle relaxation and anticonvulsant activity. Tolerance develops to some of the behavioral effects of these agents, and some degree of cross-tolerance across ethanol, benzodiazepines, and barbiturates is evident. Chronic administration of these drugs results in physical dependence. The pharmacological similarities that exist between these three classes of related CNS depressants suggests that a common modulatory pathway may be involved in their central action. Since benzodiazepines are now known to act through a y-aminobutyric acid (GABA) mechanism and barbiturates have a binding site in the GABA-benzodiazepine receptor complex, many recent investigations have examined the effect of ethanol on GABAergic pathways (Ticku & Kulkarni, 1988). As yet, however, its effects are not understood fully.

Development and Neuropharmacology of Ro 15-4513

A new drug has recently been developed which has been found to antagonize some of the biochemical and behavioral effects of low to moderate doses of ethanol and other related CNS depressants. This drug, known simply as Ro 15-4513, is a partial inverse agonist with the ability to bind to central (i.e., brain)

benzodiazepine receptors as determined by photoaffinity labeling (Mohler, Sieghart, Richards & Hunkeler, 1984; Sieghart, Eichinger, Richards & Mohler, 1987) and other electrophysiological evidence (Mereu, Passino, Carcangiu, Boi, & Gessa,1987). Synthesized by Hunkeler, a chemist at Hoffmann-La Roche, Ro 15-4513 (ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-[1,5-a][1,4] benzodiazepine-3-carboxylate) is the structural analogue of the classic benzodiazepine antagonist Ro 15-1788, a compound that has been found to displace benzodiazepines from their binding sites and counteract certain biochemical, electrophysiological and behavioral effects of these agents. Unlike Ro 15-4513, however, Ro 15-1788 produces no protection against the actions of ethanol (Hunkeler, Mohler, Pieri, et al., 1981). Very little has been written about partial inverse agonists, although it is reported that such agents have affinity for (i.e., the capacity to bind to) particular receptors, but cannot stimulate those receptors to produce the same maximal effect as a full agonist, regardless of concentration. In addition, partial inverse agonists may antagonize the action of these more potent agonists (Goth, 1984).

Early reports from researchers at Hoffmann-La Roche revealed that Ro 15-4513 was capable of antagonizing some of the biochemical and behavioral effects of several CNS depressants, including ethanol (Bonetti, Polc, & Pieri, 1984; Bonetti, Burkard, Gabl, & Mohler, 1985; Polc, 1985). This latter

finding was substantiated in a report published shortly thereafter by Suzdak, Glowa, Crawley, Schwartz, Skolnick and Paul (1986), who found that Ro 15-4513 "potently and selectively" antagonized the ability of ethanol to stimulate GABA receptor-mediated uptake of $^{36}Cl^-$-labeled chloride into isolated rat brain vesicles. Moreover, they reported that pretreating rats with Ro 15-4513 completely blocked the anticonflict and intoxicating actions of ethanol. Both the in vitro and in vivo effects of Ro 15-4513 in antagonizing the actions of ethanol in this investigation were prevented by the benzodiazepine antagonist Ro 15-1788, suggesting that Ro 15-4513 may produce its antagonism by interacting with ethanol at the central GABA-benzodiazepine receptor ionophore complex.

As discussed by Ticku and Kulkarni (1988), the GABA-benzodiazepine receptor complex consists of several sites of drug action, including GABA receptors, benzodiazepine receptors, a picrotoxin site and a barbiturate site. A possible mode of ethanol action involving chloride coupling has been envisaged as also being a part of this receptor complex (i.e., an ethanol modulatory site). This complex helps to understand how the benzodiazepines, barbiturates, and ethanol produce similar pharmacolgoical effects, as well as how Ro 15-4513 produces its antagonism. The pharmacological effects which are common to these three classes of drugs are believed to be produced by the action of these drugs on their respective sites of action, namely the benzodiazepine

receptors, the barbiturate site, and the ethanol modulatory site. In addition, the GABA receptors are activated by the inhibitory neurotransmitter, GABA, an endogenous substance which is known to be involved in the central control of motor coordination (Wachtel & Anden, 1978 as reported in Ticku & Kulkarni, 1988). A functional relationship is believed to exist between GABA neurotransmission and motor impairment during ethanol intoxication, with decreases in GABA producing a decrease in the motor impairment induced by ethanol, and increases in GABA enhancing ethanol-induced impairment (Ticku & Kulkarni, 1988). Ro 15-4513 is hypothesized to produce its antagonism of ethanol (as well as of related CNS depressants) by acting at the benzodiazepine receptor part of this receptor complex. However, the data on this are not conclusive and several other possiblities have been proposed. Reviews of the posited effects of Ro 15-4513 at the neurological and biochemical levels are provided by Suzdak et al. (1986) and by Ticku and Kulkarni (1988), and Hunt (1985) provides a complete review of the GABAergic actions of ethanol.

Some understanding of the neuropharmacology and biochemical actions of Ro 15-4513 and the drugs it is purported to antagonize is useful in attempting to understand studies that have examined at a behavioral level the interaction of Ro 15-4513 and ethanol, reviewed in the following section. In that section and throughout the manuscript, a conceptual position is

taken consistent with that explicated in the behavioral pharmacology literature (e.g., Thompson & Schuster, 1968; Weiss & Laties, 1976). Such an analysis uses the principles of operant psychology as initially formulated by Skinner (1938, 1953, 1969) to account for behavior, including drug self-administration. The primary aspects of such an approach have been cogently expressed elsewhere (e.g., Thompson & Pickens, 1969, 1972; Thompson & Schuster, 1968; Weiss & Laties, 1969). In brief, this approach views the abusive use of drugs as a behavioral problem, with the variables controlling it the same as those controlling any behavior. Drugs are treated as environmental stimuli capable of affecting behavior in a manner analogous to other, more extensively studied, classes of stimuli, such as food, colored key lights and electric shock. Approaching drugs as stimuli makes it possible to profit from previous studies of the variables affecting behavior.

REVIEW OF RELATED LITERATURE

Overview of Behavioral Studies Conducted to Date

Among the actions of ethanol studied in combination with Ro 15-4513 are motor impairment, spontaneous motor activity, anticonflict activity, hypothermia, anticonvulsant activity, lethality and the increase in seizure activity observed as part of the withdrawal syndrome. Oral ethanol self-administration, the discriminative stimulus properties of ethanol, and schedule-controlled responding have also been evaluated in the presence of Ro 15-4513. In reviewing research in theses areas, an effort has been made to present the studies in the order in which they were conducted. This format provides an historical view of the development of Ro 15-4513 and the manner in which the results of earlier investigations were received by the research community.

Motor Impairment, Anticonflict Activity, and Hypothermia

Among the well-known behavioral effects of ethanol (as well as other CNS depressants) is impairment of motor coordination (ataxia). Numerous behavioral assays have been developed in recent years to investigate the effects of various drugs on motor impairment, and the following section reviews studies published to date which have employed several of these

assays in an attempt to investigate the effect of Ro 15-4513 on ethanol-induced motor impairment. The studies conducted by Bonetti and his colleagues were the first reported investigations of the effects of Ro 15-4513 on motor impairment induced by ethanol and other CNS depressants. These researchers employed the horizontal wire test to assess behavioral impairment. In this test subjects (typically mice or rats) are lifted by the tail and allowed to grasp a horizontal wire with their forepaws. The subjects are then released, and the number failing to heave themselves onto the wire is noted. Drugs which produce sedation and muscle relaxation are particularly active in this test. The results of these investigations revealed that Ro 15-4513 (3 mg/kg) reversed completely the behavioral impairment induced in mice by the benzodiazepine diazepam (3 mg/kg), by the barbiturate phenobarbitone (100 mg/kg) (Bonetti et al., 1984), and by ethanol (3 g/kg in mice, 6 g/kg in rats). The ED_{50} dose of Ro 15-4513 for blocking ethanol-induced motor impairment was 0.84 mg/kg in mice and 8.62 mg/kg in rats (Bonetti et al.,1985). (The ED_{50} is the point at which 50% of the subjects show the desired effect.) The antagonism of both the phenobarbitone and ethanol effects by Ro 15-4513 was lastingly abolished by Ro 15-1788 (3 mg/kg) when administered both before or after Ro 15-4513, suggesting that the ataxic effects of both ethanol and phenobarbitone are mediated via the benzodiazepine receptor complex.

Polc (1985) followed up these preliminary reports by comparing the effects of Ro 15-4513 with those of another partial inverse agonist, FG 7142, on ethanol- and phenobarbitone-induced reductions in locomotor activity in rats. In this study, Ro 15-4513 (3-30 mg/kg) alone produced no effect on locomotor activity. However, Ro 15-4513 antagonized the reduction of motility caused by ethanol (3 g/kg) without affecting the depressant effect of phenobarbitone (100 mg/kg). As noted by the author, this selective action of Ro 15-4513 against ethanol is supported by the finding that FG 7142 (10-100 mg/kg) failed to antagonize the ethanol-produced decrease of locomotor activity. The reasons for the failure of Ro 15-4513 to antagonize the behavioral effects of phenobarbitone in this investigation are unclear.

Support for the findings reported by Polc (1985) is provided by Suzdak et al. (1986), who used two behavioral measures of ethanol intoxication to investigate the effect of Ro 15-4513 on ethanol-induced intoxication: An anticonflict paradigm and the behavioral rating scale developed by Marjchrowicz (1975). Relatively low doses of ethanol have been found to produce an anticonflict (i.e., antipunishment) action in a variety of species (Glowa & Barrett, 1976), an action often attributed to the anxiolytic (sedative) effects of this drug. In the version of the anticonflict paradigm employed by Suzdak et al. (1986), the drinking behavior of water-deprived rats was suppressed by

punishment (i.e., response-dependent electric shock to the feet). Subjects given a low dose of ethanol (1 g/kg) showed an increase in punished responding, as did those that received the barbiturate pentobarbital (4 mg/kg). The ethanol-induced increase in punished responding was blocked by pretreating the rats with Ro 15-4513 (3 mg/kg), although Ro 15-4513 had no effect on the increased responding induced by pentobarbital. In addition, pretreatment with Ro 15-4513 (2.5 mg/kg) reduced the level of gross intoxication induced by 2 g/kg ethanol (defined as the presence of general sedation, accentuated staggered gait, and impaired righting reflex), as measured by Majchrowicz's scale. These effects of Ro 15-4513 in antagonizing both of these measures of ethanol-induced intoxication were prevented by pretreating the rats with Ro 15-1788. Consistent with Polc's (1985) observation that Ro 15-4513 alone had no effect on locomotor activity, Ro 15-4513 had no effect on punished or nonpunished responding at doses as high as 10 mg/kg.

Another investigation of the effects of Ro 15-4513 on ethanol-induced behavioral impairment was conducted by Hoffman, Tabakoff, Szabo, Suzdak and Paul (1987) who examined the ability of Ro 15-4513 to reverse the incoordinating and hypothermic effects of ethanol in mice. The incoordinating effects of ethanol were quantified using an accelerating rotarod treadmill; hypothermia was assessed via rectal temperature. Under these procedures, Ro 15-4513 (10 mg/kg, but not 5 mg/kg)

reversed the incoordinating effect of ethanol (1.5 and 2.0 g/kg), but had no effect on ethanol-induced hypothermia. Ro 15-4513 (10 mg/kg) also reversed the hypothermic effect of pentobarbital, and at a higher dose (20 mg/kg) reversed the incoordinating effect of this barbiturate. In addition, Ro 15-4513 alone (10 and 20 mg/kg) had no effect on Rotarod performance or on body temperature. The finding that Ro 15-4513 failed to block ethanol-induced hypothermia suggests that ethanol-induced hypothermia may not be mediated by the GABA-benzodiazepine receptor complex.

Although these data confirm the findings previously discussed concerning the ability of Ro 15-4513 to antagonize ethanol-induced motor impairment, the finding that Ro 15-4513 antagonized pentobarbital-induced motor impairment supports the findings of Bonetti et al. (1984), but contradicts the reports by Polc (1985) and Suzdak et al. (1986) that Ro 15-4513 failed to antagonize pentobarbital-induced decreases in motility and increases in punished responding in rats. The reasons for these conflicting results are unknown, but it is possible that pentobarbital produces antianxiety effects and/or incoordination through different mechanisms in mice and rats.

In a further attempt to determine if Ro 15-4513 differed from other benzodiazepine receptor inverse agonists in its ability to antagonize the behavioral effects of ethanol, Lister (1987a) compared the effects of Ro 15-4513 with those of another

benzodiazepine inverse agonist, FG 7142, on the behavior of mice in a holeboard test by examining the interactions of these two compounds with ethanol. The holeboard apparatus used in this study had four holes 3 cm in diameter equally spaced in the floor. Infrared photocells in the walls of the box and directly beneath each hole provided automated measures of locomotor activity, the number of exploratory head-dips made, and the duration of head-dipping. The results of this study revealed that both Ro 15-4513 (1.5 and 3.0 mg/kg) and FG 7142 (10-20 mg/kg) reversed the reductions in the number of head dips caused by a relatively low dose of ethanol (2 g/kg), and the higher doses of these two drugs also partially reversed the locomotor stimulant action of ethanol. Ro 15-4513 (0.75-3.0 mg/kg) given alone decreased both the number of head dips and the duration of head dipping, a finding reported in previous reports by Lister (1987b, 1987c). Like Ro 15-4513, FG 7142 decreased the number of head dips and duration of head dipping.

These data were among the first to contradict the statement made in earlier reports that Ro 15-4513 was a "selective" ethanol antagonist (Polc, 1985; Suzdak et al. 1986) . This statement was based, in part, on the findings that Ro 15-4513 failed to block the behavioral impairment induced by pentobarbital, and that of several other full and partial inverse benzodiazepine receptor agonists tested (e.g., B-CCM, B-CCE, FG 7142), Ro 15-4513 alone reversed ethanol-stimulated chloride

uptake into synaptic vesicles and ethanol-induced intoxication. It is impossible to determine the reasons for these reported differences, but more recent investigations generally support the findings that (a) Ro 15-4513 is not unique in its ability to antagonize the behavioral effects of ethanol, and (b) Ro 15-4513 antagonizes the effects of CNS depressants other than ethanol. One such investigation was conducted by Lister (1987c), who found that Ro 15-4513 (1.5 mg/kg) antagonized the reduction in the number of head dips produced by diazepam (2 mg/kg), ethanol (2 g/kg) and pentobarbital (30 mg/kg).

In addition to showing that Ro 15-4513 was capable of blocking some of the effects of low to moderate doses of ethanol, these early behavioral investigations suggested that Ro 15-4513 had the potential to be developed for clinical use in the treatment of ethanol abuse. Moreover, the discovery that Ro 15-4513 operated at the GABA-benzodiazepine receptor complex provided researchers with a useful tool for investigating the cellular basis of ethanol's behavioral actions. The studies which follow represent recent attempts to assess the behavioral effects of Ro 15-4513 on ethanol-induced behaviors other than motor impairment to gain further insight into the range of ethanol's effects that may be mediated by this receptor complex.

Self-Administration

In addition to its motor incoordinating, sedating and antianxiety effects, another of ethanol's well-known behavioral actions is its ability to maintain behavior leading to its administration. By definition, when behavior is strengthened as a result of contingent presentations of a stimulus, that stimulus is said to be functioning as a positive reinforcer (Skinner, 1938). There is little doubt that ethanol functions to maintain ethanol self-administration in many humans; under the appropriate conditions, nonhumans can also be induced to initiate and maintain behavior leading to the administration of ethanol (Griffiths, Bigelow & Henningfield, 1980). An investigation of the effects of Ro 15-4513 on oral ethanol reinforcement has been reported by Samson, Tolliver, Pfeffer, Sadeghi, and Mills (1987). Using a sucrose-fading procedure, these investigators initiated ethanol self-adminstration in rats. Under this procedure, rats were maintained on free access to both food and water, and initially trained to press a lever to obtain a 20% sucrose solution. When presentation of the sucrose solution was maintaining responding, low ethanol concentrations were added to the solution. Over the course of approximately 25 sessions, the solution presented as reinforcement was gradually reduced in sucrose concentration until a 10% ethanol solution with no sucrose was presented. The results of this investigation reveal

that Ro 15-4513 (0.1-3.0 mg/kg) produced a dose-related suppression of ethanol responding, and corresponding reduction in ethanol self-administration.

Lethality

The studies reported thus far have generally supported the initial observation that Ro 15-4513 reverses some of the behavioral effects of low to moderate doses of ethanol. The extent to which Ro 15-4513 will antagonize the effects of higher doses of ethanol has received little attention, however. Fadda, Mosca, Colombo, and Gessa (1987) were the first to report the results of an investigation of the ability of Ro 15-4513 to block the lethal effects of ethanol. According to these authors, pretreatment with Ro 15-4513 (10 mg/kg) "totally protected against the intoxication and mortality induced by massive doses of ethanol" (5-15 g/kg). (Intoxication was assessed using the Majchrowicz rating scale ;Majchrowicz, 1975); mortality was assessed at one week from intoxication.) These results, however, have not been replicated. For example, in an attempt to replicate this effect, Poling , Schlinger, and Blakely (1989) pretreated rats with Ro 15-4513, followed by a lethal dose of ethanol (5.4 g/kg i.p.) ten minutes later. The number of rats that had died in each group (members of each group received either 0, 1, 3, 10. or 30 mg/kg Ro 15-4513) was recorded 1, 2, 3, 4, 8, 16, and 24 hours

after ethanol administration. The results of this study revealed that Ro 15-4513 (at all doses tested) failed to block the lethal effects of ethanol, a finding substantiated by an unpublished report by Haefely from Hoffman-La Roche (reported in Kolata, 1986). The failure of Ro 15-4513 to antagonize the lethal action of ethanol suggests that unlike the low to moderate dose behavioral effects of ethanol, ethanol-induced lethality is not mediated by the GABA-benzodiazepine receptor complex.

Discriminative Stimulus Properties of Ethanol

The purpose of an investigation reported by Rees and Balster (1988) was (a) to determine if Ro 15-4513 would block ethanol's discriminative stimulus effects and (b) to investigate the selectivity of Ro 15-4513 to block the discriminative stimulus effects of other CNS depressants. In this study, different groups of mice were trained to discriminate 1.0 or 1.5 g/kg ethanol (groups 1 and 2), 20 mg/kg of pentobarbital (group 3) or 10 mg/kg of oxazepam (group 4) from saline in a two-lever operant task. Stimulus generalization tests were conducted with Ro 15-4513 alone (0.01-20 mg/kg) and in combination with the training drugs. The results of this investigation reveal that the discriminative stimulus effects of ethanol and oxazepam, but not those of pentobarbital, were blocked by Ro 15-4513. Moreover, when given alone, Ro 15-4513 decreased overall response rates in

a dose-related fashion, a finding evident in all four training groups. The authors note that although ethanol, barbiturates, and benzodiazepines share discriminative stimulus properties under many conditions, the selective blockade of the stimulus effects of ethanol and oxazepam by Ro 15-4513 provides further evidence that their actions may be mediated by different cellular mechanisms.

Schedule-Controlled Responding

Woudenberg and Slangen (1988) investigated the effects of Ro 15-4513 alone and in combination with ethanol (1.25 g/kg) on schedule-controlled responding in rats. Under a simple fixed-ratio ten (FR 10) schedule of food delivery, both ethanol and Ro 15-4513 (5 mg/kg) reduced the rate of responding when administered alone. In combination, Ro 15-4513 (5 mg/kg) failed to antagonize the rate-reducing effects of ethanol; instead, the response-suppressing effects of Ro 15-4513 were additive with those of ethanol, resulting in a lower response rate than when either drug was administered alone. In addition, Ro 15-4513 alone (0.625-10 mg/kg) was found to dose-dependently reduce responding under a tandem variable-interval 40-second (VI 40-sec) FR 10 schedule of food delivery. The failure to antagonize the effect of Ro 15-4513 by doses of Ro 15-1788 up to 60 mg/kg in this experiment suggests that the strong response suppression

caused by Ro 15-4513 is not mediated by the benzodiazepine receptor. Substantiation of this suggestion awaits further investigation.

Seizure Activity

One of the earliest reports of the behavioral effects of Ro 15-4513 revealed that at doses as low as 1 mg/kg Ro 15-4513 increased the convulsive effects of pentylenetetrazole (PTZ) in mice (Bonetti et al., 1984). Based on the findings of this preliminary report, Lister and Nutt (1988) conducted a further investigation of the proconvulsant effects of Ro 15-4513. To induce seizures, chemical convulsants were infused intravenously, a method reported by the authors to be reliable and sensitive to both pro- and anti-convulsant drugs. Chemical convulsants with different sites of pharmacological action were chosen, including agents acting at the GABA-benzodiazepine receptor complex (e.g., PTZ, bicuculline), glycine receptors (strychnine), 5-hydroxytryptamine (5-HT) receptors (quipazine) and adenosine receptors (caffeine). The latency to the onset of repeated myoclonic jerking of head and forelimbs was used for determining seizure thresholds, except for strychnine where the onset of tonic seizure activity was used. The results of this investigation revealed that Ro 15-4513 (5 mg/kg) significantly lowered seizure thresholds to PTZ, bicuculline, and the convulsant benzodiazepine Ro 5-3663, but failed to alter seizure

thresholds to picrotoxin, strychnine, caffeine, and quipazine. Consistent with the report by Bonetti et al. (1984), at no dose administered (i.e., 0.75-6.0 mg/kg Ro 15-4513) was spontaneous seizure activity observed. As noted by the authors, the lowering of seizure thresholds to bicuculline, PTZ, and Ro 5-3663 and the lack of interactions with the other convulsants is consistent with the suggestion that Ro 15-4513 is a partial inverse agonist at the benzodiazepine receptor.

In a related investigation, Nutt and Lister (1987) examined the specificity of Ro 15-4513 as an antagonist of the anticonvulsant effects of diazepam, pentobarbital and ethanol. Bicuculline was used to induce seizures, and latency to the onset of repeated myoclonic jerking of head and forelimbs was used for determining seizure thresholds. In addition to significantly lowering seizure threshold to bicuculline, Ro 15-4513 completely reversed the anticonvulsant effects of diazepam, but failed to antagonize the anticonvulsant effects of either pentobarbital or ethanol. Based on the absence of a statistically significant interaction between Ro 15-4513 and either of these agents, the authors suggested that the ability of Ro 15-4513 to antagonize the behavioral effects of ethanol (as well as of pentobarbital) may be due to a functional opposition of behavioral effects rather than a receptor-mediated interaction.

A recent investigation has been undertaken to study the selectivity of Ro 15-4513 in reversing the anticonvulsant

property of ethanol by comparing the effects of this agent against pentobarbital, known to also facilitate GABAergic transmission, and the partial inverse agonist, FG 7142. Kulkarni and Ticku (1989) used bicuculline and picrotoxin for inducing chemoconvulsions in rats. Onset of myoclonic jerks, tonic extensor phase, mortality time and percent protection against mortality were measured. The results of this investigation reveal that pretreatment with Ro 15-4513 blocked the protective effect of ethanol against bicuculline-induced tonic extension and mortality, an action sensitive to reversal by Ro 15-1788. However, onset of myoclonic jerks and duration of clonus were not significantly altered. Ro 15-4513 also reversed partially the protective effect of pentobarbital against bicuculline- and picrotoxin-induced convulsions. FG 7142 failed to reverse the protective effect of ethanol and pentobarbital against bicuculline-induced tonic extension although it reversed the effect against onset and mortality. FG 7142 had no effect on the protective effect against picrotoxin-induced convulsions. These observations suggest that Ro 15-4513 is a stronger antagonist of ethanol effects than the other partial inverse agonist, FG 7142.

Several studies have investigated whether the proconvulsant effects of Ro 15-4513 are also observed in mice undergoing ethanol withdrawal. As noted by Lister and Karanian (1987), such a finding would severely restrict the clinical use of Ro 15-4513 as an alcohol antagonist. In one such investigation,

Lister and Karanian (1987) exposed mice to ethanol vapors in inhalation chambers. The ethanol vapor concentration in the chamber was increased over the first 24 hours, and maintained at a constant level for the remaining 48 hours. Three injections of 4-methyl pyrazole, an alcohol dehydrogenase inhibitor, were administered at 24-hour intervals, following which the mice were removed from the chambers and ethanol withdrawal was assessed. The results of this study revealed a greater incidence of seizures in mice withdrawing from ethanol if they were treated with Ro 15-4513 than if they received vehicle, suggesting that it may be dangerous to use Ro 15-4513 as an ethanol antagonist in alcoholic people.

A follow-up investigation of these findings has recently been reported by Becker and Anton (1989). The exacerbation of ethanol withdrawal by Ro 15-4513 reported by Lister and Karanian (1987) was observed at only one point in time (i.e., 6.5 hours post-ethanol withdrawal) and only in DBA/2 mice, a strain that is relatively seizure-prone to a variety of pharmacologic treatments. Thus it is not clear whether this effect is strain-specific and whether the exacerbation of ethanol withdrawal by Ro 15-4513 varies as a function of time following ethanol withdrawal.

In the study reported by Becker and Anton (1989), one group of mice was continuously exposed to ethanol vapor for 72 hours in an inhalation chamber; the remaining control mice were housed in

an identical chamber for the same period of time, but in the absence of ethanol. Ethanol intoxication was induced by administering an intraperitoneal (IP) injection of ethanol, and blood alcohol levels (BALs) were stabilized by daily injections of the alcohol dehydrogenase inhibitor pyrazole. At the end of the 72-hour exposure period, mice were removed from the chamber and withdrawal behavior was scored. Ro 15-4513 was given at various times during this withdrawal phase. When administered immediately following chronic ethanol exposure (0 hours post ethanol exposure), Ro 15-4513 did not influence the withdrawal response. However, when given later (3, 5, and 8 hours postethanol withdrawal), Ro 15-4513 significantly increased the severity of the withdrawal response in ethanol-exposed mice. This exacerbation was completely reversed by pretreatment with the benzodiazepine receptor antagonist Ro 15-1788, suggesting mediation through the GABA-benzodiazepine receptor complex. These data indicate that the benzodiazepine inverse agonist, Ro 15-4513, is capable of exacerbating, but not precipitating, ethanol withdrawal, a finding which severely limits the potential clinical use of Ro 15-4513 as an amethystic agent.

Summary

Although Ro 15-4513 generated considerable excitement as a possible selective amethysitic agent, the studies reviewed

above indicate that Ro 15-4513 (a) blocks some effects of drugs other than ethanol, and (b) fails to block some effects of ethanol. Moreover, the proconvulsant activity of Ro 15-4513 severely limits its clinical utility as an ethanol antagonist.

The remainder of this manuscript primarily describes novel investigations of the effects of Ro 15-4513 on schedule-induced ethanol self-administration in rats and on schedule-maintained behavior in pigeons.

EXPERIMENT 1: EFFECTS OF RO 15-4513 ON SCHEDULE-INDUCED ETHANOL INTAKE IN RATS

INTRODUCTION

A report of the effects of Ro 15-4513 on ethanol-maintained responding in rats has recently appeared in the literature (Samson et al., 1987). As previously described, in this study oral ethanol self-administration was initiated through the use of a sucrose-fading procedure, and at no time were the three subjects food- or water-deprived. Under these conditions, pretreatment with Ro 15-4513 (0.3-3.0 mg/kg) produced a dose-related suppression of ethanol-maintained lever-pressing, an action attributed to a decrease in the reinforcing efficacy of ethanol.

The sucrose-fading procedure is a recent development for initiating oral ethanol self-administration by nonhuman subjects such as monkeys and rats. A major problem in studies of oral drug self-administration in nonhumans is that subjects initially reject most drug solutions, very likely because they have an aversive taste. This problem has been largely overcome through the use of inducing procedures such as the sucrose-fading procedure and the schedule-induced polydipsia (SIP) procedure, which uses intermittent delivery of small amounts of food to initiate drinking (Meisch, 1976; Meisch & Thompson, 1972). Falk (1961) was the first to report that food-deprived rats exposed to

schedules of intermittent food delivery ingested excessive amounts of water. In sessions lasting approximately three hours and under a fixed-interval 90-second (FI 90-sec) schedule of food delivery, Falk's rats drank an average of 92 ml of water, compared with their pre-experimental daily intake of 27 ml. The first report of schedule-induced ethanol intake appeared in 1961, shortly after Falk originally reported the phenomenon. In that year, Lester (1961) found that food-deprived rats receiving food pellets on a fixed-time 55-second (FT 55-sec) schedule consumed enough 5.6% (weight/volume) ethanol solution to show behavioral intoxication at the end of 3-hour test sessions. Since then, schedule-induced drinking has been extensively studied and employed in studies of oral ethanol self-administration, studies which have demonstrated that after exposure to inducing procedures, ethanol characteristically functions as a reinforcer in its own right (e.g., Meisch & Thompson, 1974).

The purpose of the present experiment was to assess (a) the generality of the findings reported by Samson et al. (1987) by using a schedule-induced drinking procedure, and (b) the chronic effects of Ro 15-4513 on oral ethanol self-administration. In the present study, the effects of Ro 15-4513 on water intake, as well as ethanol intake, were examined. Samson et al. (1987) only evaluated the effects of Ro 15-4513 on ethanol intake, hence it is not clear from their results whether the drug selectively reduced

the reinforcing effects of ethanol, or simply reduced drinking *per se*.

METHOD

Subjects

Four adult male Sprague-Dawley rats obtained from the Upjohn Company (Kalamazoo, MI) were used in this study. Free-feeding weights as established prior to experimental testing were as follows: Subject 1 weighed 435 gms, Subject 2 weighed 445 gms, Subject 3 weighed 425 gms, and Subject 4 weighed 410 gms. Subjects were gradually reduced to 80% of their respective free-feeding weights, a weight maintained by daily feedings following experimental sessions. Subjects were housed two to a group in stainless steel hanging rodent cages (47 cm long, 31 cm wide and 20 cm deep) located in a colonly room with controlled temperature (74-76^{o} F) and lighting (dark from 7:00 p.m. to 7:00 a.m.).

Apparatus

Two aluminum operant conditioning chambers, measuring approximately 24 cm wide, 36 cm long, and 20 cm high, were used for behavioral testing. A response lever centered horizontally on the front wall of each chamber 7.5 cm above the floor was present, but inoperative in this study. A pellet trough into which 45-mg Noyes rat pellets could be delivered was located 3 cm below and to the left of the lever. Constant ambient illumination

was provided by a 7-w white light (houselight) located 5 cm above the lever. A drinking bottle was horizontally centered and secured to the outer left side wall; the spout protruded 2 cm into the chamber through a small hole located 2.5 cm above the floor. The drinking bottles were calibrated in ml, which allowed the amount of fluid consumed to be easily recorded. When subjects contacted the spout with lips or tongue, an electrical circuit was completed and a lick recorded. Each operant chamber was enclosed in a sound attenuating box equipped with a fan for ventilation and masking noise. Electromechanical equipment located adjacent to the operant chambers recorded licks and arranged scheduled food deliveries.

Behavioral Procedure

Subjects were induced to self-administer water using a FT 60-sec schedule of food delivery. A session began with onset of the houselight and fan. A single 45 mg Noyes pellet was delivered every 60 seconds regardless of the subject's behavior, and the session terminated after 60 food deliveries. These conditions remained in effect until daily water consumption was stable, the criterion for which was 5 consecutive sessions with no obvious trend. Once this criterion was met, an 8% (v/v) ethanol solution was substituted for the water on alternating days. A single 60-

min session was conducted for each subject 6 days per week at about the same time each day.

Pharmacological Procedure

Once performance was stable (as defined above) under both water- and ethanol-present conditions, acute dose-response determinations were begun. Ro 15-4513 was prepared as an ultrasonified suspension in distilled water to which 2-3 drops of Tween 80 were added. Four doses were tested: 1.0, 1.8, 3.2, and 5.4 mg/kg. In all phases of the study, drug and vehicle control injections were administered intraperitoneally (ip) at an injection volume of 1 ml/kg, 15 minutes prior to experimental testing. A BDBVD design was employed (B=baseline, D=drug, V=vehicle), with water and ethanol continuing to be placed in the drinking bottle on alternating days. Drug doses were administered in random order to each subject, and each dose of Ro 15-4513 was administered twice under both ethanol- and water-present conditions.

Following completion of the acute dose-response determinations, three of the four subjects received testing under chronic drug administration procedures. A fourth subject (S3) became ill at the end of the acute regimen and was removed from the study. For the others, each received a minimum of five baseline sessions, followed by 10 consecutive sessions in which

a dose of 5.4 mg/kg Ro15-4513 was administered 15 minutes prior to testing. This procedure was repeated twice, once with water in the drinking bottle, and again with ethanol in the bottle. The order of exposure to ethanol and water was randomly determined for each subject: Subject 1 received water, then ethanol; S2 and S4 received ethanol, then water.

RESULTS

Although the number of licks per 60-minute session were recorded, this measure was not found to be reliable, due to the intermittent failure of subjects to break contact with the tube between drinks. Because of this, ml consumed is the sole dependent measure of importance. As seen in Table 1, a comparison between water and ethanol (8% v/v) consumption under baseline and vehicle control conditions reveals a clear preference for water over ethanol for two subjects (S1 and S2), but no clear preference for either liquid by the remaining two subjects. Pretreatment with Ro 15-4513 (0.6, 1.8 and 5.4 mg/kg) produced a slight suppression of water consumption relative to control conditions in S2 and S4, although this reduction was not clearly dose-dependent. At no dose of Ro 15-4513 tested was there a substantial effect on ethanol consumption, although a slight overall reduction in consumption is evident.

Figure 1 shows the effects of Ro 15-4513 on water and ethanol consumption expressed relative to vehicle control values. This figure clearly shows the slight suppression in both water and ethanol consumption observed at all doses of Ro 15-4513 tested. The only notable exception is seen at the lowest dose (0.6 mg/kg), where a slight increase in ethanol intake is evident.

As shown in Figure 2, baseline levels of water consumption prior to chronic testing were approximately the same as those

obtained during the acute phase of this experiment for S1, but were slightly reduced for S2 and S4, possibly as the result of a sequencing effect. Baseline levels of ethanol consumption were slightly reduced for S1, approximately the same for S2, and substantially reduced for S4.

The clearest result obtained from the data shown in Figure 2 is that the chronic administration of Ro 15-4513 produced a general suppression of both water and ethanol consumption, although ethanol consumption was reduced to a slightly greater extent than water consumption.

Table 1

Mean Water and Ethanol Consumption (in mls)
for Individual Subjects During Acute Dose-Response Testing

	Water Consumption (ml)					Ethanol Consumption (ml)				
	Baseline	Vehicle	0.6	1.8	5.4	Baseline	Vehicle	0.6	1.8	5.4
S1	31.3 (2.6)	30.6 (2.5)	32.0	32.5	22.0	17.2 (1.9)	14.6 (3.4)	16.0	15.0	13.0
S2	30.8 (6.0)	34.0 (4.1)	23.0	28.0	26.0	18.2 (5.2)	18.6 (2.9)	25.5	19.5	15.0
S3	14.2 (1.6)	12.0 (2.9)	9.5	13.5	12.0	10.6 (3.3)	10.7 (5.3)	10.5	6.0	13.0
S4	12.0 (2.1)	10.6 (0.5)	9.0	8.0	9.0	14.3 (1.2)	12.3 (1.2)	13.5	5.0	7.5

Note: Numbers in parentheses represent 1 standard deviation. Baseline means are based on 6 observations; vehicle means are based on 3 observations; Ro 15-4513 means are based on 2 observations at each dose.

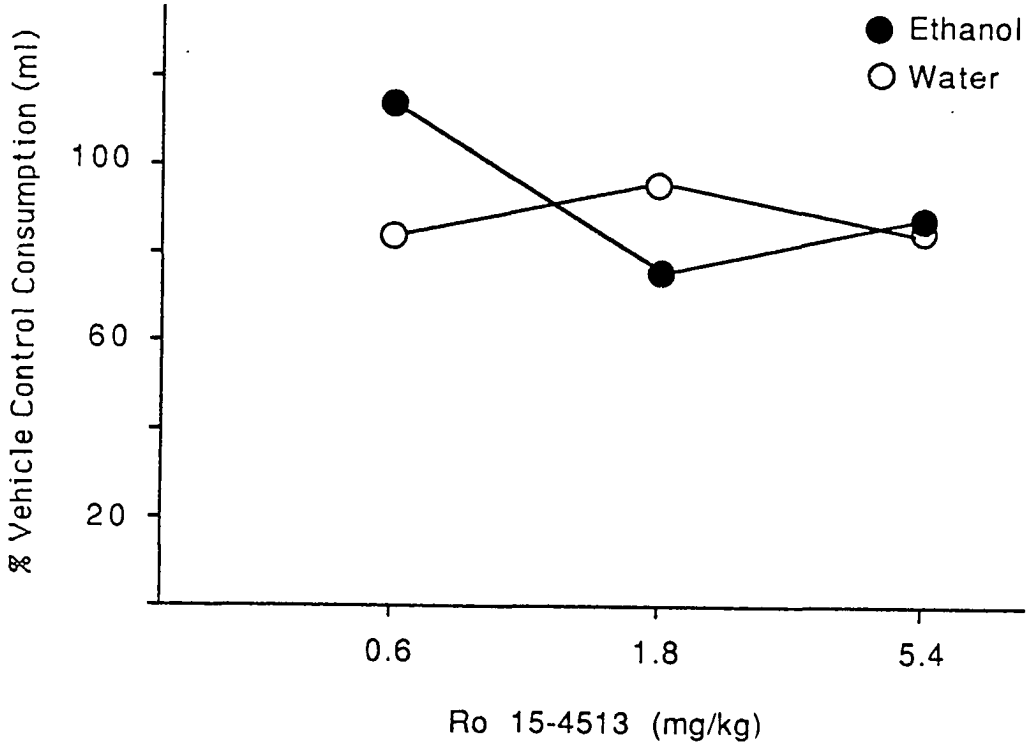

Figure 1. Group Data Showing the Effects of Acute Administrations of Ro 15-4513 on Schedule-Induced Consumption of Water and 8% Ethanol Solution. Values shown are expressed relative to vehicle control.

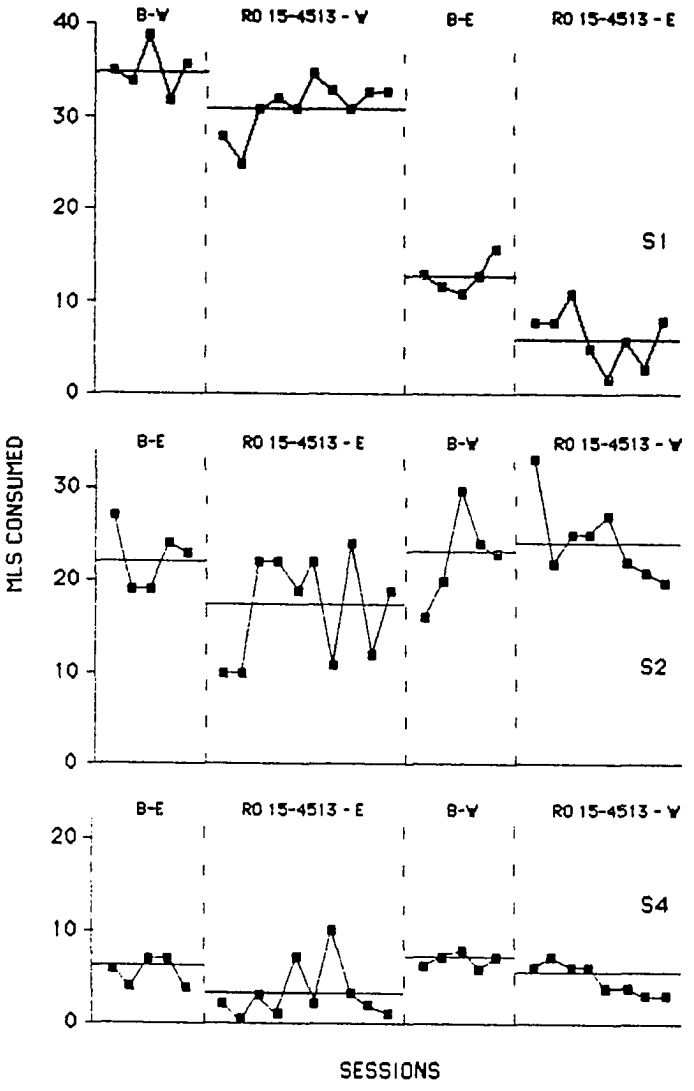

Figure 2. Individual Data Showing the Effects of Chronic Administration of Ro 15-4513 on Schedule-Induced Consumption of Water and 8% Ethanol Solution.

Figure 2. Individual Data Showing the Effects of Chronic Administration of Ro 15-4513 on Schedule-Induced Consumption of Water and 8% Ethanol Solution. Data at B-W represent five consecutive sessions under baseline conditions with water in the drinking bottle; data at B-E represent five consecutive sessions under baseline conditions with an 8% ethanol solution in the bottle. Data at Ro 15-4513-W represent ten consecutive days of chronic administration of 5.4 mg/kg Ro 15-4513 with water in the drinking bottle; data at Ro 15-4513-E represent ten consecutive days of chronic administration of 5.4 mg/kg Ro 15-4513 with an 8% ethanol solution in the bottle. The horizontal line within each condition represents the mean number of mls consumed by each subject during that condition.

DISCUSSION

The results of the present experiment reveal that the acute administration of Ro 15-4513 did not differentially affect water or ethanol consumption, although a slight overall decrease in consumption of both liquids was noted. Under chronic administration, Ro 15-4513 again reduced consumption of both fluids, with ethanol consumption reduced to a slightly greater extent than water consumption. These results suggest that the primary effect of Ro 15-4513 in the present investigation was to produce a general suppression of fluid consumption.

Samson et al. (1987) previously reported that Ro 15-4513 reduced ethanol intake, and the present results are in agreement with that finding. However, in the present study, the drug reduced schedule-induced intake of water, as well as ethanol. This suggests that under the present procedure, although not necessarily under that used by Samson et al.(1987), Ro 15-4513 did not *selectively* reduce ethanol consumption. Interestingly, the reversal of ethanol's antipunishment effects by Ro 15-4513 can be interpreted in terms of general drug-induced response suppression: The reversal of ethanol's antipunishment effects by Ro 15-4513 (Suzdak et al., 1986) involves a reduction in responding when Ro 15-4513 is given. Such an analysis cannot, however, account for the effects of Ro 15-4513 on the discriminable properties of ethanol (Rees & Balster, 1988), nor

for the effects of the drug in non-operant assays in which rate is not a dependent variable.

EXPERIMENT 2: EFFECTS OF RO 15-4513 ON SCHEDULE-MAINTAINED RESPONDING IN PIGEONS

INTRODUCTION

The results of experiment 1 are interesting primarily in indicating that Ro 15-4513 is in some cases behaviorally active in its own right. Schedule-induced drinking, however, provides for a relatively crude assay of drug effects. In an attempt to explore further the behavioral effects of Ro 15-4513 alone, the present study examined the acute and chronic effects of the drug on the responding of pigeons under a multiple fixed-ratio 25 interresponse-time-greater-than-6 -seccnds (mult FR 25 IRT > 6-s) schedule of food delivery. This schedule characteristically engenders high rates under one component (i.e., the FR) and low rates under the other (i.e., the IRT > t), and has a demonstrated sensitivity to many kinds of drugs (e.g., McKearney & Barrett, 1978). To date, nothing has been reported concerning (a) the behavioral effects of Ro 15-4513 in pigeons, (b) the behavioral effects of chronic administrations of Ro 15-4513, and (c) the effects of Ro 15-4513 under schedules of reinforcement that engender low rates of responding. The reported study provides information in each of these areas.

METHOD

Subjects

Three adult female White Carneau pigeons, maintained at 80% of free-feeding weights, served as subjects. All subjects had experience responding under multiple schedules of food delivery, but had no drug history. During periods outside experimental sessions, they were individually housed with unlimited access to grit and water.

Apparatus

Experimental testing was conducted in three computer-controlled operant conditioning chambers, measuring 32 cm long, 36 cm high, and 35 cm wide. In each chamber, three response keys 2.5 cm in diameter were located 23 cm from the bottom of the front wall, approximately 5.5 cm apart. Only the left and right keys, each of which could be illuminated in red or blue-green, were lighted and operative in this study. A minimum of 0.2 g pressure was required for key operation. An aperture centered horizontally on the front wall 7.5 cm above the floor allowed access to a hopper filled with mixed grain when the hopper was raised. When raised, the hopper was illuminated by a 7-W white bulb. A 7-W white bulb (houselight) centrally mounted 33 cm above the chamber floor provided ambient illumination and a

white noise generator provided masking sound. Programming of experimental events and data collection were accomplished through the use of a Digital Equipment Corp. (Maynard, MA) PDP8/A computer using interfacing and software (SUPERSKED) supplied by State Systems Inc. (Kalamazoo, MI).

Behavioral Procedure

Because all subjects had histories of food-maintained key pecking, initial training was not required and subjects were exposed from the onset of the study to the mult FR 25 IRT > 6-s schedule of food delivery. Under this schedule, red key illumination was correlated with the FR 25 component and blue-green key illumination was correlated with the IRT > 6-s component. Food was delivered for 3 s following every 25th keypeck under the FR 25 component, and following the first response emitted at least 6 s from the previous food delivery or the onset of blue-green key illumination under the IRT > 6-s component. Components alternated at 4-min intervals, with the initial component selected at random each session. Red key illumination was always presented on the left key, whereas blue-green illumination was always presented on the right key. A single 24-min session was conducted for each bird 6 days per week at about the same time each day.

Pharmacological Procedure

Subjects were exposed to the mult FR 25 IRT > 6-s schedule until the response rates of the individual birds were stable under both components. The criterion for stability was three consecutive sessions in which the response rate in each individual session was within 10% of the mean rate of responding across those three sessions. Once this criterion was met, acute dose-response determinations were begun. Four doses of Ro 15-4513 (1.0, 1.8, 3.2, and 5.4 mg/kg), suspended in a vehicle of distilled water with Tween 80 (2 drops/10 ml) added , were evaluated. In all phases of the study, drug (and vehicle control) injections were administered intramuscularly (IM) at an injection volume of 1 ml/kg, 15 minutes prior to testing. During acute dose-response determinations, each subject received all doses on two occasions, in random order. All drug administrations were separated by a minimum of three sessions in which responding was stable as defined above; one of these sessions was preceded by a control (vehicle) injection.

Following completion of the acute dose-reponse determinations, all subjects received control injections prior to at least five consecutive sessions. Subjects then received 5.4 mg/kg Ro 15-4513 prior to each of 15 consecutive sessions. Following the fifteenth day of chronic exposure, subjects were injected with a challenge dose of 10 mg/kg.

RESULTS

Figure 3 shows overall response rates for individual subjects under all experimental conditions. In the absence of drug, all subjects responded at a much higher rate under the FR 25 component than under the IRT > 6-s component. In all subjects, mean response rates under the FR component were below the control value at all doses of Ro 15-4513. However, drug rates differed very little from the control value at 1 mg/kg for all subjects and at 1.8 mg/kg for subject 2. At all other doses, rates under the FR differed from control rates by more than 15%. Overall, subject S2 appeared to be less sensitive to Ro 15-4513 than the other two pigeons. For all subjects, IRT > 6-s response rates were not systematically affected by acute administrations of any of the doses.

These findings are also evident in Figure 4, which shows grouped response rates (expressed relative to vehicle control) across the first, second and third presentations of each component schedule during acute dose-response determinations. As shown here, the suppression of responding evident under the FR 25 component is dose-related, with little change in response rate occurring across the three presentations of this schedule during drug sessions. As also shown, response rates under the IRT > 6-sec schedule were not reduced relative to vehicle control

rates at any dose of Ro 15-4513 tested and remained fairly constant across the three exposures.

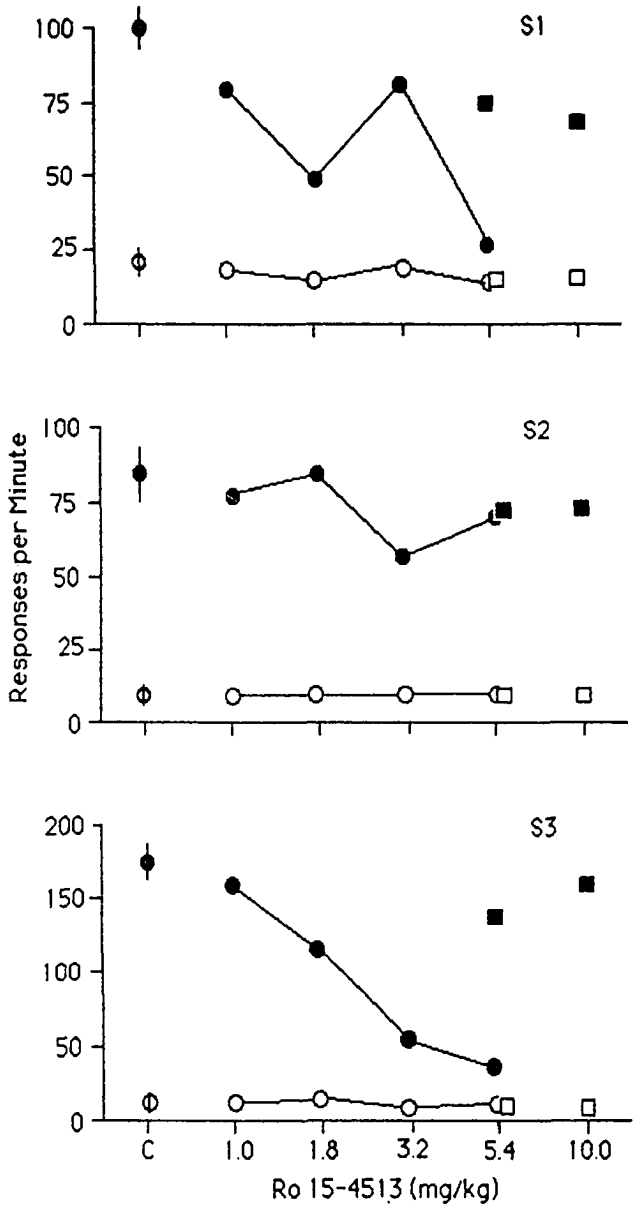

Figure 3. Individual Data Showing the Effects of Acute and Chronic Administration of Ro 15-4513 on Schedule-Maintained Responding.

Figure 3. Individual Data Showing the Effects of Acute and Chronic Administration of Ro 15-4513 on Schedule-Maintained Responding. Closed circles represent FR 25 response rates; open circles represent IRT > 6-sec response rates. Data at C represent mean response rates across all control sessions; vertical lines represent plus and minus one standard deviation. Connected drug data points (circles) represent the mean of two acute administrations of the listed dose. The unconnected points (squares) at 5.4 mg/kg indicate performance on the fifteenth session of chronic exposure to that dose. The 10.0 mg/kg dose was given as a challenge dose after chronic exposure to 5.4 mg/kg.

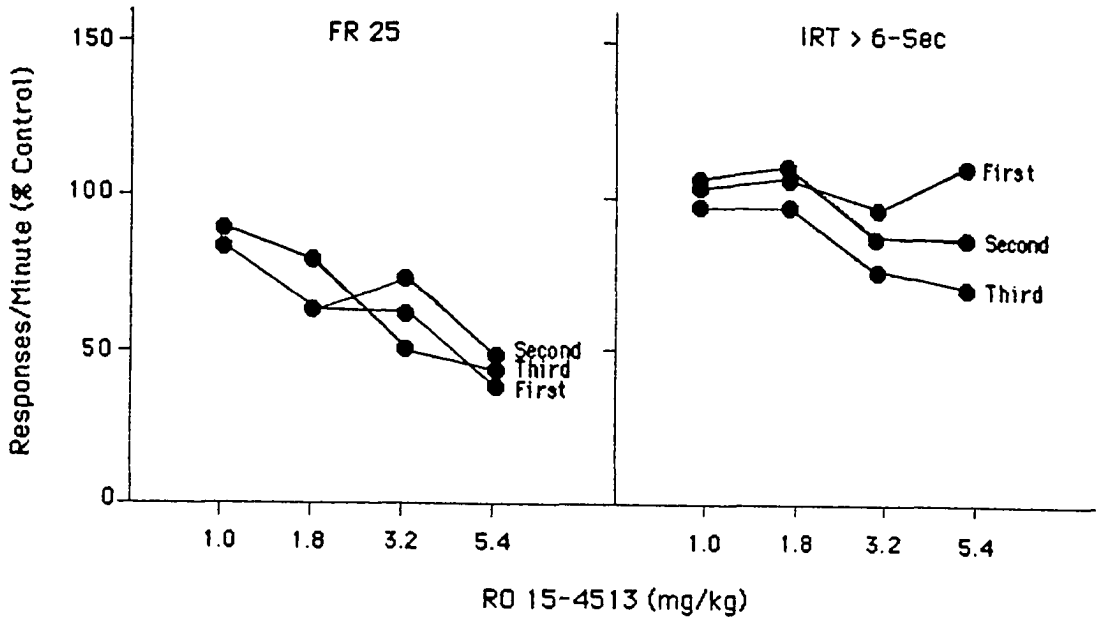

Figure 4. Group Data Showing the Effects of Acute Administration of Ro 15-4513 on the Temporal Pattern of Responding during each Component Schedule. During each session, the two component schedules alternated at 4-minute intervals, resulting in three presentations of each schedule during the 24-minute session. Response rates at each dose of Ro 15-4513 are expressed relative to response rates obtained under control conditions during the first, second and third presentation of each component schedule.

The two subjects that were most sensitive to Ro 15-4513 (S1, S3) developed tolerance to the rate-reducing effects of the drug when it was administered chronically at a dose of 5.4 mg/kg. For those subjects, response rates under the FR schedule were much higher during the final session of chronic exposure than during acute exposure. The FR 25 response rate for S2 at the end of chronic exposure to 5.4 mg/kg was comparable to the rate observed when this dose was given acutely. Administration of a dose of 10 mg/kg following the chronic regimen revealed FR response rates that approximated those seen at chronic 5.4 mg/kg for all subjects. This finding suggests that tolerance developed to the rate-decreasing effect of Ro 15-4513. For all subjects, chronic exposure to 5.4 mg/kg produced no detectable change in IRT > 6-s response rates.

Table 2 shows mean food deliveries per session under all experimental conditions. Because rate of reinforcement is proportional to (i.e., 4% of) rate of responding under the FR 25 component, the effects of Ro 15-4513 on mean food deliveries per session under this component were equivalent to its effects on response rates. There is no simple relation between rate of responding and number of food deliveries under the IRT > 6-s schedule, and the effects of Ro 15-4513 under this component were highly variable across the three subjects. For subject S1, mean number of reinforcers earned per session under the IRT > 6-s component increased when Ro 15-4513 was administered. For

subject S2, this measure decreased as a function of Ro 15-4513 administration. For subject S3, it was generally unaffected by the drug.

Table 2

Mean Number of Reinforcers Earned Per Session for Individual Subjects During Acute and Chronic Dose-Response Testing

Drug Dose (mg/kg)	FR 25 Component	IRT > 6-s Component
Subject 1		
0	49 (3.2)a	4 (2.5)a
1.0	41	10
1.8	25	18
3.2	41	0
5.4	14	23
5.4 (chronic)	37b	9b
10.0	35	5
Subject 2		
0	42 (4.0)	50 (5.6)
1.0	39	38
1.8	42	38
3.2	31	39
5.4	35	35
5.4 (chronic)	36	55
10.0	37	53
Subject 3		
0	77 (3.6)	33 (5.1)
1.0	71	36
1.8	55	34
3.2	30	30
5.4	20	28
5.4 (chronic)	64	49
10.0	72	24

aThese values represent the mean of all control sessions; the value in parentheses is one standard deviation.

bThese values represent the final (fifteenth) session of chronic exposure.

DISCUSSION

Previous observations with rats have revealed that acute administrations of Ro 15-4513 do not produce changes in behavior that are evident in gross observations (e.g., Hoffman et al., 1987; Suzdak et al., 1986). Nonsystematic observations of subjects in the present study also failed to reveal gross changes in behavior when the drug was administered, under both acute and chronic conditions. Prior to, during, and after experimental sessions, the pigeons did not appear to be ataxic or unresponsive to environmental stimuli. They were, however, affected by the drug, which substantially reduced response rates under the FR component. That the drug did so indicates that Ro 15-4513 is behaviorally active in pigeons at relatively low doses. A previous report has demonstrated similar sensitivity in rats, in which doses of Ro 15-4513 comparable to those used in the present study reduced response rates under a tandem variable-interval 40 sec fixed-ratio 10 schedules of food delivery (Woudenberg and Slangen, 1988). That schedule, like the FR component in the present study, engendered relatively high response rates in the absence of drug.

In contrast, the IRT > 6-s component employed in the present study engendered low response rates in the absence of drug. Response rates under this component were unaffected by doses of Ro 15-4513 that substantially reduced FR responding.

That this occurred suggests that the effects of Ro 15-4513 on schedule-controlled responding may be related to response rate: The drug appears to reduce high-rate operant responding at doses that leave low-rate operant responding intact. Because the FR and IRT components differ in several regards other than the rates engendered, further research will be required to determine if the effects of Ro 15-4513 are actually rate-dependent.

GENERAL DISCUSSION

The discovery that a newly synthesized compound, Ro 15-4513, antagonized some of the behavioral effects of ethanol in nonhumans caught the imagination of some members of the research community. Many researchers in the U. S. were first introduced to Ro 15-4513 in volume 234 of *Science* magazine. It was here that the report by Suzdak and his colleagues appeared, as well as an article written by Kolata (1986) that provided an informative lay introduction to Ro 15-4513 and its posited actions. Kolata included in her article a picture showing the effects of Ro 15-4513 on ethanol-induced intoxication. Two rats appear in the photograph, one lying on its back, the other standing normally facing the photographer. The caption next to these animals explains that the rat on its back has passed out from ethanol intoxication; the one standing normally had the same amount of ethanol, but after it had passed out, it was given Ro 15-4513 and within two minutes was behaving soberly (Kolata, 1986). The message is clear: An amazing discovery had been made in the pharmacological treatment of ethanol intoxication.

Although early claims of Ro 15-4513's antagonism against the behavioral impairment produced by ethanol have been nearly unanimously supported by more recent data, it is unlikely that Ro 15-4513 will be developed for clinical use. There are several obvious reasons for this. First, the proconvulsant activity of Ro

15-4513 makes it a potentially dangerous drug for those at risk for having seizures, including chronic drinkers who may experience seizure activity if they suddenly reduce the amount of ethanol they consume. Second, Ro 15-4513 has a short duration of action. To be of clinical use, a longer acting derivative would need to be developed. Third, Ro 15-4513 does not consistently block the lethal effects of ethanol. Because Ro 15-4513 has been shown to block the discriminable properties of ethanol, a person pretreated with the drug might, in an attempt to produce subjective intoxication, drink a great deal of ethanol, and die of the resultant overdose. Fourth, Ro 15-4513 apparently does not prevent the physical damage that accompanies long-term ethanol use.

Although it is generally accepted that Ro 15-4513 binds to benzodiazepine receptors, the precise mechanism underlying the ability of Ro 15-4513 to antagonize some of the neuropharmacological and behavioral actions of ethanol, and to affect behavior when given alone, as in the present studies, has not been established. Moreover, the behavioral mechanisms of action of the drug have been largely ignored. As noted by Thompson:

> By behavioral mechanism of drug action, we refer to a description of a drug's effect on a given behavioral system (locus) expressed in terms of some more general set of environmental principles regulating behavior. Specifying the behavioral mechanism(s) responsible for an observed effect involves: a) identifying the environmental variables

> which typically regulate the behavior in question, and b) characterizing the manner in which the influence of those variables is altered by the drug. (1981, p.3)

Although the precise *behavioral* mechanisms through which Ro 15-4513 alone affects responding, and alters the behavioral effects of ethanol, are unknown, additional work at this level of analysis appears to be justified. Providing a neuropharmacological explanation of how a drug or drug combination produces its behavioral actions presupposes understanding of these actions, and such understanding is incomplete with respect to Ro 15-4513 and ethanol.

APPENDIX A

INVESTIGATOR CERTIFICATION

Title of Project Effects of RO15-4513 on schedule-controlled responding in pigeons

If any of the above procedures are changed, I will submit a new protocol.

I understand that any failure to comply with the *Animal Welfare Act*, the provisions of the *DPHS Guide for the Care and Use of Laboratory Animals* and requirements set down by the IACUC may result in the suspension of my animal studies.

Signature: Principal Investigator | *Department* | *Date*

REVIEW BY THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

_____Disapproved X Approved _____ Approved with the provisions listed below

Provisions:
or
Explanation _____

IACUC Chairperson | *Date*

Researcher's Acceptance of Provisions:

Signature: Principal Investigator | *Date*

IACUC Chairperson Final Approval | *Date*

Approved IACUC Number 6027

Revised June, 1988

INVESTIGATOR CERTIFICATION

Title of Project Effects of RO15-4513 on oral ethanal self-administration in rats

If any of the above procedures are changed, I will submit a new protocol.

I understand that any failure to comply with the *Animal Welfare Act*, the provisions of the *DPHS Guide for the Care and Use of Laboratory Animals* and requirements set down by the IACUC may result in the suspension of my animal studies.

Signature: Principal Investigator — Department: Psychology — Date: 1/10/89

REVIEW BY THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

_____Disapproved X Approved _____ Approved with the provisions listed below

Provisions:
or
Explanation _____

IACUC Chairperson — Date: 1-12-89

Researcher's Acceptance of Provisions:

Signature: Principal Investigator — Date

IACUC Chairperson Final Approval — Date

Approved IACUC Number 27

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