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Effect of 5-HT_{1B} Receptor Ligands on Self-administration of Ethanol in an Operant Procedure in Rats

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TOMKINS, D. M. AND M. F. O'NEILL. *Effect of 5-HT_{1B} receptor ligands on self-administration of ethanol in an operant procedure in rats.* PHARMACOL BIOCHEM BEHAV **66**(1) 129–136, 2000.—Recent evidence suggests that 5-HT_{1B} receptor activation modifies ethanol's reinforcing, intoxicating and discriminative stimulus effects. The present study further explored the role played by 5-$HT_{1A/1B}$ receptors by examining their influence on oral ethanol self-administration. Male Wistar rats were trained on an FR 4 schedule to obtain a reinforcer of 0.1 12% w/v ethanol solution. Once responding was stable, the effect of the 5-$HT_{1A/1B}$ agonist RU24969 alone and in combination with the 5-HT_{1B} antagonist GR127935 or the 5-HT_{1A} antagonists (+) WAY100135 and (+) WAY100635 was assessed. The effect of RU24969 on ethanol's pharmacokinetic profile and on operant oral saline self-administration was also examined to assess if alterations in oral ethanol self-administration were due to nonspecific effects on level pressing. For comparison, we examined the effect of another 5-$HT_{1A/1B}$ agonist, CGS12066B, on oral ethanol self-administration. Both RU24969 (0.1 to 1 mg/kg) and CGS12066B (0.1 to 1 mg/kg) significantly suppressed oral ethanol self-administration. Administration of GR127935 (1 mg/kg), significantly reversed the effects elicited by RU24969, whereas neither WAY100635 (1 mg/kg) nor (+)WAY100135 (1 mg/kg) had any effect. The effects of lower doses of RU24969 on oral ethanol self-administration were selective as oral saline self-administration and blood ethanol levels were not altered by these doses. These data demonstrate that 5-HT_{1B} receptor activation suppresses oral ethanol self-administration. These studies provide further evidence that 5-HT_{1B} receptors play a modulatory role in ethanol's behavioral effects. © 2000 Elsevier Science Inc.

Ethanol self-administration 5-HT_{1B} RU24969 CGS12066B GR127935 Saline self-administration Blood ethanol levels

A SUBSTANTIAL body of evidence implicates the central serotonergic system in regulating many of ethanol's behavioral, physiologic, and biochemical effects (20,34,41). Until recently, the potential involvement of 5-HT_{1B} receptors in mediating many of these effects has not been extensively examined due to the lack of agents combining high 5-HT_{1B} affinity with good selectivity for these receptors. However, mounting evidence suggests that 5-HT_{1B} receptors are important for modifying the reinforcing (3,18,29), intoxicating (3), and discriminative stimulus effects of ethanol (9,10,35) as well as regulating its voluntary intake (3,17,18,31).

Recent evidence supporting a role of 5-HT_{1B} receptors in mediating ethanol's effects comes from various genetic studies. Research using the high and low ethanol preferring P and NP rat lines have reported fewer 5-HT_{1B} receptors in discrete brain regions, including the lateral and medial septum and lateral nucleus amygdala, of the P rats compared with the NP rats (17). The authors suggest that differences in 5-HT_{1B} receptor densities in these areas, which hare been previously linked with reinforcement processes, may be one of the determining factors of ethanol preference. Furthermore, quantitative trait loci (QTL) mapping studies have found provisional support for a QTL on mouse chromosome 9 near the 5-HT_{1B} receptor gene (36), which is linked with ethanol-drinking phenotypes, ethanol-induced conditioned place preference, and ethanol-induced taste aversion (2,27,32). In addition, initial

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findings using null mutant mice lacking the 5-HT_{1B} receptor gene reported that these mice did not differ from the wild type in terms of basal locomotor activity and food or water intake, but they did consume more ethanol when allowed 24-h access (3). The authors demonstrated that the knockout mice accepted higher ethanol concentrations (20%) and consumed pharmacologic and behavioral relevant amounts of ethanol (approximately 11 g/kg/day compared to 4 g/kg/day consumed by the wild-type mice) (3). This was not due to changes in taste perception or thirst because the acceptance of both sweet and bitter solutions was similar between the mouse strains. More recent research efforts, however, have not consistently confirmed these original findings (4).

The studies described above support a role played by 5-HT_{1B} receptors in regulating ethanol consumption. These findings are in agreement with previous pharmacologic studies in which agonists displaying modest 5-HT_{1B} receptor selectivity have consistently been reported to attenuate voluntary ethanol intake (1,12,19). Thus the 5-HT_{1B} receptor agonists TFMPP, mCPP, and CP-94,253 have been shown to reduce ethanol intake (1,12,16,19). The relevancy of these findings for human alcoholics is supported by the recent report that suggests that a locus predisposing for antisocial alcoholism may be linked to the 5-HT_{1B} receptor gene (13). These intriguing human data strongly support the need for further research in this area.

The aim of the present study was to further evaluate the impact of 5-HT_{1B} receptor manipulations on oral ethanol self-administration. Because many of the agents used as 5-HT_{1B} agonists also possess 5-HT_{1A} agonist activity, we used the selective 5-HT_{1B} and 5-HT_{1A} antagonists to investigate the relative contribution of these two receptor types in altering ethanol intake. To determine if alterations in oral ethanol self-administration were due to nonspecific effects induced by the agonist used, we conducted two parallel studies. To rule out the possibility that the 5-HT_{1B} receptor–mediated changes in oral ethanol self-administration were due to a nonspecific disruption of operant responding comparison studies assessed the effects of the same manipulations on responding for an alternative reinforcer, a 0.9% saline solution. This novel approach was adopted as in preliminary studies we demonstrated that the response profile generated by animals self-administering 0.9% saline on an FR4 schedule of reinforcement is similar to that recorded from rats responding on the same schedule for a 12% ethanol solution. Because rats voluntarily self-administer saline in the absence of food or water deprivation, in a manner similar to that for ethanol and the mechanisms regulating its intake are different from those controlling ethanol intake (23,34) support its inclusion as a good control for assessing nonspecific drug effects. In the second study, we assessed the possibility that RU24969 administration alters oral ethanol self-administration via changing the kinetic profile of ethanol.

METHOD

Animals and Housing

Male Wistar rats (Charles River, Canada), weighing approximately 250 g at the start of the studies, were individually housed in hanging wire mesh cages with food and water available ad libitum except where stated otherwise. They were maintained on a 12-h light/dark cycle in an environmentally controlled room (lights on at 1900h, temperature: 22°–24°C, humidity: 30% to 60%). The experimental procedures employed conformed to the guidelines laid down by the Canadian Council on Animal Care and were approved by the Animal Care Committee at the Centre for Addictions and Mental Health.

Operant Self-Administration Procedure

Testing was carried out in 16 chambers measuring 28-cm long, 21-cm wide, and 21-cm high (Med. Associates, Georgia, VT). Each chamber contained a solenoid operated liquid dispenser calibrated to deliver 0.1 ml of fluid into a recessed dish positioned 3 cm above floor level and two response levers (4.5-cm wide and 7-cm above the chamber floor), the centers of which were located 6.5 cm either side of the dish. Each chamber was housed in a sound-attenuating box equipped with a ventilation fan and illuminated by a house light. The apparatus was controlled by a microcomputer interface (Med. Associates) linked to a 386sx IBM computer.

For both the oral ethanol self administration and oral saline self-administration procedures the rats had free access to both food and water except during the 30-min operant test sessions at which point only the fluid acting as the reinforcer was available to the animal. For the ethanol studies, in addition to water the rats were given 24 h home cage ethanol access for 3 weeks during which time the ethanol concentration was gradually increased from 3% to 12% w/v at weekly intervals. At this point, home cage ethanol access is ceased and the rats are then placed daily in the operant boxes for 30 min and trained to press the active lever for a 12% (w/v) ethanol solution on an FR1 schedule. The response requirement is switched to FR2 and finally to FR4 when reliable responding at each schedule was achieved. Responding on the inactive lever was recorded but had no programmed consequences. When stable responding had been established, the studies using peripheral pharmacologic manipulations were started. For the saline self-administration studies, in addition to water the rats were given 24-h home cage access to a 0.9% saline solution for 1 week. At this point home cage saline access was stopped and the rats were then placed daily in the operant boxes for 30 min and trained to press the active lever for a 0.9% solution on an FR1 schedule. The response requirement is switched to FR2 and finally to FR4 when reliable responding at each schedule is achieved. When responding has stabilized the drug studies were commence. For both procedures test sessions were conducted whenever 3 consecutive days occurred in which there was neither an increasing nor decreasing trend in the number of ethanol reinforcers obtained.

Study 1. Effect of Acute 5-HT_{1B} Agonist Administration on Oral Ethanol Self-administration

Male Wistar rats ($n = 8$) were initially trained to consume ethanol as outlined above. When the response pattern had stabilized, the effect of the acute administration of RU24969 (0.1 to 1 mg/kg IP 30 min pretreatment) on self-administration behavior was evaluated. A Latin square design was employed such that each animal received each dose in a balanced order. Each treatment day was separated from the next by at least 3 days. In a second group of animals ($n = 9$), the effect of the acute administration of CGS12066B (0.1 to 1 mg/kg IP 30 min pretreatment) on self-administration behavior was evaluated in a similar manner.

Study 2. Effect of Selective 5-HT Receptor Antagonists on RU24969-induced Suppression of Oral Ethanol Self-administration

Because RU24969 has agonist activity at both 5-HT_{1B} and 5-HT_{1A} receptors (11) the present study was designed to determine if the effects observed in Study 2 were due to 5-HT_{1B} receptor activation. Thus, the ability of three selective antago-

nists, GR127935 (5-HT_{1B}), WAY100635 (5-HT_{1A}) and (+)-WAY100135 (5-HT_{1A}) to reverse the RU24969's effects on oral ethanol self-administration was examined. Three separate groups of rats were trained to self-administer ethanol. In the first group, the ability of GR127935 (1 mg/kg IP, $n = 5$), to produce a rightward shift in the dose response curve to RU24969 (0.1 to 1 mg/kg IP) was assessed. A Latin square design was employed with GR127935 administered 30 min prior to RU24969 such that each animal received each dose in a balanced order. Each treatment day was separated from the next by at least three days. In two separate group of rats, the ability of the 5-HT_{1A} antagonists, WAY100635 (1 mg/kg, IP $n = 17$) and (+) WAY100135 (1 mg/kg IP, $n = 10$) administered 30 min prior to RU24969 (0.1 to 1 mg/kg) was assessed in a similar manner. An additional group of rats ($n = 4$) were employed to determine whether GR127935 (1 mg/kg) would reverse the effects of 0.5 mg/kg RU24969 on oral ethanol self-administration.

Study 3. Effect of RU24969 on Oral Saline Self-administration

Male Wistar rats ($n = 8$) were initially trained to self-administer 0.9% saline as outlined above. When the response pattern had stabilized, the effect of the acute administration of RU24969 (0.1 to 1 mg/kg IP 30 min pretreatment) on self-administration behavior was evaluated. A Latin square design was employed such that each animal received each dose in a balanced order. Each treatment day was separated from the next by at least 4 days.

Study 4. Effect of RU24969 on the Pharmacokinetic Profile of Experimenter-administered Ethanol in Male Wistar Rats

Rats were fasted overnight and then divided into 2 treatment groups: vehicle ($n = 10$) and 1 mg/kg RU24969 ($n = 11$). Each rat received its allocated treatment and 30 min later received a 0.5 g/kg bolus dose of ethanol (10% w/v by gavage. Blood samples (50 μl) were taken from the tip of the tail of each rat at 15, 30, 45, 60, and 75 min post ethanol administration. Blood ethanol levels were determined by gas-liquid chromatography technique with n-butanol as internal standard (14).

Drugs and Injections

RU24969 and GR127935 were a gift from Eli Lilly Research Ltd., U.K., (+) WAY100135 was a gift from Wyeth UK, WAY100635 was purchased from RBI, MA, USA, and CGS12066B was purchased from Tocris, MO, USA. All drugs except GR127935 were dissolved in 0.9% sterile saline. GR127935 was suspended in 0.9% saline with a drop of methyl cellulose and sonicated. RU24969 and CGS12066B were administered as a 30-min pretreatment by the intraperitoneal (IP) route. The antagonists GR127935, (+) WAY100135 and WAY100635 were administered IP 30 min prior to the administration of RU24696. All doses are expressed as the salt.

Data Analysis

In studies 1 and 3, which examined the impact of acute agonist administration on ethanol and saline self administration, a one-way repeated measures analysis was conducted, with agonist dose as the within factor. In the CGS12066B study, data for two animals receiving the 0.1 mg/kg dose were excluded due to technical difficulties, therefore a univariate analysis was conducted. For both the antagonist interaction studies described in study 2 and the ethanol kinetic study, a two-way analysis of variance was employed. One blood sample from the vehicle group at time 15 min and two samples from the vehicle group at time 30 min could not be analyzed as insufficient blood was collected. When appropriate, comparisons between treatment groups were made using planned contrasts.

RESULTS

Study 1. Effect of Acute 5-HT_{1B} Agonist Administration on Oral Ethanol Self-administration

Rats in the RU24969 treatment group were consuming 1.2 ± 0.14 g/kg ethanol prior to the start of the study. Acute administration of RU24969 (0.1 to 1 mg/kg) significantly attenuated the total number of ethanol reinforcers obtained ($F(7, 21) = 9.93$, $p < 0.001$) during the 30-min test session (Fig. 1). Post hoc analysis showed that all doses of RU24969 reduced ethanol motivated responding in a dose-dependent manner. At the highest dose tested (1 mg/kg), hyperactivity was noted in some of the animals. Rats in the CGS12066B treatment group were consuming 0.78 ± 0.09 g/kg ethanol prior to the start of the study. Similarly, acute CGS12066B administration significantly attenuated the total number of ethanol reinforcers obtained ($F(8, 22) = 4.86$, $p < 0.01$) during the 30-min test session (Fig. 1). Post-hoc analysis showed that both the 0.1 and 1 mg/kg dose significantly reduced ethanol motivated responding.

Study 2. Effect of Selective 5-HT Receptor Antagonists on RU24969-induced Suppression of Oral Ethanol Self-administration

Pretreatment with either 5-HT_{1A} antagonist, WAY100635 or (+) WAY100135, did not reverse the effects of RU24969 on ethanol motivated responding (Fig 2). Statistical analysis

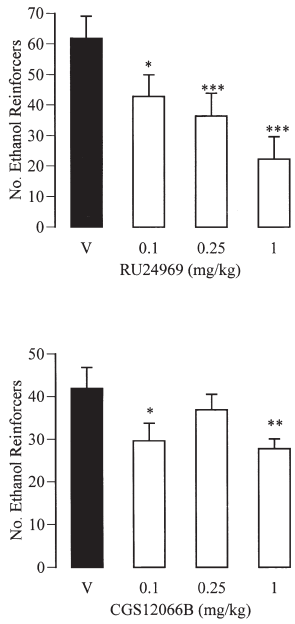

FIG. 1. Effect of RU24969 (upper panel) and GGS12066B (lower panel) on the number of ethanol (12% w/v) reinforcers earned on an FR4 schedule of reinforcement during a 30 min operant test session. Data are expressed as the mean ±SEM. Significant differences from the vehicle treatment condition are represented by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

confirmed the previous results that RU24969 significantly attenuates oral ethanol self-administration ($F(3, 48) = 27.73$, $p < 0.001$ and $F(3, 27) = 5.44$, $p < 0.01$ for the WAY100635 and (+) WAY100135 groups respectively) but there was no significant effect of the antagonist ($F(1, 48) = 2.78$, N.S. and $F(1, 27) = 0.01$, N.S. for the WAY100635 and (+) WAY100135 groups respectively). In contrast, the selective 5-HT_{1B} antagonist GR127935 reversed RU24969's effect on oral ethanol self-administration (Fig. 3). In the first study, statistical analysis confirmed that RU24969 significantly attenuated oral ethanol self-administration ($F(3, 12) = 5.70$, $p < 0.05$). Post hoc analysis revealed that 1 mg/kg RU24969 significantly suppressed ethanol self-administration; however, while the 0.25 mg/kg dose reduced ethanol motivated responding by approximately 50% this failed to reach statistical significance ($p = 0.09$). There was an overall effect of GR127935 in this study ($F(1, 12) = 6.81$, $p = 0.059$). Post hoc analysis revealed a significant difference in ethanol self-administration behavior following treatment with vehicle—0.25 mg/kg RU24969 and GR127935 + 0.25 mg/kg RU24969, however, pretreatment with GR127935 did not reverse the effects induced by 1 mg/kg RU24969. Because the 0.25 mg/kg dose of RU24969 failed to significantly suppress ethanol self-administration in this study when tested alone, the interpretation that GR127935 reversed this effect is compromised. In a follow-up study, we assessed to ability of GR127935 to reverse the suppression in oral ethanol self-administration elicited by 0.5 mg/kg RU24969 (Fig. 3). As anticipated 0.5 mg/kg RU24969 significantly reduced ethanol motivated responding by approximately 70% ($p = 0.001$), which was reversed by pretreatment with 1 mg/mg GR127935 ($p = 0.026$).

Study 3. Effect of RU24969 on Saline Self-administration

The response profile generated in rats responding for 0.9% saline was similar to that observed in ethanol self-administering rats. Thus, the rate of responding, latency to initiate responding and the mean inter-reinforcer interval parameters derived from the response records are not statistically different between these two groups of animals (data not shown). Saline administering animals generally earned more reinforcers per session than their ethanol self-administering counterparts. Acute administration of RU24969 significantly attenuated the total number of saline reinforcers obtained ($F(3, 18) = 6.47$, $p < 0.01$) (Fig. 4). Post hoc tests revealed that this effect was attributable to a significant suppression in responding at the 1 mg/kg dose alone. As in the ethanol study, hyperactivity was noted in some of the animals at this dose level.

Study 4. Effect of RU24969 on the Pharmacokinetic Profile of Experimenter-administered Ethanol in Male Wistar rats

The blood ethanol concentrations achieved in male Wistar rats following oral administration of 0.5 g/kg ethanol solution were within the expected range compared with those previously reported. Overall analysis of variance failed to reveal any significant effect of RU24969 on the pharmacokinetic profile of ethanol ($F(1, 73) = 0.0001$, N.S.) (Table 1).

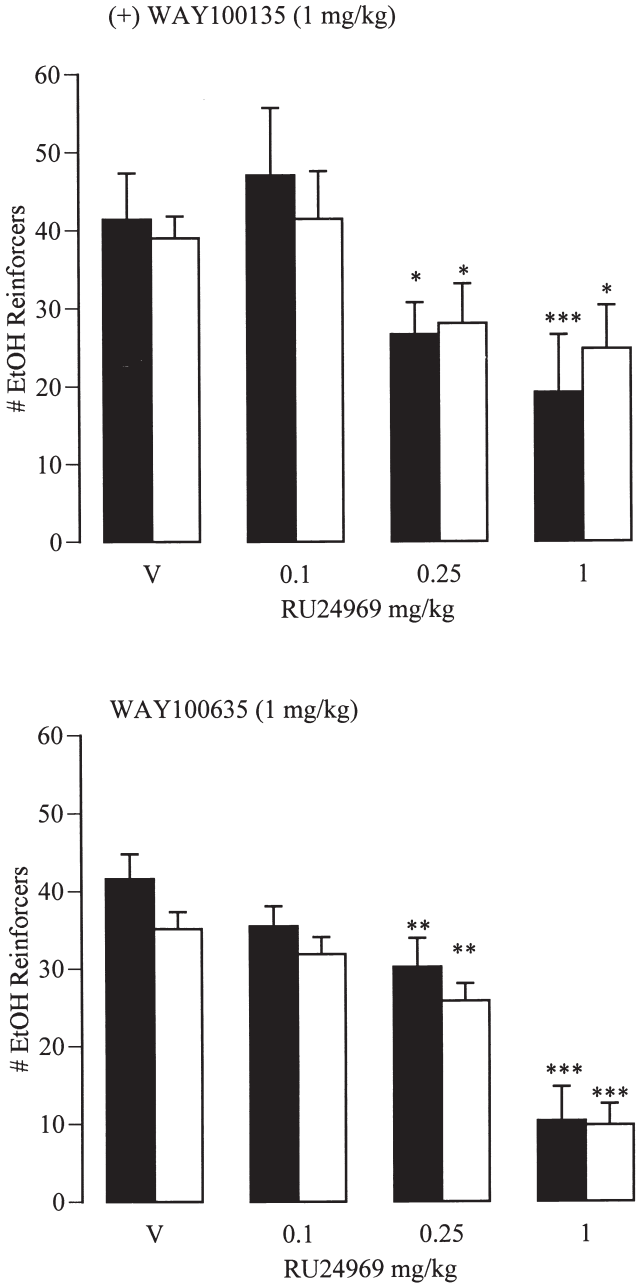

FIG. 2. Effect of (+) WAY100135 (upper panel) and WAY100635 (lower panel) on RU24969-induced suppression of ethanol self-administration. Data are expressed as the mean (±SEM) number of ethanol (12% w/v) reinforcers earned on an FR4 schedule of reinforcement during a 30-min operant test session. The white bars represent data in which the antagonist was administered 30 min prior to RU24969 administration, whereas the black bars represent pretreatment with the antagonist vehicle. Significant differences from the vehicle treatment condition are represented by $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

DISCUSSION

The results demonstrate that peripheral administration of the 5-HT_{1B} agonist RU24969 and the partial 5-HT_{1B} agonist CGS12066B suppressed oral ethanol self-administration. The effect of RU24969 on oral ethanol self-administration was reversed by the selective 5-HT_{1B} antagonist GR127935 and not by the 5-HT_{1A} antagonists WAY100635 or (+) WAY100135, thus further underlining a role for 5-HT_{1B} receptors in mediating the effect of RU24969 in the suppression of ethanol self-

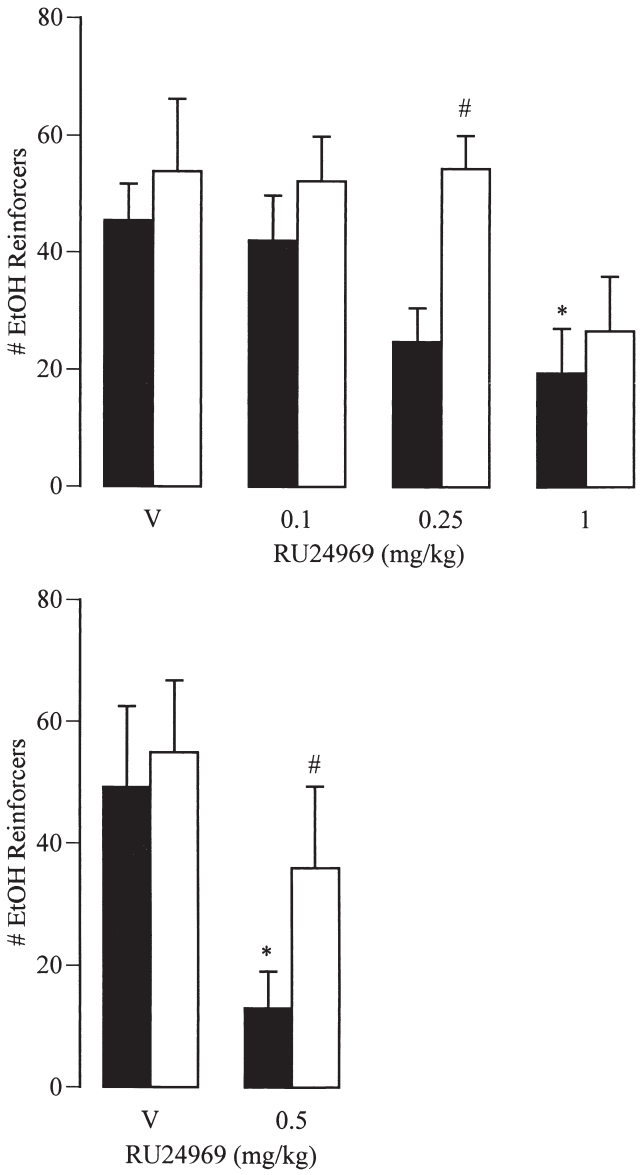

FIG. 3. Effect of GR127935 (1 mg/kg) on RU24969-induced suppression of ethanol self-administration. Data are expressed as the mean (±SEM) number of ethanol (12% w/v) reinforcers earned on an FR4 schedule of reinforcement during a 30 min operant test session. The white bars represent data in which GR127935 was administered 30 min prior to RU24969 administration, whereas the black bars represent pretreatment with the antagonist vehicle. Significant differences from the vehicle/vehicle treatment condition are represented by $*p < 0.05$, significant differences between RU24969 treatment cycles with or without GR127935 are represented by $\#p < 0.05$.

administration. Control studies suggest that at moderate doses, RU24969's ability to attenuate ethanol-reinforced responding is not due to a generalized suppression of operant responding or altered ethanol kinetics. Taken together, these data support an involvement of 5-HT$_{1B}$ receptors in regulating ethanol self-administration behavior.

In the first study, we demonstrated that both RU24969 and CGS12066B reduced oral ethanol self-administration behavior. These data are consistent with previous findings in which

TABLE 1

EFFECT OF PRETREATMENT WITH 1 mg/kg RU24969 ON BLOOD ETHANOL LEVELS ACHIEVED FOLLOWING ORAL ADMINISTRATION OF 0.5 g/kg OF ETHANOL

Time (min)	Vehicle	RU24969
15	23.3 ± 1.4	28.6 ± 4.5
30	32.5 ± 2.7	27.5 ± 3.0
45	26.2 ± 2.2	23.6 ± 2.1
60	15.3 ± 2.3	16.0 ± 1.9
75	12.0 ± 4.0	12.5 ± 2.3

Data represents the mean blood ethanol levels expressed as mg/dl ± SEM.

a range of agents with modest selectivity at the 5-HT$_{1B}$ receptor suppress ethanol intake in both two bottle choice and operant ethanol self-administration paradigms (1,12,19). In the case of RU24969, a clear dose-dependent effect was observed. At the highest dose tested, 1 mg/kg, informal observations revealed that mild hyperactivity was elicited in some of the animals that may have influenced the animals ability to operantly respond for ethanol. This observation is consistent with previous research demonstrating that RU24969 produces hyperactivity, with 1.25 mg/kg being the minimum effective dose to elicit persistent increases in locomotion, and has also been shown to suppress response rates (21,24,26,39). However, it is unlikely that the ability of RU24969 to attenuate ethanol motivated responding at lower doses is due to changes in locomotor behavior as hyperactivity was not observed. CGS12066B, at doses that did not elicit any obvious signs of hyperactivity, also reduced oral ethanol self-administration. These data suggest that 5-HT$_{1B}$ receptor agonists reduce ethanol self-administration in a specific manner. This is further supported by the observation that operant responding for an alternative reinforcer, saline, was not attenuated at moderate RU24969 doses.

Although a recent study reported a significant and marked reduction in ethanol self-administration following the administration of another selective 5-HT$_{1B}$ agonist, CP-94,253, in contrast to our findings, they raised concerns over its specificity in producing this effect (16). They considered that for a drug effect to be specific in reducing ethanol self-administration in the two lever, ethanol versus water choice self-admin-

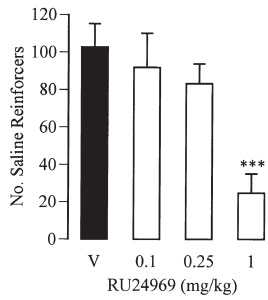

FIG. 4. Effect of RU24969 on the number of saline (0.9%) reinforcers earned on an FR4 schedule of reinforcement during a 30-min operant test session. Data are expressed as the mean ± SEM. Significant differences from the vehicle treatment condition are represented by $***p < 0.001$.

istration procedure employed, a concomitant reduction in ethanol preference should be observed, whereas selectivity was defined as a reduction in ethanol responding in the absence of an effect on total responding. Using these criteria, CP-94,253 did not elicit selective or specific effects because it altered ethanol preference only at the highest dose tested, which also had a marked effect on total responding. However, closer inspection of the data revealed no significant reduction in responding on the water lever, and indeed a trend to enhanced responding was recorded. Therefore the reduction in total responding was solely attributable to CP-94,253s effect on ethanol-motivated responding and therefore suggests that the criteria employed be viewed with caution. Because the rats in this particular study consistently exhibited low responding on the water reinforced lever, the ability to assess nonspecific drug effects may have been limited by a floor effect.

In the present study, a saline-solution reinforcer was employed to assess nonspecific drug effects. Alternative reinforcers including water food and sucrose or saccharin were considered for this particular study, but for various reasons they were discounted. In the case of food and water, self-administration is generally elicited by subjecting the animals to a mild deprivation state, which results in very high levels of responding. In instances in which deprivation states are not employed, as is the case of the study described above (16), responding for these reinforcers are consistently low. Thus interpretation of the data generated from such studies would be compromised by the fact that basal levels of responding in these animals are not comparable to those observed in ethanol self-administering rats, and therefore issues of rate dependency are not addressed. Although comparison of drug-induced alterations in ethanol self-administration with their effects on the self-administration of sweet solutions represents important controls, we did not consider them to be the best controls for assessing nonspecific effects on operant behavior due to difficulties in interpretation. For example, ethanol and saccharin self-administration may be similarly attenuated by 5-HT_{1B} agonist administration; however, this could suggest either that these receptors regulate consummatory behavior in general and not ethanol specifically or that the drug manipulation impedes the animal from operant responding. In order to circumvent these issues, saline self-administration was assessed because it is voluntarily consumed by rats, whereas the mechanisms regulating its intake appear to be independent of those controlling ethanol intake (23,34). Furthermore, the response profile generated by animals self-administering 0.9% saline on an FR4 schedule of reinforcement is similar to that recorded from rats responding on the same schedule for 12% ethanol. Thus the rate of responding, latency to initiate responding, and the mean inter-reinforcer interval parameters derived from the response records are not statistically different between these two groups of animals. In the present study, RU24969 only suppressed saline motivated responding at the highest dose tested. It is therefore unlikely that the effects of RU24969 over the low-to-moderate dose range on ethanol self-administration are due to nonspecific effects on operant responding. We propose that self-administration of a 0.9% saline solution represents a good alternative control for assessing nonspecific drug effects on ethanol self-administration.

Experimental evidence suggests that ethanol consumption may be regulated in part by pharmacokinetic as well as pharmacodynamic factors. For example, reduced ethanol absorption rates have been linked with increased ethanol consumption (15). It is therefore feasible that a pharmacologic agent that modifies voluntary ethanol intake could do so by altering ethanol's kinetic profile. Because the effect of 5-HT_{1B} receptor agonists on blood ethanol levels achieved following oral ethanol administration have not previously been examined, we conducted a pharmacokinetic study to more clearly ascertain the mechanism of action by which 5-HT_{1B} receptors may regulate ethanol intake. For this study, we examined the ability of a high dose of RU24969 (1 mg/kg) to alter ethanol's pharmacokinetic profile following oral administration of a low ethanol dose. The ethanol dose used was selected to reflect the amount of ethanol typically self-administered within 10 min of the start of the ethanol operant session. RU24969 did not alter the blood ethanol levels following ethanol administration. These data support that RU24969, and likely other 5-HT_{1B} agonists, do not alter ethanol self-administration via an alteration in ethanol kinetics.

RU24969 is the most commonly used agonist for examining the role of 5-HT_{1B} receptors in controlling many different behaviors, as well as the neurochemical consequences of 5-HT_{1B} receptor activation (7,11,24,26). Although RU24969 shows similar affinity for both 5-HT_{1B} and 5-HT_{1A} receptors (22) many of its behavioral and neurochemical effects have been attributed to its 5-HT_{1B} receptor activity (6,7,24,26). However, because both 5-HT_{1A} and 5-HT_{1B} receptors have been implicated in modifying ethanol intake (3,16,17,33,40) an additional study was conducted to determine which of these receptor subtypes was mediating RU24969's effects on ethanol self-administration. We examined the ability of three selective antagonists, GR127935 (5-HT_{1B}), WAY100635 (5-HT_{1A}), and (+) WAY100135 (5-HT_{1A}) to reverse RU24969's effects. GR127935 is the most selective 5-HT_{1B} antagonist available (37,38). Its inclusion in this study is a major strength because previous work in this area has been hindered by the lack of specific antagonists to clearly demonstrate that the influence of the less selective 5-HT_{1B} agonists on ethanol mediated behaviors is in fact via activation of the 5-HT_{1B} receptor (16). GR127935 is a potent and selective 5-$HT_{1B/1D}$ antagonist that shows approximately 1000-fold selectivity for the 5-HT_{1B} receptor compared with the 5-HT_{1A}, 5-HT_{2C}, and 5-HT_{2A} receptors, with little or no affinity for several other receptors (37). Although GR127935 was shown to be a partial agonist in cell lines expressing high numbers of recombinant human 5-$HT_{1D}\alpha$ and β receptors (42), in isolated tissue preparations and whole animal studies no agonist activity has been shown (24,28,38), suggesting that under physiologic conditions, GR127935 acts as a full 5-$HT_{1B/1D}$ antagonist. In the present study we demonstrated that GR127935 completely reversed the suppression of ethanol self-administration elicited by a moderate dose of RU24969. Although GR127935 failed to reverse the effects of the 1 mg/kg RU24969, this is likely due to the dose used, because previous research has demonstrated that a higher dose of 10 mg/kg GR127925 attenuated both the hyperactivity and anti-immobility effects of RU24969 (24). In contrast neither WAY100635, a 5-HT_{1A} antagonist that shows good selectivity for this receptor subtype (8), or (+) WAY100135, which has recently been reported to possess partial 5-HT_{1B} agonist activity (5), as well as 5-HT_{1A} antagonist activity reversed RU24969-induced suppression of ethanol self-administration. Interestingly, WAY100635 has been reported to attenuate RU24969-induced behavioral syndrome in rats at comparable antagonist doses (0.03 to 1.25 mg/kg) to that used in the present study (1 mg/kg) (25). In light of this observation, the impact of RU24969 administration on ethanol reinforced behavior is not likely due to nonspecific behavioral effects as WAY100635 pretreatment failed to reverse these effects. Taken together, these data confirm

that RU24969 reduces ethanol motivated responding via activation of 5-HT_{1B} and not 5-HT_{1A} receptors, and further support a role played by 5-HT_{1B} receptors in regulating ethanol consumption.

Because 5-HT_{1B} knockout mice have been reported to consume greater amounts of ethanol than wild-type mice (3) (but see (4,30)) it would seem likely that administration of a selective 5-HT_{1B} antagonist would enhance ethanol self-administration. However, GR127935, when administered alone, did not effect ethanol intake in this study. This lack of effect may reflect that 5-HT_{1B} receptors do not exert a tonic influence over ethanol self-administration behavior. Alternatively, chronic rather than acute blockade of the 5-HT_{1B} receptor site may be necessary for an alteration in ethanol self-administration to be expressed. However, we have only tested a single dose of GR127935 and therefore the lack of effect we have observed on ethanol self-administration following GR127935 administration may reflect a dose issue.

In summary, these data further support that 5-HT_{1B} receptors play an important role in regulating ethanol intake. The mechanism via which these effects are mediated are not certain; however, it is unlikely to be due to nonspecific effects of the 5-HT_{1B} agonist used, because the effect on ethanol self-administration was reversed by a selective 5-HT_{1B} antagonist, but not by either of the 5-HT_{1A} antagonists employed. In addition, RU24969 did not elicit similar changes in responding for the alternative reinforcer, saline, or alter ethanol's kinetic profile; therefore, general disruption of operant behavior or altered kinetics appear unlikely explanations of these data. One potential mechanism is that 5-HT_{1B} activation leads to an enhancement of ethanol's reinforcing effects. This is supported by the observation that the 5-HT_{1B} knockout mice exhibit a subsensitivity to ethanol's rewarding effects as assessed using the conditioned place preference paradigm (29). However, in order to confirm this hypothesis additional studies are necessary.

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