Ethanol self-administration in freely feeding and drinking rats: effects of Ro15-4513 alone, and in combination with Ro15-1788 (flumazenil)

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Abstract. Recent work in our laboratory demonstrated that Ro15-4513, a partial inverse benzodiazepine (BDZ) agonist, decreases ethanol (ETOH) self-administration in rodents under fluid deprivation conditions. The present study further examined the effects of Ro15-4513 (2.5 and 5.0 mg/kg) alone and in combination with Ro15-1788, (flumazenil) (8.0 and 16.0 mg/kg), a BDZ receptor antagonist on ETOH self-administration in freely feeding and drinking rats. Animals were trained to consume ETOH (11% v/v) using a limited access procedure. Measurements were taken at 10- and 60-min intervals. Ro15-4513 (2.5 and 5.0 mg/kg) markedly attenuated ETOH consumption at both intervals. The antagonistic actions of Ro15-4513 were completely blocked by the higher dose of flumazenil at both intervals; the lower dose failed to antagonize the Ro15-4513-induced reduction of ETOH intake. When flumazenil was given alone, both doses reduced ETOH self-administration at 60 min; although the magnitude of the antagonism was comparable to that of Ro15-4513 only with the highest does of flumazenil (16.0 mg/kg). Neither Ro15-4513 nor flumazenil alone or in combination significantly altered water intake at any of the tested doses. Rats pretreated with Ro15-4513 showed a substantial reduction in blood ethanol concentration (BEC) compared with the Tween-80 vehicle condition at the 10-min interval. However, the BEC of animals given Ro15-4513 in combination with flumazenil were similar to rats given Tween-80 vehicle. The present study extends our previous research by demonstrating that Ro15-4513 and flumazenil attenuate ETOH self-administration in non-food or water deprived rats. These studies suggest that the suppressant effects of Ro15-4513 and flumazenil on ETOH self-administration are associated with actions at the BDZ site of the GABA_A receptor complex. These data are discussed in relation to the possible mechanism(s) by which Ro-15-4513 and flumazenil exert their antagonism on ETOH self-administration.

Key words: Ro15-4513 – Ro15-1788 – GABA-BDZ – Flumazenil – Ethanol – Self-administration – Rats

Ethanol (ETOH) is one of society's most abused drugs. It is classified as a depressant drug (Rall 1990); however, depending on the dose and time after exposure it can increase as well as decrease activity. Many individuals find the consumption of ETOH-containing beverages pleasurable. These effects reinforce alcohol-seeking behavior and are undoubtedly important factors in alcohol abuse and alcoholism (Lewis 1990). While there is considerable agreement that ETOH functions as a reinforcer in both humans and animals (Samson and Grant 1990; Myers et al. 1991), the basis of these effects is not clearly understood. Substantial research suggests that ETOH produces positive reinforcement by generating euphoria on the one hand (Lewis and June 1990; Lukas et al. 1991; Myers and Dolinsky 1991; Weiss and Koob 1991), or by producing anxiety reduction on the other (Koob et al. 1984; Dalterio et al. 1988; Lewis 1990). It is probable that ETOH reinforcement is due to a combination of these effects and perhaps other factors (Lewis 1990).

Oral ETOH self-administration paradigms have been important tools to study the neuropharmacology of ETOH seeking behavior and reward (Weiss and Koob...
Recent work has implicated the GABA-benzodiazepine (BDZ) systems in ETOH reinforcement (McBride et al. 1988; Samson et al. 1989; June et al. 1991, 1992; June and Lewis 1994). This interest was originally stimulated by work from two lines of research. First, substantial research suggested that a major mechanism of action in the anti-conflict properties (as well as other pharmacological effects) of ETOH, BDZs and barbiturates, may be mediated via the GABA-BDZ barbiturate receptor complex (Cott et al. 1976; Goldstein 1978; for review see Hunt 1983; Koob et al. 1984, 1986). Second, inverse BDZ agonists have been found to antagonize a number of behavioral (for review see Harris and Lal 1988) and neurochemical (Suzdak et al. 1986; Harris et al. 1988; Mehta and Ticku 1988) actions of ETOH. In particular, considerable attention has focused on the imidazobenzodiazepine inverse agonist Ro15-4513, (ethyl-8-azido-5,6-dihydro-5-methyl-oxo-4H-imidazo (1,5-alpha), (1,4) benzo-diazepine-3-carboxylate), a structural analog of the benzodiazepine receptor antagonist, Ro15-1788 (flumazenil). While somewhat controversial (Suzdak et al. 1986, 1988; Britton et al. 1988; Lister and Nutt 1988a,b; Misslin et al. 1988), this research has contributed substantially to our understanding of how ETOH may be exerting its actions via the GABA-BDZ receptor complex (Harris et al. 1988; Ticku and Kulkarni 1988; Harris 1991; Morrow et al. 1991).

Several studies have investigated the role of Ro15-4513 in ETOH self-administration paradigms (Samson et al. 1987, 1989; McBride et al. 1988; June et al. 1991, 1992; Rassnick et al. 1993; June and Lewis 1994). McBride et al. (1988) demonstrated that Ro15-4513 selectively attenuates ETOH intake in alcohol preferring (P) and non-prefering (NP) rats given a two-bottle choice test between ETOH and water under deprivation conditions. Others have shown that Ro15-4513 antagonizes ETOH-reinforced responding in outbred rats (Samson et al. 1987, 1989; Rassnick et al. 1993) during a free choice operant task. We (June et al. 1991, 1992) have demonstrated that Ro15-4513 attenuates ETOH consumption in outbred rats given a choice between sweetened ETOH and water solutions or hamsters given ETOH alone (June and Lewis 1994). Together, these studies suggest that the suppressive effects of Ro15-4513 on ETOH intake are associated with actions at the GABA-BDZ receptor complex.

However, our previous findings with outbred animals (June et al. 1991, 1992) using fluid deprivation and ETOH adulteration procedures can be questioned, since it is not clear whether the animals were consuming ETOH for its pharmacological effects, or for the hedonic value of thirst reduction, or the positive reinforcement of sweeteners (for review see Samson 1987; Weiss and Koob 1991). As previously suggested, fluid deprivation and ETOH adulteration to not represent optimal ETOH initiation procedures in rodents (Samson 1987; Weiss and Koob 1991).

One method of obtaining oral ETOH self-administration in outbred rats without the use of sweeteners or fluid deprivation has been the limited access paradigm (MacDonnell and Marcucella 1979; Brown et al. 1982; Stewart and Grupp 1984; Linseman 1987, 1990). The procedure entails providing freely feeding and drinking rats daily limited access to gradually increasing ETOH concentrations. The limited access paradigm produces sustained ETOH intake which results in measurable BEC levels (Gill et al. 1986; Linseman 1987). The paradigm also permits the experimenter to schedule drinking sessions during a time when the pretreatment drug is maximally effective (Linseman 1990). Thus, the model represents a useful method to study the effects of compounds with rapid onset of effects and short duration of action.

The primary objective of the present study was to further examine the role of the GABA-BDZ receptor complex in mediating ETOH reinforcement in outbred Sprague Dawley rats using the limited access paradigm to initiate ETOH drinking behavior. Specifically, the ability of the inverse BDZ agonist Ro15-4513 to suppress ETOH drinking was examined at doses (2.5 and 5.0 mg/kg) previously shown to be effective in fluid deprivation studies (June et al. 1991, 1992). However, unlike most of the previous work that has investigated the effects of Ro15-4513 across a 30-min test session (Samson et al. 1987, 1989; but see McBride et al. 1988; June et al. 1991, 1992; Rassnick et al. 1993), the present study extended the consumption interval to 60 min to determine more specifically the time course of Ro15-4513’s actions on ETOH intake. Second, because no study has examined the interaction of Ro15-4513 and ETOH with the BDZ receptor antagonist flumazenil in freely feeding and drinking rats, the ability of flumazenil to reverse the Ro15-4513 antagonism was also investigated. Finally, because of the discrepant findings in the literature regarding the ability of flumazenil to alter some of the actions of ETOH (for review see Chan et al. 1988, 1991; also Fie et al. 1989), we examined the ability of flumazenil alone (8.0 and 16.0 mg/kg) to attenuate/block the chronic self-administration of ETOH in freely feeding and drinking rats.

Materials and methods

Subjects

Subjects were 40 experimentally naive male Sprague Dawley rats approximately 3–4 months of age, weighing between 301–355 g at the start of the experiment. Animals were individually housed in wire-mesh stainless steel cages at an ambient temperature of 22 °C on a 12:12 reversed light:dark cycle, with the dark period beginning at 0700 hours. All test sessions were conducted during the dark phase of the cycle between 1100 and 1300 hours. A single red light bulb illuminated the experimenter’s corner of the colony room to aid in data collection procedures. All animals were given ad lib access to both food (Purina Rat Chow) and water throughout the experiment. The only exception being that animals received no food during their 1-h experimental drinking session.

Drugs and solutions

Ro15-4513 and Ro15-1788 (both donated as gifts from Drs. Eigenmann and Haefely Hoffmann La-Roche, Basel, Switzerland), were prepared as an emulsion by power agitation (Fisher Scientific Mixer) in 4% Tween-80 vehicle and mixed with a 0.8% sodium chloride solution to a fixed volume. All Ro15-4513 and Ro15-1788 solutions were made immediately prior to injections. Injections were administered intraperitoneally (IP) and given in a volume of 1.0 ml/kg body weight.