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An investigation of the role of 5-HT_{2C} receptors in modifying ethanol self-administration behaviour

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Abstract

We have previously reported that the 5-HT uptake blocker and releaser, dexfenfluramine, attenuates ethanol intake, and that this may be mediated via a 5-HT_{2C} receptor mechanism. Our goals were to further determine the contribution made by this receptor subtype in mediating the reduction in ethanol self-administration induced by dexfenfluramine using the selective 5-HT_{2C} antagonist, SB242,084. Additionally, we wanted to compare dexfenfluramine's effects on ethanol motivated responding with those elicited by the 5-HT_{2C} receptor agonist Ro60-0175. In male Wistar rats trained to self-administer a 12% w/v ethanol solution on an FR-4 schedule, both dexfenfluramine (0.05–2.5 mg/kg ip) and Ro60-0175 (0.1–1 mg/kg sc) produced a significant dose-dependent reduction in ethanol self-administration, which was reversed by SB242,084 (0.5 mg/kg ip). Interestingly, SB242,084 alone (0.1–1 mg/kg ip) significantly increased ethanol motivated responding in both high and low ethanol drinking animals. While dexfenfluramine had no effect on ethanol's kinetic profile, the selective 5-HT_{2C} agents used had opposing effects, with the agonist Ro60-0175 decreasing and the antagonist SB242,084 increasing blood ethanol levels. Since there were incongruent drug effects on ethanol self-administration and blood ethanol levels, these data support a role for 5-HT_{2C} receptors in modifying ethanol intake independent of their effects on blood ethanol kinetics. Furthermore, 5-HT_{2C} receptors may exert a tonic control over ethanol self-administration behaviour, since agonist and antagonist administration had opposing effects on this behaviour. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: 5-HT_{2C} receptors; Dexfenfluramine; Ro60-0175; SB242,084; Ethanol self-administration; Blood ethanol levels

1. Introduction

An important role played by central serotonergic systems in modulating many of the behavioural effects of ethanol, including its self-administration, has been recognized for sometime (Sellers et al., 1992; McBride et al., 1993). Studies have demonstrated that manipulations that enhance central 5-HT function attenuate voluntary ethanol intake across species, although there are some inconsistencies in the literature (LeMarquand et al., 1994a,b). The reasons for these inconsistencies may reflect the complexity of the serotonergic system, with at least 14 mammalian 5-HT receptor subtypes identified to date (Boess and Martin, 1994). Therefore, there has been an increasing focus to ascertain the functional role of these different 5-HT receptor subtypes in modifying the behavioural and physiological effects of ethanol (Romach and Tomkins, 1995). Previously, we have shown that the ability of the 5-HT releaser, dexfenfluramine, to suppress ethanol intake may be mediated via 5-HT_2 receptors, possibly the 5-HT_{2C} receptor subtype (Higgins et al., 1992). Due to a paucity of selective 5-HT_2 receptor agents available at that time, however, this could not be shown definitively. With the recent development of more selective pharmacological probes for the 5-HT_{2C} receptor subtype (Martin et al., 1998; Kennett et al., 1997), the relative contribution played by this receptor in the regulation of ethanol intake may be examined more fully.

In addition to the original data described above, there are a number of compelling reasons for undertaking further re-

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search to explore the role of the 5-HT_{2C} receptor subtype in regulating ethanol intake. Firstly, autoradiographical and immunohistochemical studies have reported notable levels of 5-HT_{2C} mRNA and protein in various brain regions implicated in reward related behaviours, including regions of the amygdala, cortex, nucleus accumbens shell and ventral tegmental area (Pompeiano et al., 1994; Abramowski et al., 1995; Eberle-Wang et al., 1997; Clement et al., 2000). Furthermore, 5-HT_{2C} receptors have been reported to exert a modulatory influence over dopamine neurotransmission, possibly via an alteration in GABAergic function (Prisco et al., 1994; Di Matteo et al., 1998; Di Matteo et al., 1999; Di Giovanni et al., 2001; Lucas and Spampinato, 2000). This is noteworthy for the present investigation given that dopamine is one of the major neurotransmitter systems linked to reinforcement processes (Koob, 2000; Koob and Bloom, 1988; Robinson and Berridge, 2000). Thus, peripheral administration of the 5-HT_{2C} agonist, Ro60-0175 decreases, while the 5-HT_{2C} antagonist, SB242,084 increases extracellular dopamine levels in the nucleus accumbens and frontal cortex (Millan et al., 1998; Di Matteo et al., 1999). Activation of 5-HT_{2C} receptors within the VTA has also been shown to inhibit dopamine cell firing, while the selective 5-HT_{2C} antagonist has the opposite effect (Millan et al., 1998; Di Matteo et al., 1999). Taken together, these data suggest that the 5-HT_{2C} receptor exerts an important modulatory influence over mesolimbic dopamine function and therefore may have an important impact on ethanol motivated behaviour.

While selective agents for the 5-HT_{2C} receptor only recently became available, drugs with mixed agonist activity at 5-HT receptor subtypes further support a role for the 5-HT_{2C} receptor in regulating ethanol intake. Thus, mixed 5-$HT_{1B/2C}$ agonists, such as *m*CPP and TFMPP, have consistently been reported to suppress ethanol self-administration, both in two bottle choice and operant test procedures, although their behavioural selectivity for modifying ethanol intake is a matter of controversy (Higgins et al., 1992; Buczek et al., 1994; Wilson et al., 1998; Maurel et al., 1999a,b). Furthermore, in the case of *m*CPP, there are conflicting results from antagonist interaction studies regarding the relative involvement of the 5-HT_{2C} receptor in mediating these effects (Buczek et al., 1994; Maurel et al., 1998, 1999a,b). Interestingly, there is some evidence to suggest that *m*CPP's ability to generalize to an ethanol cue in rats trained to discriminate modest ethanol doses can be blocked by the selective 5-$HT_{2C/2B}$ antagonist, SB206,553 (Kennett et al., 1996), suggesting that some of ethanol's effects are mediated via this receptor subtype (Maurel et al., 1998). Another 5-HT agonist that has been widely investigated is DOI, which has similar affinities for the 5-HT_{2A} and 5-HT_{2C} receptor subtypes (Middlemiss and Tricklebank, 1992). Studies to date suggest that this agent selectively suppresses ethanol intake and motivated responding (McBride et al., 1990; Wilson et al., 1998; Maurel et al., 1999a,b), and while antagonist studies suggest that this may be mediated via its interaction with the 5-HT_{2A} receptor (Maurel et al., 1999a,b), an additional role played by the 5-HT_{2C} receptor has yet to been ruled out. In addition to the data suggesting that the ability of the 5-HT releaser, dexfenfluramine, to suppress ethanol intake is mediated via the 5-HT_{2C} receptor (Higgins et al., 1992), these observations support the need for further research in this area.

The aim of this series of studies was to further clarify the potential involvement of 5-HT_{2C} receptors in regulating ethanol intake. In the first set of experiments, we compared the behavioural profile elicited by acute administration of dexfenfluramine on ethanol self-administration behavior by male Wistar rats with that seen following the administration of the 5-HT_{2C} receptor agonist, Ro60-0175 under identical experimental conditions. Ro60-0175, an *N*-methy-ethylamine-indole, is often described as a selective 5-HT_{2C} receptor agonist, since many of its effects in vivo are reported to be mediated by this receptor subtype (Millan et al., 1997; Martin et al., 1998; Dekeyne et al., 1999), however, it also exhibits almost equivalent affinity and efficacy at the closely related 5-HT_{2A} and 5-HT_{2B} receptor subtypes (Martin et al., 1998; Porter et al., 1999). Therefore, to clearly demonstrate the involvement of 5-HT_{2C} receptors in mediating the suppressant effects of both dexfenfluramine and Ro60-0175 on ethanol motivated responding, it was important to demonstrate that their effects could be reversed by a selective 5-HT_{2C} antagonist. In the second series of experiments, the effect of the selective 5-HT_{2C} receptor antagonist, SB242,085, administered alone, and in combination with dexfenfluramine and Ro60-0175, on ethanol self-administration was examined. The selection of SB242,084 for these studies was based on its high affinity (pK_I 9.0) and 100-fold selectivity for the 5-HT_{2C} receptor subtype, in addition to its ability to penetrate the blood brain barrier (Kennett et al., 1997). A previous study reported that fenfluramine did not alter ethanol metabolism in Fawn-hooded rats, and therefore its ability to suppress ethanol intake was due to enhancement of serotonergic activity and not alteration in ethanol kinetics (Rezvani and Grady, 1994). Thus, a final study was included to assess the effect of dexfenfluramine, and the newer agents, Ro60-0175 and SB242,084, alone and in combination, on blood ethanol levels.

2. Methods

2.1. 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 19:00 h, temperature: 22–24 °C, humidity: 30–60%) and allowed a 1-week acclimatization period to the animal facilities prior to the start of any of the procedures.

2.2. Operant ethanol self-administration procedure

Training the animals to operantly respond for ethanol consisted of three elements. Initially, the animals had 24-h home cage access to both an ethanol solution and water for a 3-week period. The concentration of the ethanol solution presented was increased each week from 3% to 6% and finally to a 12% w/v ethanol solution. On the third day of home cage access to the 6% w/v ethanol solution food training was initiated. During this time, the rats continued to have free access to water and ethanol, except for the time spent in the operant chambers, but their daily food intake was restricted to 20 g. Food training was conducted in eight operant chambers measuring 28 cm long, 21 cm wide and 21 cm high (Med. Associates, Georgia, VT). Each chamber contained a food pellet dispenser able to deliver 45 mg Noyes food pellets 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 centres 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, VT) linked to a 386sx IBM computer. Initially, each response on the active lever resulted in a delivery of a food pellet (fixed ratio, FR-1), while responses on the inactive lever were recorded but had no consequences. The sessions lasted 15 min or until the rat had earned 50 reinforcers. The reinforcement schedule was increased to FR-5 and eventually FR-10 when the rat earned 50 reinforcers on 2 consecutive days. The rats received a total of 8 days of food operant training. The ad libitum food regimen was then recommenced and home cage ethanol access was stopped. Two days following the last food training session, operant responding for a 12% w/v ethanol solution was initiated.

Ethanol self-administration sessions were performed in 16 operant chambers similar in construction to those used for the food training except that 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. The rats were placed daily in the operant boxes for 30 min and trained to press the active lever for a 12% (w/v) ethanol solution on a FR-1 schedule. The response requirement was switched to FR-2 and finally to FR-4 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 drug studies were started.

2.3. Study 1: Effect of acute dexfenfluramine and Ro60-0175 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 dexfenfluramine (0, 0.5, 1 and 2.5 mg/kg ip, 60 min pretreatment) on self-administration behaviour 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. Following the data analysis of this study, a second experiment was conducted to determine the effects of lower dexfenfluramine doses (0, 0.05, 0.1 and 0.5 mg/kg ip) on ethanol motivated responding. This was assessed in 11 previously drug naïve rats. In a third group of male Wistar rats ($n = 10$), the effect of the acute administration of Ro60-0175 (0, 0.1, 0.3 and 1 mg/kg sc, 30 min pretreatment) on self-administration behaviour was evaluated in a similar manner.

2.4. Study 2: Effect of selective 5-HT_{2c} receptor antagonist, SB242,084 on dexfenfluramine- and RU24969-induced suppression of oral ethanol self-administration

This study was designed to determine if the effects observed in Study 1 were due to 5-HT_{2C} receptor activation. Thus, the ability of the selective 5-HT_{2C} antagonist, SB242,084, to reverse the effects of dexfenfluramine and Ro60-0175 on oral ethanol self-administration was examined. Two separate groups of experimentally naïve rats were trained to self-administer ethanol. In the first group, the ability of SB242,084 (0.5 mg/kg ip, $n = 6$), to reverse the effects of 0.5 mg/kg dexfenfluramine on ethanol self-administration was assessed. A Latin square design was employed, with SB242,084 administered 20 min prior to dexfenfluramine, such that each animal received each drug combination in a balanced order. Each treatment day was separated from the next by at least 3 days. In a second group of animals ($n = 8$), the ability of the SB242,084 (0.5 mg/kg ip) administered 20 min prior to Ro60-0175 (0.5 mg/kg sc) was assessed in a similar manner. Analysis of the data from these studies suggested that SB242,084 increased ethanol self-administration behaviour. To examine this further, an additional group of rats ($n = 4$) that did not acquire pharmacologically relevant levels of ethanol self-administration (< 0.3 g/kg) were employed to determine whether SB242,084 dose dependently increases ethanol self-administration (0, 0.1, 0.5 and 1 mg/kg ip, 40 min pretreatment) in low ethanol drinking rats.

2.5. Study 3: Effect of dexfenfluramine, Ro60-0175 and SB242,084 on the pharmacokinetic profile of experimenter-administered ethanol in male Wistar rats

Rats were fasted overnight prior to the study. Each rat received its allocated treatment and when the relevant pretreatment time had elapsed the rats received a 0.8 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 and 60 min post-ethanol administration. Blood ethanol levels were determined by gas–liquid chromatography technique (LeBlanc, 1968) with propanol-2 as

internal standard. The effect of dexfenfluramine on ethanol blood levels was examined in 10 drug naïve male Wistar rats. The rats were divided into two groups, one group received 0.5 mg/kg dexfenfluramine 60 min prior to the ethanol administration, the remaining animals were administered saline (1 ml/kg). One week later, the study was repeated, with the animals receiving the second treatment. In a similar manner, the effects of 0.5 mg/kg Ro60-0175 administered as a 30-min pretreatment was examined in a separate group of 10 drug naïve rats. In a group of 32 rats, the influence of the combined administration of the selective 5-HT_{2C} antagonist, SB242,084 (0.5 mg/kg), and either dexfenfluramine (0.5 mg/kg), Ro60-0175 (0.5 mg/kg) or saline (1 ml/kg) on blood ethanol levels achieved following oral administration of 0.8 g/kg ethanol was examined.

2.6. Drugs

Ro60-0175 ((*S*)-2-(chloro-5-fluoro-indol-1-yl)-1-methyl-ethylamine 1:1 $C_4H_4O_4$) and SB242,084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl]indoline) were synthesised within the Chemistry department at F. Hoffmann-La Roche, Basel. Dexfenfluramine hydrochloride was a gift from Servier. Ro60-0175 and dexfenfluramine were dissolved in 0.9% saline. SB242,084 was prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid. All drug doses are expressed as that of the salt.

2.7. Statistical analysis

Data were analysed by one or two way repeated measures ANOVA using Statsview software. Post hoc comparisons were carried out with Neuman–Keuls test. In all cases, the accepted level of significance was taken at $P<.05$.

3. Results

3.1. Study 1: Effect of acute dexfenfluramine and Ro60-0175 administration on oral ethanol self-administration

In the initial dexfenfluramine study, the rats selected for the study exhibited stable and selective responding on the ethanol appropriate lever (total presses on the active lever: 162.1 ± 29.9; total presses on the inactive lever; 0.8 ± 0.3), to self-administer pharmacologically relevant levels of ethanol (0.76 ± 0.14 g/kg). Acute dexfenfluramine administration (0.5–2.5 mg/kg) significantly and dose dependently reduced the total number of ethanol reinforcers obtained [$F(3,21)=20.47$, $P<.001$] during the 30-min test session (Fig. 1). Post hoc analysis showed that all doses of dexfenfluramine reduced ethanol motivated responding [$F(3,21)=18.8$, $P<.001$], with none of the animals responding for ethanol at the 2.5 mg/kg dose level. This decrease in ethanol self-administration was due, in part, to an earlier termination in ethanol motivated responding [$F(3,21)=12.37$, $P=.001$] and a modest increase in the latency to earn the first reinforcer (Fig. 1). There was no significant effect of dexfenfluramine on response rate in those animals that did respond for ethanol [$F(2,10)=1.33$, N.S., Table 1], or responding on the inactive lever [$F(3,21)=2.08$, N.S., data not shown]. In the second study to determine the lowest effective dose of dexfenfluramine to attenuate ethanol self-administration, there was a significant overall treatment effect on ethanol intake [$F(3,30)=7.1$, $P=.001$], number of ethanol reinforcers earned [$F(3,30)=7.20$, $P<.001$] and latency to earn the first ethanol reinforcer [$F(3,30)=4.57$, $P<.01$], while the total

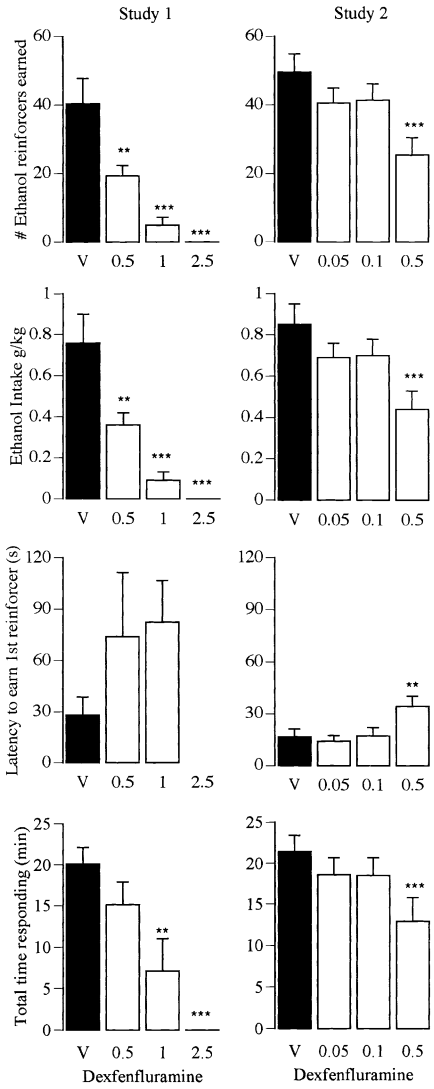

Fig. 1. Effect of dexfenfluramine administration on ethanol self-administration maintained on a FR-4 schedule of reinforcement. In two separate groups of animals, dexfenfluramine (0,0.5, 1 and 2.5 mg/kg ip, $n=8$ and 0, 0.05, 0.1 and 0.5 mg/kg ip, $n=10$) was administered such that each rat received all treatments in a randomised manner 60 min prior to the initiation of the test session. Significant differences from the vehicle control condition are indicated by ** $P<.01$ and *** $P<.001$.

Table 1
Effect of dexfenfluramine (0.01–2.5 mg/kg ip) and Ro60–0175 (0.1–1 mg/kg sc) on the rate of responding during 30 min operant ethanol self-administration sessions where behaviour was maintained on an FR-4 schedule of reinforcement

Drug treatment	Dose (mg/kg)	Response rate (responses/min)
Dexfenfluramine	0	8.8 ± 2.0
	0.5	6.3 ± 1.2
	1.0	4.9 ± 1.6
	2.5	ND
Dexfenfluramine	0	10.6 ± 1.6
	0.05	10.2 ± 1.7
	0.1	9.9 ± 1.4
	0.5	9.4 ± 1.3
Ro60-0175	0	12.4 ± 3.0
	0.1	11.5 ± 1.5
	0.3	11.9 ± 2.6
	1.0	6.9 ± 1.7

Data is presented as the mean ± S.E.M. where the response rate was calculated as the total number of lever presses/(time to last response − latency to first response) expressed per minute. ND signifies where none of the animals pressed either lever during the ethanol self-administration session and therefore a response rate could not be calculated for this treatment condition.

time spent responding for ethanol just failed to reach statistical significance [$F(3,30) = 2.76$, $P = .059$]. There was no effect of dexfenfluramine on response rate [$F(3,30) = 0.19$, N.S., Table 1]. Post hoc analysis revealed that only the 0.5 mg/kg dose of dexfenfluramine significantly reduced ethanol motivated responding (Fig. 1). Under control conditions, responding on the inactive lever during the operant session was low (1.1 ± 0.5) and this was not altered by dexfenfluramine administration [$F(3,30) = 0.34$, N.S., data not shown].

Rats selected for the acute Ro60-0175 challenge study exhibited stable and selective responding on the ethanol appropriate lever (total presses on the active lever: 129.4 ± 20.4; total presses on the inactive lever; 0.7 ± 0.4), to self-administer pharmacologically relevant levels of ethanol (0.62 ± 0.11 g/kg). Acute Ro60-0175 administration produced similar effects on ethanol self-administration as that observed following dexfenfluramine administration (Fig. 2). Ro60-0175 (0.1–1 mg/kg sc) significantly altered ethanol intake [$F(3,27) = 18.07$, $P = .001$], number of ethanol reinforcers earned [$F(3,27) = 18.42$, $P = .001$] and total time spent responding for ethanol [$F(3,27) = 6.50$, $P = .0019$]. Post hoc analysis showed that both the 0.3 and 1 mg/kg doses reduced ethanol motivated responding, with three of the animals failing to respond for ethanol at the 1 mg/kg dose level. There was no effect of Ro60-0175 on response rate in those animals that did respond for ethanol [$F(3,24) =$

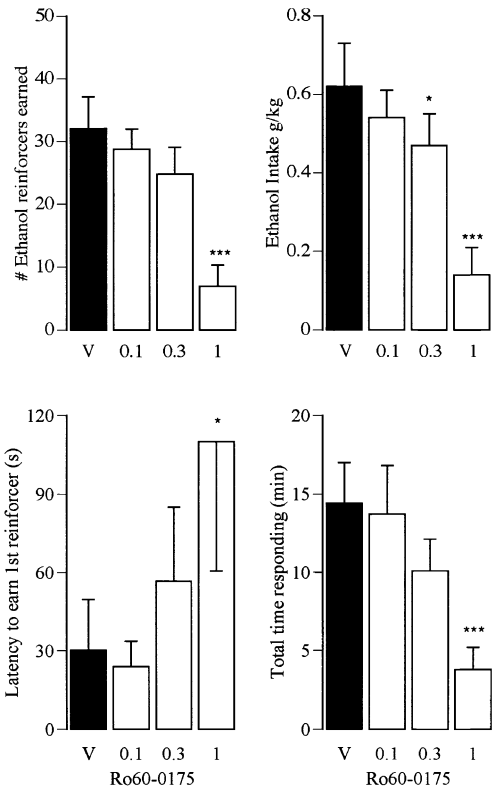

Fig. 2. Effect of Ro60-0175 administration (0, 0.1, 0.3 and 1 mg/kg sc, 30 min pretreatment, $n = 11$) on ethanol self-administration maintained on a FR-4 schedule of reinforcement. Significant differences from the vehicle control condition are indicated by $^{*}P < .05$ and $^{***}P < .001$.

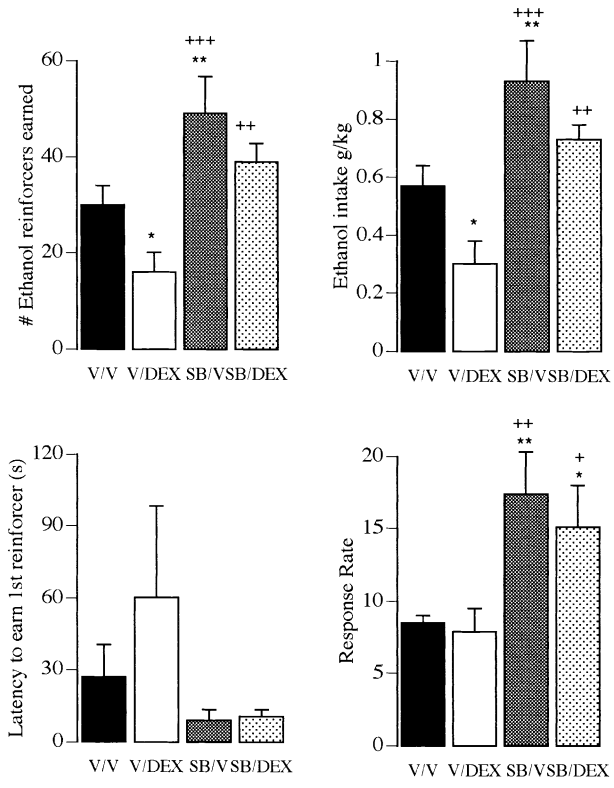

Fig. 3. Effect of SB242,084 administration (0.5 mg/kg ip) on dexfenfluramine (0.5 mg/kg ip) induced suppression of ethanol self-administration maintained on a FR-4 schedule of reinforcement ($n = 6$ male Wistar rats). Significant differences from the vehicle/vehicle control condition are indicated by $^{*}P < .05$ and $^{**}P < .01$ and from the vehicle/dexfenfluramine combination are indicated by $^{+}P < .05$, $^{++}P < .01$ and $^{+++}P < .001$.

1.06, N.S., Table 1] or responding on the inactive lever [$F(3,27) = 1.89$, N.S., data not shown].

3.2. Study 2: Effect of the selective 5-HT$_{2C}$ receptor antagonist, SB242,084 on dexfenfluramine- and Ro60-0175-induced suppression of oral ethanol self-administration

Pretreatment with the selective 5-HT$_{2C}$ antagonist, SB242,084, reversed dexfenfluramine's effects on ethanol self-administration behaviour (Fig. 3). Statistical analysis confirmed that dexfenfluramine reduced ethanol intake [$F(1,5) = 7.06$, $P = .045$] and the number of ethanol reinforcers earned [$F(1,5) = 6.85$, $P = .047$], but did not alter responding on the inactive lever [$F(1,5) = 1.18$, N.S.]. There was an overall effect of SB242,084 in this study on measures of ethanol intake [$F(1,5) = 18.7$, $P = .0075$], number of ethanol reinforcers earned [$F(1,5) = 17.7$, $P = .0085$] and response rate [$F(1,5) = 20.4$, $P = .0063$]. Post hoc tests revealed that the dexfenfluramine-induced suppression of ethanol self-administration was reversed by SB242,084 (Fig. 3), however, SB242,084 pretreatment alone significantly enhanced ethanol self-administration and the rate of responding on the active, but not the inactive lever.

In a similar manner, SB242,084 reversed the effects of Ro60-0175 on ethanol self-administration (Fig. 4). Statistical analysis confirmed that Ro60-0175 reduced ethanol intake [$F(1,7) = 5.79$, $P = .047$] and that SB242,084 had an overall significant effect on ethanol intake [$F(1,7) = 39.3$, $P = .0004$], number of ethanol reinforcers earned [$F(1,7) = 39.32$, $P = .0004$] and response rate [$F(1,7) = 10.7$, $P = .014$], but did not alter responding on the inactive lever [$F(1,7) = 2.74$, N.S.]. Post hoc analyses showed that SB242,084 reversed the effects of Ro60-0175, however, SB242,084 pretreatment alone significantly enhanced ethanol self-administration and the rate of responding on the active, but not the inactive lever, as observed in the above study.

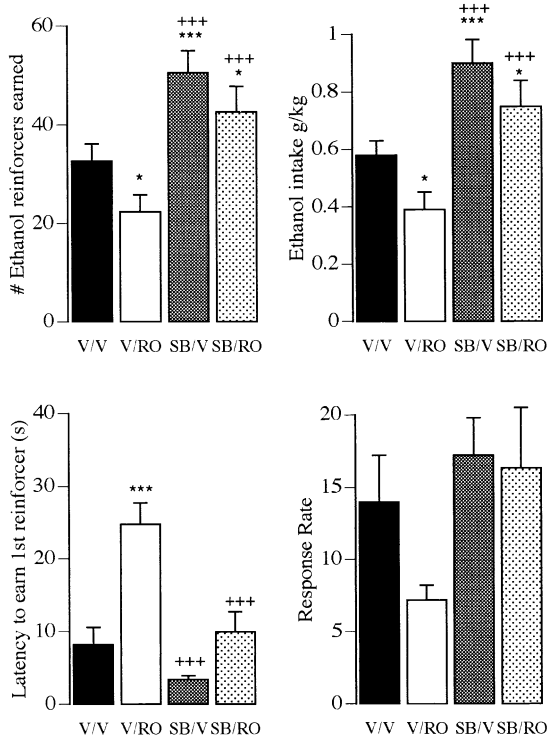

Fig. 4. Effect of SB242,084 administration (0.5 mg/kg ip) on Ro60-0175 (0.5 mg/kg sc) induced suppression of ethanol self-administration maintained on a FR-4 schedule of reinforcement ($n = 8$ male Wistar rats). Significant differences from the vehicle/vehicle control condition are indicated by $^{*}P < .05$ and $^{***}P < .001$ and from the vehicle/dexfenfluramine combination are indicated by $^{+++}P < .001$.

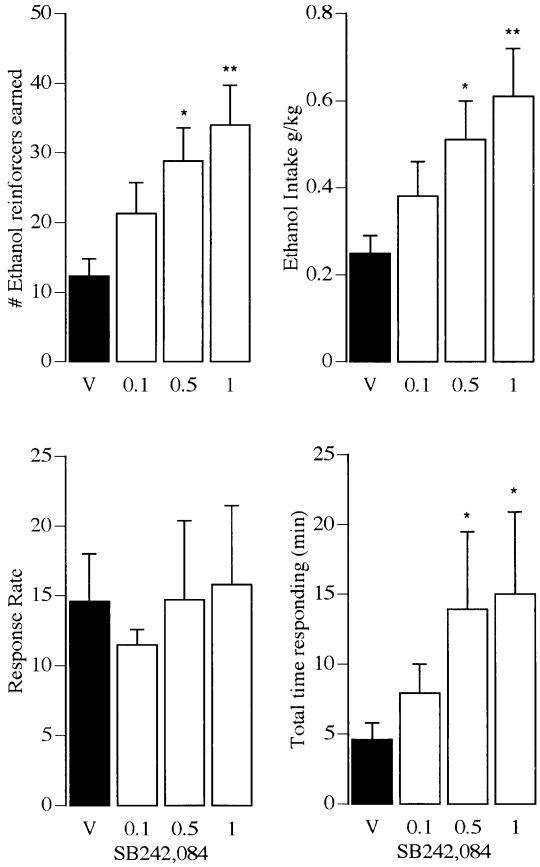

Fig. 5. Effect of SB242,084 administration on ethanol self-administration maintained on a FR-4 schedule of reinforcement in low ethanol drinking rats. SB242,084 (0,0.1, 0.5 and 1 mg/kg ip, $n = 4$) was administered such that each rat received all treatments in a randomised manner 30 min prior to the initiation of the test session. Significant differences from the vehicle control condition are indicated by $^{*}P < .05$ and $^{**}P < .01$.

In the final study examining the effects of SB242,084 pretreatment on ethanol self-administration behaviour in low ethanol consuming animals, statistical analyses revealed a significant potentiation in ethanol intake that was dose dependent [$F(3,9) = 4.02$, $P = .045$] (Fig. 5). Post hoc tests showed that both the 0.5 and 1 mg/kg doses tested accounted for this overall significance. In contrast to the effects seen in high ethanol drinking rats, there was no significant effect of SB242,084 on response rates [$F(3,9) = 0.52$,

Table 2
Effect of dexfenfluramine (0.5 mg/kg), Ro60-0175 (0.5 mg/kg) and SB242,084 (0.5 mg/kg), administered either alone or in combination, on the time course of blood ethanol levels achieved (mg/dl) following oral administration of 0.8 g/kg dose of ethanol

	Time (min)			
Drug treatment	15	30	45	60
Vehicle	42.6 ± 2.5	74.8 ± 3.8	78.0 ± 1.9	73.3 ± 1.7
Dexfenfluramine	42.0 ± 5.5	66.6 ± 4.2	73.5 ± 2.1	74.0 ± 1.5
Vehicle	61.2 ± 6.6	77.7 ± 3.8	83.4 ± 2.0	79.9 ± 1.6
Ro60-0175	21.9 ± 2.0***	38.2 ± 4.7***	48.4 ± 4.1***	55.4 ± 3.9***
Vehicle/vehicle	34.8 ± 4.7	47.8 ± 4.2	53.5 ± 2.3	53.6 ± 3.5
SB242,084/vehicle	51.4 ± 2.9	67.3 ± 1.9**	66.0 ± 2.9*	60.5 ± 2.9
SB242,084/dexfenfluramine	48.5 ± 3.4	64.3 ± 2.3**	60.6 ± 2.6	57.8 ± 1.7
SB242,084/Ro60-0175	36.5 ± 6.9	56.2 ± 4.2	63.1 ± 3.1	61.4 ± 3.9

Data is presented as the mean blood ethanol levels achieved ± S.E.M. expressed as mg/dl. Significant differences from the vehicle control conditions are indicated by * $P < .05$, ** $P < .01$ and *** $P < .001$.

N.S.]. Furthermore, the increase in responding was limited to the ethanol reinforced lever since responding on the inactive level was not altered [$F(3,9) = 0.42$, N.S.].

3.3. Study 3: Effect of dexfenfluramine, Ro60-0175 and SB242,084 on the pharmacokinetic profile of experimenter-administered ethanol in male Wistar rats

Statistical analysis of the effects of dexfenfluramine and Ro60-0175 on blood ethanol levels revealed a difference between these two agents. While dexfenfluramine had no effect on ethanol's kinetic profile [$F(1,27) = 0.8$, N.S.], Ro60-0175 significantly attenuated blood ethanol levels achieved over the 1-h sampling period [$F(1,27) = 53.8$, $P = 0.0001$] (Table 2). In the second study, examining the effects of the selective 5-HT_{2C} antagonist, SB242,084 on blood ethanol levels, when administered alone, or in combination with dexfenfluramine or Ro60-0175, statistical analysis revealed a significant effect of drug condition on blood ethanol levels [$F(3,73) = 5.5$, $P = .004$]. Post hoc tests revealed that SB242,084 reversed the effects of Ro60-0175, however, when administered alone, or in combination with dexfenfluramine, SB242,084 significantly increased blood ethanol levels (Table 2).

4. Discussion

The present results suggest that 5-HT_{2C} receptors exert a tonic influence over ethanol self-administration behaviour by male Wistar rats. This is supported by the observations that peripheral administration of the 5-HT releaser, dexfenfluramine, and the 5-HT_{2C} agonist, Ro60-0175, suppressed ethanol self-administration and that this suppression induced by both of these drugs was reversed by the administration of the selective 5-HT_{2C} antagonist, SB242,984. In addition, SB242,084 administration alone enhanced ethanol self-administration in both high and low ethanol self-administering animals. These findings are consistent with our previous data that implicated the 5-HT_2 receptor, possibly the 5-HT_{2C} receptor subtype, in mediating the effects of dexfenfluramine (Higgins et al., 1992). In addition, other serotonergic agents with intrinsic activity at the 5-HT_{2C} receptor subtype (e.g., *m*CPP, TFMPP, DOI) have been reported to suppress ethanol intake at doses that do not alter lever press response rates or locomotor activity (McBride et al., 1990; Higgins et al., 1992; Buczek et al., 1994; Wilson et al., 1998; Maurel et al., 1999a,b). Furthermore, dexfenfluramine and Ro60-0175 altered the profile of ethanol motivated responding in a similar manner, suggesting that their effects are elicited by a common mechanism, that was typified by an early termination in responding at doses that did not alter response rate.

Ro60-0175, is often described as a selective 5-HT_{2C} receptor agonist, however, it also exhibits almost equivalent affinity and efficacy at the closely related 5-HT_{2A} and 5-HT_{2B} receptor subtypes (Martin et al., 1998; Porter et al., 1999), which may have contributed to the present findings. This is supported by autoradiographic mapping studies that have suggested that lower densities of 5-HT_{2A} binding sites in discrete brain regions are correlated with an increased propensity to consume ethanol (McBride et al., 1993; Ciccocioppo et al., 1997, 1999; but see Korpi et al., 1992). Furthermore, pharmacological studies conducted by Maurel et al. (1999a,b) have proposed a selective role played by the 5-HT_{2A} receptor in regulating ethanol intake. However, we have demonstrated that the effects of Ro60-0175 on ethanol self-administration were reversed by SB242,084, an antagonist that has been reported to exhibit 100- and 158-fold selectivity for the 5-HT_{2C} receptor over the 5-HT_{2A} and 5-HT_{2B} receptor subtypes respectively (Kennett et al., 1997). These data suggest that the effects of Ro60-0175 on ethanol intake are mediated via activation of the 5-HT_{2C} receptor which is in concordance with findings from other behavioural studies using this agonist (Millan et al., 1997; Martin et al., 1998; Dekeyne et al., 1999) and with our previous data with dexfenfluramine (Higgins et al., 1992).

Previously, it has been reported that dexfenfluramine does not alter locomotor behaviour at doses relevant to the present study (Wilson et al., 1998), while doses of Ro60-0175 that suppress ethanol intake do not induce catalepsy or modify performance in tests of motor co-ordination (Grottick et al., 2000). Therefore, it is unlikely that a general

disruption of motor behaviour could account for the suppression in ethanol self-administration produced by either of these agents. Administration of SB242,084 alone, enhanced ethanol motivated responding, an effect that is also unlikely to be due to nonspecific changes in motor behaviour. This is supported by the reports that SB242,0984, at similar or higher doses than those used in the present study, did not significantly increase locomotor activity behaviour (Kennett et al., 1997; Hutson et al., 2000; Higgins et al., 2001). In the case of dexfenfluramine, we have also ruled out a possible influence of altered ethanol pharmacokinetics in underlying its ability to reduce ethanol intake, which is consistent with previous findings in Fawn-hooded rats (Rezvani and Grady, 1994). In contrast, the 5-HT_{2C} agonist, Ro60-0175 decreased, while the selective 5-HT_{2C} antagonist, SB242,084 increased blood ethanol levels under similar experimental conditions. Since we observed bidirectional effects with more selective 5-HT_{2C} agonist and antagonist treatments, there is a possibility that this is a 5-HT_{2C} mediated effect. Although, there has been no direct evidence that 5-HT_{2C} receptors occur in peripheral tissue (Hoyer et al., 1994), a recent study by Javid and Naylor (1999) suggests that gastrointestinal motility may be under the influence of 5-HT_{2C} receptors, which in turn may alter ethanol absorption. Alternatively, drug-induced alterations in peripheral blood flow may have contributed to these findings since the blood samples used in the analyses were drawn from the tail. There is clear evidence that 5-HT administration causes vasoconstriction of the rat tail artery, and that is susceptible to reversal by preferential 5-HT_{2A} antagonists (Kaumann and Frenken, 1988; Pertz and Elz, 1995). There is, however, currently no evidence to suggest a 5-HT_{2C} or 5-HT_{2B} receptor mediated effect on blood flow in the rat tail. Further assessment of other selective agents at this receptor subtype, as well as the demonstration of their distribution in peripheral tissue, and/or their effects on peripheral blood flow are necessary to confirm a 5-HT_{2C} mediated effect on ethanol pharmacokinetics and elucidate the mechanism underlying this effect.

These blood ethanol data raise the possibility that the alterations in ethanol self-administration behaviour are secondary to the influence of both Ro60-0175 and SB242,084 on ethanol pharmacokinetics. Experimental evidence to date, however, has not demonstrated a clear relationship between alterations in ethanol pharmacokinetics and ethanol drinking behaviour. Thus, pharmacological manipulations that reduce blood ethanol levels, have been reported to decrease (Buczek et al., 1998), increase (Linseman, 1989) or have no effect (Linseman, 1989) on ethanol self-administration behaviour. Pharmacological manipulations that increase blood ethanol levels have also produced conflicting effects on ethanol self-administration, with both increases and decreases in ethanol intake being reported (Waller et al., 1982; Boyle et al., 1998; Williams et al., 1998; Gentry et al., 1983; Gentry, 1985; Aragon et al., 1993). In light of these conflicting data, which may be a result of the differing experimental conditions employed, it is difficult to predict the contribution made by alterations in ethanol pharmacokinetics on ethanol intake in the present study. Evidence suggests, however, that 5-HT_{2C} receptor-mediated changes in ethanol self-administration cannot solely be accounted for by altered ethanol pharmacokinetics. Thus, while dexfenfluramine and Ro60-0175 elicit similar changes in ethanol motivated responding, namely an early termination in responding, both of which are reversed by SB242,084 administration, they have dissimilar effects on blood ethanol levels. Furthermore, this altered behavioural profile of an early termination of motivated responding has been observed for other drug and nondrug reinforcers, following dexfenfluramine and Ro60-0175 administration, for which altered gastrointestinal motility may not necessarily be implicated. For example, dexfenfluramine has been reported to suppress responding for heroin, cocaine and food (Blundell, 1984; Higgins et al., 1994; Wang et al., 1995; Glowa et al., 1997; Clifton et al., 2000), while Ro60-0175 has been reported to suppress food intake (Clifton et al., 2000) and reduce break points of cocaine maintained on a progressive ratio schedule (Grottick et al., 2000). Taken together, these data suggest that 5-HT_{2C} receptors play a role in modifying motivated responding for a wide range of reinforcers in a similar manner, and not ethanol specifically, and that these effects are unlikely to be attributable to a general alteration in measures of performance or kinetics.

The fact that SB242,084 increased ethanol self-administration when administered alone raises the possibility that its ability to attenuate the effects of both dexfenfluramine and Ro60-0175 on ethanol self-administration may be due to functional antagonism, as opposed to pharmacological antagonism at the level of the 5-HT_{2C} receptor as proposed. This explanation however, seems unlikely based on the pharmacological evidence that 5-HT_{2C} receptors exert a tonic influence over some neurochemical systems (Prisco et al., 1994; Di Matteo et al., 1998; Millan et al., 1998; Di Matteo et al., 1999; Lucas and Spampinato, 2000), and that the drugs used exhibit high affinity for this receptor subtype (Martin et al., 1998, Porter et al., 1999, Kennett et al., 1997). Additional studies, that identify lower doses of SB242,084, that reverse dexfenfluramine's and Ro60-0175's effects on ethanol self-administration but do not influence ethanol intake alone are required to further support this. The observation that SB242,084 does alter ethanol intake suggests that the 5-HT_{2C} receptor subtype exerts a tonic influence over ethanol self-administration. The mechanism via which 5-HT_{2C} receptors regulates ethanol motivated responding is still not known, however, the discrete localization of this receptor subtype within some brain regions implicated in reinforcement processes is intriguing (Pompeiano et al., 1994; Abramowski et al., 1995; Eberle-Wang et al., 1997; Clement et al., 2000). Furthermore, 5-HT_{2C} receptors have been shown to exert a tonic influence over mesolimbic dopamine function (Prisco et al., 1994; Di Matteo et al., 1998; Millan et al., 1998; Di

Matteo et al., 1999; Lucas and Spampinato, 2000). Since this dopaminergic pathway has been shown to play an important role in regulating many of the behavioural effects of psychoactive substances, including ethanol, it is conceivable that the effects reported in this study are mediated, in part, by this system. This is supported by the fact that discrete nucleus accumbens injections of dopamine agonists, increase, while dopamine antagonists decrease ethanol motivated responding (Hodge et al., 1992; Samson et al., 1993). These findings are concordant with the present study since the 5-HT_{2C} antagonist, SB242,084, increases both nucleus accumbens dopamine release and ethanol self-administration behaviour (Di Matteo et al., 1999), while the converse is true for the effects of the 5-HT_{2C} agonist, Ro60-0175 (Millan et al., 1998, Di Matteo et al., 2001). Further studies are necessary to more directly examine any relationship between 5-HT_{2C} receptor-mediated modulation of mesolimbic dopaminergic function and ethanol self-administration behaviour.

In summary, the present series of studies demonstrate that 5-HT_{2C} receptors exert tonic control over ethanol self-administration behaviour by male Wistar rats, and that this receptor subtype plays an important role in the suppressant effects of the 5-HT releaser, dexfenfluramine, on ethanol motivated responding. The effects of dexfenfluramine and the selective 5-HT_{2C} agents on ethanol self-administration exhibit a similar profile, both in terms of effective doses and alterations in behavioural responding, to their effects on the self-administration of other drug and nondrug reinforcers. This suggests that the 5-HT_{2C} receptor represents an important neural substrate for regulating motivated behaviours in general.

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