ELSEVIER

European Journal of Pharmacology 318 (1996) 257–265

ejp

Effects of a range of dopamine receptor agonists and antagonists on ethanol intake in the rat

Jordi S. Silvestre *, Michael F. O'Neill [1], Andrés G. Fernández, José M. Palacios

Department of Pharmacology, Research Centre, Laboratorios Almirall, Cardener 68–74, Barcelona 08024, Spain

Received 29 February 1996; revised 10 October 1996; accepted 15 October 1996

Abstract

The aim of this study was to assess the effects of a range of dopaminergic agents on consumption of an ethanol solution (10% ethanol, 3% glucose) in rats. A two-bottle, free-choice paradigm was used following induction of ethanol consumption and preference in standard laboratory rats. The model used provides a robust and reliable level of ethanol oral administration in normal laboratory rats. Both ethanol intake and preference were reduced by a dopamine D_1 receptor partial agonist, SFK 38393 ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride), in a dose-dependent manner. The dopamine D_2/D_3 receptor agonist 7-OH-DPAT ((±)-7-hydroxy-*N*,*N*-(di-*n*-propyl-2-aminotetralin)) at the lowest dose of 0.01 mg/kg increased both ethanol intake and preference. At higher doses (0.03–0.1 mg/kg) no significant effects were found. The dopamine D_1 receptor antagonist SCH 23390 (*R*-(+)-7-chloro-2,3,4,5-tetrahydro-3-methyl-1-phenyl-1*H*-3-benzazepine-8-ol), dopamine D_2/D_3 receptor antagonist raclopride and 5-HT_2/D_2 receptor antagonist risperidone did not affect ethanol consumption, although all at high doses induced a significant decrease in water intake, indicating a non-specific decrease in consummatory behavior with these compounds. These results suggest the involvement of the dopaminergic system in ethanol intake and ethanol reinforcement with dopamine D_1 and D_2/D_3 receptors playing opposing roles. Blockade of dopamine D_2 receptors had no selective effect on ethanol consumption and ethanol preference.

Keywords: Ethanol preference; Dopamine; SKF 38393; Raclopride; 7-OH-DPAT (7-hydroxy-2-(di-*n*-propylamino)tetralin); Risperidone; SCH 23390; (Rat)

1. Introduction

The reinforcing properties of ethanol are believed to be mediated at least in part by the mesocorticolimbic dopamine system (Samson and Harris, 1992). This system has also been implicated in the mediation of the rewarding properties of other drugs of abuse (Koob, 1992). While it has been suggested that only part of the regulatory process controlling ethanol drinking directly involves those pathways (Samson et al., 1993), it has been shown that oral self-administration of ethanol (Weiss et al., 1992) and systemic administration of low doses of ethanol (Imperato and Di Chiara, 1986; Yoshimoto et al., 1991) both increase extracellular dopamine levels and stimulate dopamine release respectively in the nucleus accumbens. It has recently been shown that ethanol-preferring selected C57BL/6J mice (C57) show reduced dopamine content and turnover in the terminals of the mesolimbic and mesostriatal dopamine neurons (George et al., 1995). Furthermore, George et al. (1995) have also shown that increasing synaptic dopamine levels led to decreases in ethanol preference and consumption in C57 mice, suggesting that reduced dopaminergic function in limbic dopamine pathways is an important factor predisposing to the liability for increased ethanol preference and intake.

Chronic ethanol treatment produces changes in the B_{max} values of dopamine receptors. Alcoholic liquid-diet treatment produced significant changes in dopamine D_1 receptor (Hamdi and Prasad, 1993; Lograno et al., 1993; Pellegrino and Druse, 1992) and dopamine D_2 receptor densities in different brain regions of the rat. A lower density of dopamine D_2 receptors has been shown in several brain regions in ethanol-preferring rats compared to non-preferring or unselected rats (Hamdi and Prasad, 1992; Korpi et al., 1987; Stefanini et al., 1992).

* Corresponding author. Fax: (34-3) 291-3532.

[1] Present address: Lilly Research Centre, Erl Wood Manor, Sunninghill RD, Windlesham, Surrey GU20 6PH, UK.

PII S0014-2999(96)00821-7

There are some, although inconsistent and even contradictory, findings from studies relating to the involvement of dopaminergic systems in ethanol consumption and preference. Intraventricular administration of 6-hydroxydopamine decreased ethanol preference in rats (Melchior and Myers, 1976). However, some studies have shown that 6-hydroxydopamine lesions of mesolimbic dopamine systems in rats, including nucleus accumbens, failed to alter ethanol self-administration as measured by the lever-press response for ethanol reward (Rassnick et al., 1993a). Likewise, 6-hydroxydopamine lesions of ventral striatal nucleus have shown similar negative results (Fahlke et al., 1994).

Pharmacological studies have provided similarly diverse findings. Intra-accumbens microinjections of fluphenazine, a dopamine D_2/D_3 receptor antagonist, decreased oral ethanol self-administration in rats (Rassnick et al., 1992), while microinjections of *d*-amphetamine increase ethanol-reinforced responding in rats (Hodge et al., 1992; Samson et al., 1993). This is in contrast with the effects of systemic administration of *d*-amphetamine which caused the opposite effect (Pfeffer and Samson, 1986). Microinjections into the nucleus accumbens of quinpirole, a dopamine D_2/D_3 receptor agonist, increased ethanol-reinforced responding in one study (Hodge et al., 1993) but had no effect in another study (Samson et al., 1993), while raclopride, a dopamine D_2/D_3 receptor antagonist, decreased ethanol-reinforced responding (Samson et al., 1993). However, opposite effects have been shown with sulpiride, a dopamine D_2/D_3 receptor antagonist, which enhances ethanol drinking in alcohol-preferring (P) rats (Levy et al., 1991).

Systemic administrations of either (+)-SKF 38393 or SKF 82958, two dopamine D_1 receptor partial agonists, produced reductions in ethanol intake in mice (George et al., 1995; Ng and George, 1994) and in rats genetically selected for high-alcohol intake (Dyr et al., 1993). SCH 23390, a dopamine D_1 receptor antagonist, appears also to decrease ethanol intake in genetically selected high-alcohol-drinking (HAD) rats (Dyr et al., 1993). A mixed dopamine D_1/D_2 receptor agonist, SDZ 205-152, reduced ethanol-reinforced responding in unselected rats without affecting responses for water (Rassnick et al., 1993b). Furthermore, bromocriptine, a dopamine D_2/D_3 receptor agonist, decreased both ethanol intake and preference in both an ethanol-reinforced responding task in unselected rats (Weiss et al., 1990) and ethanol intake in selected mice (Ng and George, 1994). Likewise, quinpirole, another dopamine D_2/D_3 receptor agonist, caused a decrease in ethanol drinking in both HAD rats (Dyr et al., 1993) and C57 mice (George et al., 1995). Haloperidol, a dopamine D_2-type receptor antagonist, also has been shown to decrease both ethanol preference and intake in genetically selected Sardinian alcohol-preferring (sP) rats (Panocka et al., 1993b) and non-genetically selected rats (Panocka et al., 1993a). Likewise, a 5-HT_2/D_2 antagonist, risperidone, decreased ethanol intake and preference but only in non-genetically selected rats (Panocka et al., 1993a), while it showed effects in sP rats only at the highest dose tested (10 mg/kg) (Panocka et al., 1993b).

Finally, the data from behavioral studies relating to dopamine D_3 receptors have been limited due to lack of selective dopamine D_3 receptor agents, although it has been suggested that the dopamine D_3 receptor could be involved in brain reward pathways and thus mediates drug-seeking behavior (Schwartz et al., 1994). Recently it was shown that the selective dopamine D_3 receptor agonist 7-OH-DPAT could reduce the preference for ethanol (Meert and Clincke, 1994).

The objective of this study was to compare the effects of dopamine D_1, D_2 and D_3 receptor modulators on ethanol intake in rats. The protocol chosen used a sweetened ethanol solution, oral administration procedure, with free access to both water and the ethanol solution in food-restricted rats. This model achieves high and stable levels of both ethanol consumption and preference for the freely available ethanol solution over prolonged periods of time, using non-genetically selected standard laboratory rats (Silvestre et al., 1996).

2. Materials and methods

2.1. Animals

Seventy male Sprague-Dawley rats (210–245 g, Janvier, France) were individually housed in wire mesh cages at 21–22°C and under a 12 h light-dark cycle (on 06:30 h, off 18:30 h). The animals had free access to food and water during a 5-day adaptation period before beginning the experimental phase.

2.2. Drugs

Ethanol 99.5% (Panreac) and D-(+)-glucose (Merck) were made up in solution in distilled water to 10% ethanol (v/v) with 3% glucose (w/v). Single bottles were used for either water or experimental solution. Fresh solutions were made up every 2 days.

Raclopride ((*S*)-3,5-dichloro-*N*-((1-ethyl-2-pyrrolidinyl)methyl)-2-hydroxy-6-methoxybenzamide, Astra, Sweden), SKF 38393 HCl ((±)-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride, Research Biochemicals International, USA), 7-OH-DPAT ((±)-7-hydroxy-*N*,*N*-(di-*n*-propyl-2-aminotetralin, Research Biochemicals International, USA) and SCH 23390 (*R*-(+)-7-chloro-2,3,4,5-tetrahydro-3-methyl-1-phenyl-1*H*-3-benzazepine-8-ol, Research Biochemicals International, USA) were dissolved in saline. Risperidone (3-(2-(4-(6-fluoro-1,2-benzisoxasol-3-yl)piperidin-1-yl)ethyl)-2-methyl-6,7,8,9-tetrahydro-4*H*-pyrido(1,2-*a*)pyrimidin-4-

one, synthesized in the Almirall Medicinal Chemistry Department) was suspended in a vehicle composed of 0.5% methylcellulose and 0.1% Tween-80 in distilled water. All doses refer to free base weights.

2.3. Procedure

2.3.1. Ethanol induction phase

The method used followed that previously employed by Silvestre et al. (1996), which has been based on that previously developed and validated as an animal model of alcoholism (Nadal et al., 1992; Pallarés et al., 1992). In their home cages rats had access to food and ethanol solutions restricted to 1 h per day for 15 days (10:00–11:00 h), while water was freely available for the remaining 23 h. In this 1-h period, food was made available and the water bottle was replaced by a bottle containing the ethanol solution. In this 15-day period the body weight of the animals was reduced and maintained at 80% of their theoretical weight for their age.

2.3.2. Preference induction phase

After the ethanol induction phase period, both water and ethanol solution were made available for another 15 days, in order to achieve a stable baseline of both ethanol intake and preference. Measurement of 24-h fluid intake was performed every day (between 10:00–11:00 h) when each rat was weighed. Relative positions of ethanol solution and water bottles were exchanged on a random basis to prevent the acquisition of position preferences. Food (10–12 g) was only available 1 h (10:00–11:00 h) every day. Absolute ethanol consumption (in g/kg per day) was calculated. Ethanol preference was expressed as the percentage of ethanol solution consumption against total fluid consumption.

2.4. Drug administration studies

After the preference induction phase period, rats achieved a stable baseline intake of both water and ethanol solution. The criterion for inclusion was that rats showed an ethanol consumption ≥ 6.5 g/kg per day and a preference $\geq 60\%$ during 4 consecutive days after the end of the preference induction phase (baseline consumption). On this basis, 14 rats had to be excluded from the experiments. Rats were then randomly allocated to 7 experimental groups ($n = 8$). Once subjects showed a stable baseline consumption, the animals were treated twice daily (10:00–11:00 and 16:00–17:00 h) for 4 consecutive days with drugs or suitable vehicle in a latin square counterbalanced design: raclopride (0.01, 0.1 and 1.0 mg/kg), 7-OH-DPAT (0.01, 0.03 and 0.1 mg/kg), risperidone (0.01, 0.1 and 1.0 mg/kg), SKF 38393 (1.0, 3.0 and 10.0 mg/kg) and SCH 23390 (0.01, 0.03 and 0.1 mg/kg) were compared with their vehicle-control group. All drugs were administered subcutaneously (s.c.) in a volume of 1 ml/kg.

This study was part of another extensive one, where other in-house pharmacological agents were evaluated. Results from these compounds could not be shown for reasons of commercial confidentiality; as they were included in the original analysis the degrees of freedom may differ from those expected from regarding only the groups outlined above.

2.5. General design

Each animal received two injections per day for 4 consecutive days of a single dose of a test compound. This drug treatment period was followed by a 10-day washout period where the animal was not treated and left to recover baseline ethanol consumption. There then followed a second, third and fourth 4-day period when each rat received twice daily injections of another dose of another test compound. Each drug treatment period was separated by a 10-day washout period. The design was counterbalanced so that each group received a vehicle treatment and a high, low or medium dose each of a different test compound. Thus each rat underwent four periods of drug treatment sessions each with a different dose of a different test compound.

2.6. Data analysis

Ethanol preference ratio (%) was calculated by dividing the ethanol solution consumed (ml) by the total amount of fluid (ethanol solution and water) consumed (ml). Baseline consumption and preference were calculated as the mean for the 4 days immediately prior to the beginning of drug administration. The mean consumption and preference were then calculated for the four drug administration days for each treatment group. Changes in water and ethanol consumption as well as in ethanol preference with respect to baseline consumption were calculated for each group. Finally, the change relative to baseline for each group was compared to the respective vehicle group. To account for any change in baseline consumption, the change in consumption or preference in the vehicle group was subtracted from all groups in order to show the real changes caused by the pharmacological interventions.

2.7. Statistical analysis

The normality of the data was assessed by means of the Kolmogorov-Smirnov test and homogeneity of variance was assessed by means of the Bartlett test. For comparison between groups a mixed one-way analysis of variance (MANOVA) for both simple and repeated measures with planned contrasts between groups was used with one between-factor that was drug treatment. Post-hoc tests were performed by means of t-test implemented in the Contrast options of the MANOVA instruction when a mixed one-way ANOVA was applied.

3. Results

During the preference induction phase period, rats established a stable baseline intake of both water and ethanol solution, and then of ethanol preference. At the beginning of the first drug treatment the average water intake was 57.7 ± 3.9 ml/kg per day, while the daily average intake of the 10% ethanol solution was 137.7 ± 1.2 ml/kg per day, giving a daily dose of 10.9 ± 0.6 g/kg per day. These rates of consumption gave ethanol preferences of the order of 70%.

SKF 38393 reduced ethanol preference at doses of 3.0 and 10.0 mg/kg ($F(1,32) = 4.94$ $P = 0.033$ and $F(1,32) = 36.53$ $P < 0.001$, respectively) and ethanol consumption ($F(1,32) = 4.76$ $P = 0.037$ and $F(1,32) = 10.97$ $P = 0.002$, respectively. Although both doses increased water consumption, only the highest dose reached significant values ($F(1,32) = 8.95$ $P = 0.005$) (Table 1). The effect of SKF 38393 (10.0 mg/kg) on both ethanol preference and consumption was significant on the first and second drug treatment day and baseline levels were restored immediately after drug treatment ended (Fig. 1A and B). Moreover, a trend to increase water consumption was observed on the second drug treatment day, but baseline levels were also restored immediately after drug treatment ended. However, an increase in ethanol preference with respect to the vehicle group was detected when drug treatment ended, largely caused by a decrease in water intake during the same period (Fig. 1A and C).

SCH 23390 had no significant effect on ethanol consumption at the doses tested, but did significantly decrease

Table 1
Comparison of effects of dopamine receptor agonists and antagonists on ethanol solution and water intake and ethanol preference in rats

Treatment (mg/kg, s.c.)	n	Ethanol intake (g/kg/day)	Water intake (ml/kg/day)	Ethanol preference (%)
Vehicle	47	−0.18 ± 0.54	−1.81 ± 2.55	−0.08 ± 1.68
SKF 38393				
1.0	8	−2.06 ± 1.00	−7.30 ± 4.04	−1.38 ± 1.47
3.0	7	−1.87 ± 0.28 [a]	10.82 ± 4.88	−7.64 ± 2.10 [a]
10.0	8	−3.17 ± 0.75 [b]	16.77 ± 2.14 [b]	−15.67 ± 2.16 [c]
SCH 23390				
0.01	8	−0.63 ± 0.47	−2.69 ± 1.90	−0.23 ± 0.64
0.03	7	−0.39 ± 0.43	−13.70 ± 3.80 [a]	4.60 ± 1.25 [d]
0.1	8	−0.50 ± 0.65	−24.67 ± 3.85 [c]	8.18 ± 2.15 [b]
Raclopride				
0.01	7	0.08 ± 0.47	−1.06 ± 4.94	0.73 ± 1.91
0.1	7	−0.67 ± 0.52	−13.98 ± 2.36 [b]	3.84 ± 1.21 [a]
1.0	8	−0.49 ± 1.21	−16.95 ± 3.03 [c]	4.11 ± 2.23 [a]
7-OH-DPAT				
0.01	7	2.01 ± 1.13 [a]	−7.41 ± 6.75	6.10 ± 2.54 [a]
0.03	8	−1.03 ± 0.78	0.46 ± 3.28	−1.86 ± 2.37
0.1	8	−1.57 ± 0.69	0.05 ± 5.05	−2.66 ± 2.40
Risperidone				
0.01	8	−0.15 ± 0.32	−6.57 ± 2.17	2.05 ± 1.26
0.1	8	−0.98 ± 0.83	−19.10 ± 3.55	2.44 ± 3.64
1.0	8	0.15 ± 0.41	−19.84 ± 2.73 [a]	8.83 ± 2.06 [d]

Data shown are the mean (±S.E.M.) of changes for the 4 drug treatment days from baseline consumption for each of the variables, ethanol intake, water intake and ethanol preference respectively for each of the drug treatments. The baseline was the mean of the 4 days prior to the commencement of the drug treatment. Each drug was administered twice a day for 4 days. Change relative to baseline for each group was compared to the respective vehicle group. Significant differences were calculated by planned contrasts after significant MANOVA. [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$. Trends to significance appointed by [d] $P < 0.1$.

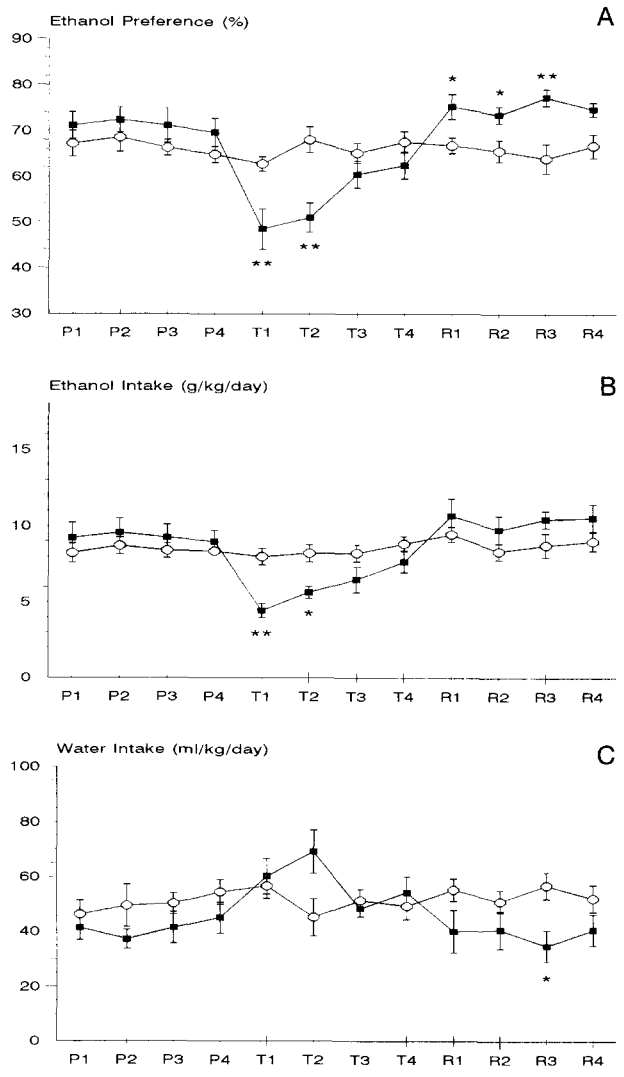

Fig. 1. Time-course of effects of SKF 38393 (10.0 mg/kg, marked by ■) on ethanol consumption and preference vs. vehicle (marked by ○) in rats ($n = 7$–8). The x-axis shows effects on: (A) ethanol preference (as percentage with respect to total fluid consumption); (B) ethanol intake (g/kg per day); and (C) water intake (ml/kg per day). The y-axis shows test sessions: P1 to P4 refer to pre-treatment baseline days; T1 to T4 refer to compound treatment days (administrated twice daily); and R1 to R4 refer to post-treatment recovering days, for each parameter measured. Significant differences calculated by means of Duncan's multiple range test following a one-way analysis of variance (ANOVA): ** $P < 0.01$, * $P < 0.05$ vs. vehicle.

water consumption. This effect was observed at doses of 0.03 and 0.1 mg/kg ($F(1,32) = 5.57$ $P = 0.024$ and $F(1,32) = 23.00$ $P < 0.001$, respectively). This effect in turn was responsible for the significant increase in preference at the highest dose tested (0.1 mg/kg) ($F(1,32) = 13.36$ $P = 0.001$) (Table 1). The effect of SCH 23390 at the dose of 0.1 mg/kg on ethanol preference was significant, increasing it from day 2 of drug treatment (Fig. 2A). However, ethanol consumption levels were unaffected (Fig. 2B). Nevertheless, the effect observed on ethanol preference for the 0.1 mg/kg-treated group was entirely due to a

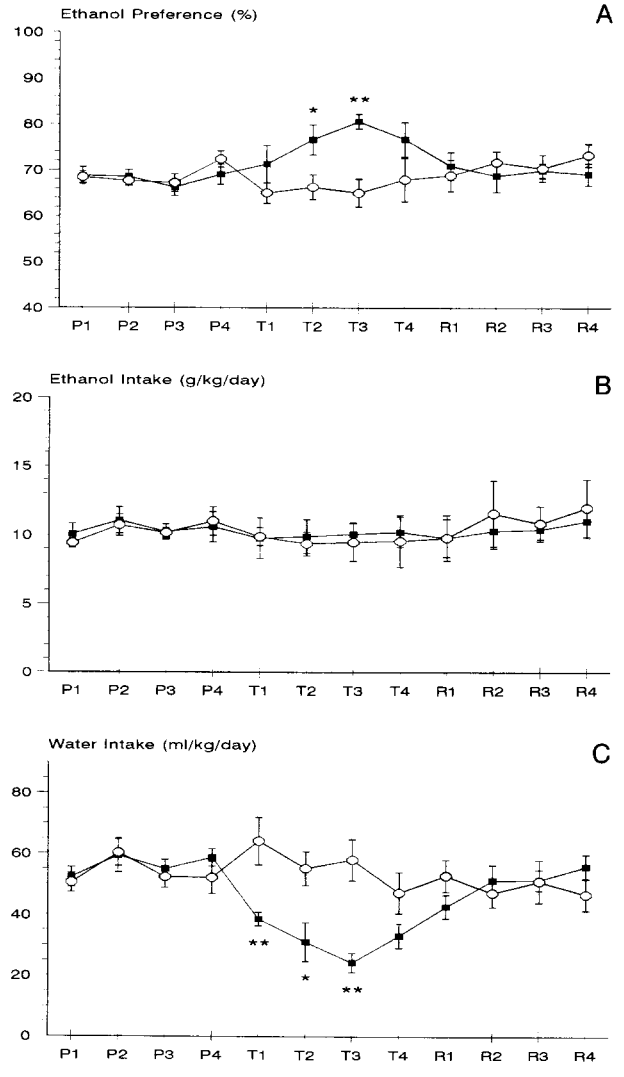

Fig. 2. Time-course of effects of SCH 23390 (0.1 mg/kg, marked by ■) on ethanol consumption and preference vs. vehicle (marked by ○) in rats ($n = 7–8$). The x-axis shows effects on: (A) ethanol preference (as percentage with respect to total fluid consumption); (B) ethanol intake (g/kg per day); and (C) water intake (ml/kg per day). The y-axis shows test sessions: P1 to P4 refer to pre-treatment baseline days; T1 to T4 refer to compound treatment days (administrated twice daily); and R1 to R4 refer to post-treatment recovering days, for each parameter measured. Significant differences calculated by means of Duncan's multiple range test following a one-way analysis of variance (ANOVA): ** $P < 0.01$, * $P < 0.05$ vs. vehicle.

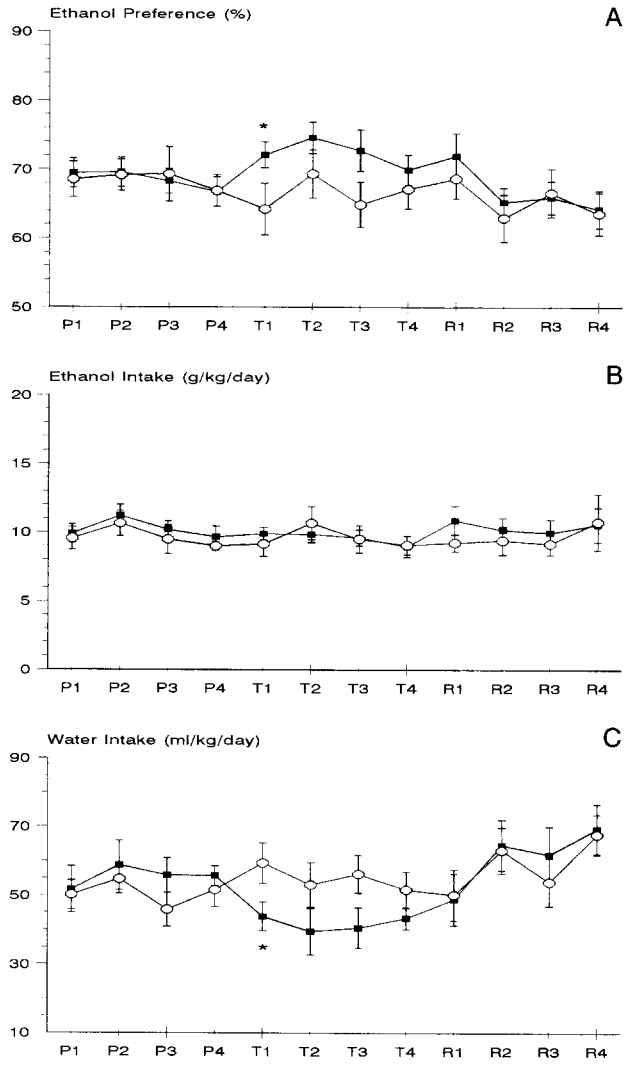

Fig. 3. Time-course of effects of raclopride (0.1 mg/kg, marked by ■) on ethanol consumption and preference vs. vehicle (marked by ○) in rats ($n = 7–8$). The x-axis shows effects on: (A) ethanol preference (as percentage with respect to total fluid consumption); (B) ethanol intake (g/kg per day); and (C) water intake (ml/kg per day). The y-axis shows test sessions: P1 to P4 refer to pre-treatment baseline days; T1 to T4 refer to compound treatment days (administrated twice daily); and R1 to R4 refer to post-treatment recovering days, for each parameter measured. Significant differences calculated by means of Duncan's multiple range test following a one-way analysis of variance (ANOVA): * $P < 0.05$ vs. vehicle.

reduction in water consumption, which was evident throughout the whole administration period (Fig. 2C). The decrease in water intake would show non-specific effects in consumption. Baseline levels of water intake were restored immediately after drug treatment ended.

Raclopride also failed to show any specific effect on ethanol consumption. However, the highest doses of raclopride (0.1–1.0 mg/kg) significantly decreased water intake ($F(1,32) = 11.70$ $P = 0.002$ and $F(1,32) = 17.05$, $P < 0.001$, respectively). As with SCH 23390, raclopride at the doses of 0.1 and 1.0 mg/kg led to an apparent increase

in ethanol preference ($F(1,32) = 5.60$ $P = 0.024$ and $F(1,32) = 5.31$ $P = 0.027$, respectively) (Table 1). Likewise, ethanol consumption levels were unaffected by raclopride on the 4 drug administration days (Fig. 3B). Raclopride at the dose of 0.1 mg/kg slightly increased ethanol preference throughout the administration period (Fig. 3A). Nevertheless, the effect observed for the 0.1 mg/kg-treated group was also due to a reduction in water consumption throughout the same administration period (Fig. 3C), which would also show that non-specific effects in consumption are emerging. Baseline levels of either ethanol preference or water intake were restored immediately after drug treatment ended.

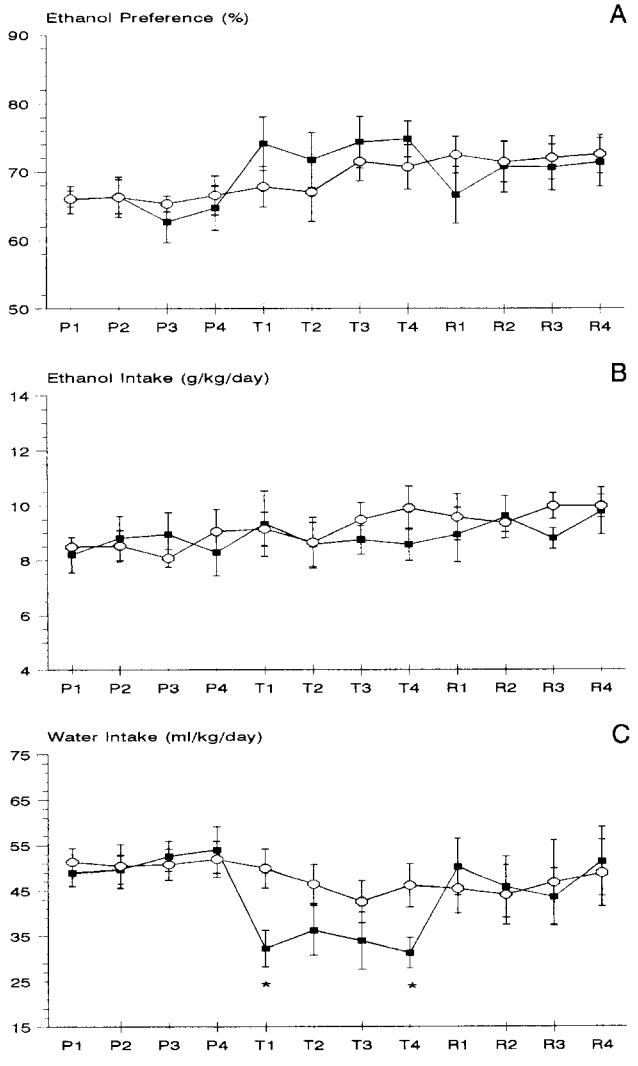

Fig. 4. Time-course of effects of risperidone (1.0 mg/kg, marked by ■) on ethanol consumption and preference vs. vehicle (marked by ○) in rats ($n = 8$). The x-axis shows effects on: (A) ethanol preference (as percentage with respect to total fluid consumption); (B) ethanol intake (g/kg per day); and (C) water intake (ml/kg per day). The y-axis shows test sessions: P1 to P4 refer to pre-treatment baseline days; T1 to T4 refer to compound treatment days (administrated twice daily); and R1 to R4 refer to post-treatment recovering days, for each parameter measured. Significant differences calculated by means of Duncan's multiple range test following a one-way analysis of variance (ANOVA): * $P < 0.05$ vs. vehicle.

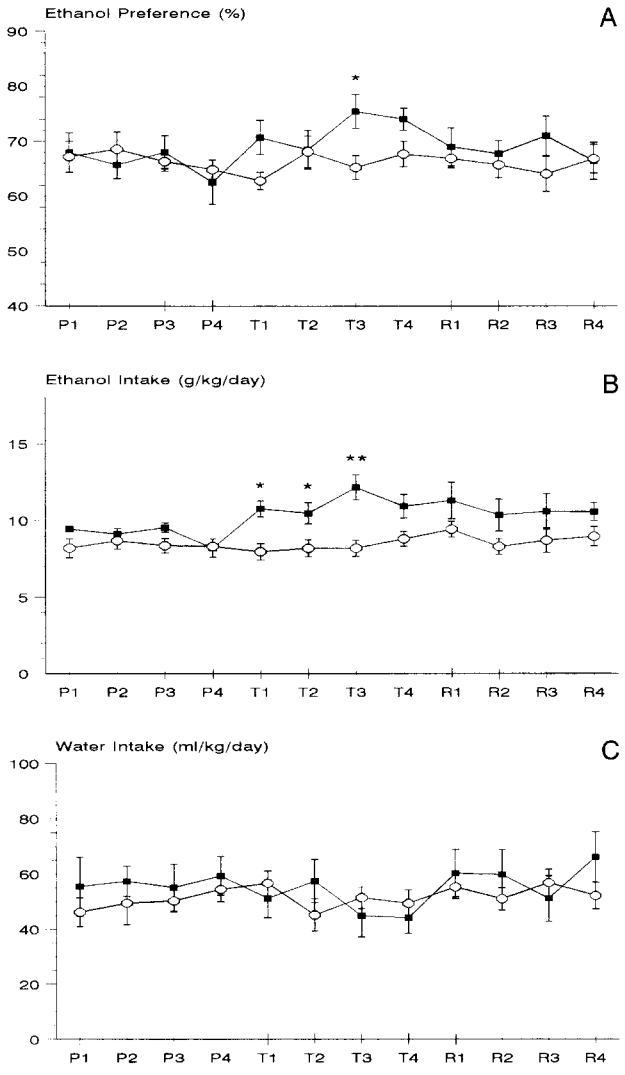

Fig. 5. Time-course of effects of 7-OH-DPAT (0.01 mg/kg, marked by ■) on ethanol consumption and preference vs. vehicle (marked by ○) in rats ($n = 7$–8). The x-axis shows effects on: (A) ethanol preference (as percentage with respect to total fluid consumption); (B) ethanol intake (g/kg per day); and (C) water intake (ml/kg per day). The y-axis shows test sessions: P1 to P4 refer to pre-treatment baseline days; T1 to T4 refer to compound treatment days (administrated twice daily); and R1 to R4 refer to post-treatment recovering days, for each parameter measured. Significant differences calculated by means of Duncan's multiple range test following a one-way analysis of variance (ANOVA): ** $P < 0.01$, * $P < 0.05$ vs. vehicle.

Neither ethanol consumption nor ethanol preference were affected by risperidone at any dose tested. However, a significant decrease in water intake ($F(1,35) = 6.77$ $P = 0.013$) was only seen at the highest dose tested (1.0 mg/kg), which is reflected by a non-significant ($F(1,35) = 3.67$ $P = 0.064$) increase in preference for ethanol at this dose (Table 1). Neither evolution of ethanol consumption nor ethanol preference were affected by risperidone at the highest dose tested (1.0 mg/kg) (Fig. 4A and B).

However, risperidone significantly decreased water intake with respect to the vehicle-treated group on the second and fourth drug treatment days and baseline levels were restored immediately after drug treatment ended (Fig. 4C). As with SCH 23390 and raclopride, this decrease in water intake would show that non-specific effects in consumption are emerging.

7-OH-DPAT induced a significant increase in both ethanol consumption ($F(1,32) = 4.56$, $P = 0.040$) and ethanol preference ($F(1,32) = 5.90$ $P = 0.021$) but only at the lowest dose tested (0.01 mg/kg). A non-significant and simultaneous decrease in water intake was also observed. No effects were obtained in the groups receiving the remaining doses tested (Table 1). The effects of 7-OH-DPAT at the dose of 0.01 mg/kg on ethanol consumption were significant from the first drug treatment day (Fig. 5B), while effects on ethanol preference were significant from the third drug treatment day (Fig. 5A) and baseline levels were restored immediately after drug treatment ended. However, water intake levels were unaffected by 7-OH-DPAT on the 4 drug administration days with respect to the vehicle-treated group (Fig. 5C).

4. Discussion

As described previously (Silvestre et al., 1996) the model used – a continuous two-bottle free-access procedure in food-restricted animals – established high levels of ethanol consumption (10 g/kg per day) with a stable preference for ethanol (70%) over prolonged periods of time in unselected rats. Therefore, it seems a suitable model to assess a drug's effect on ethanol consumption in rats. Food deprivation increases self-administration of compounds that are abused by humans and have rewarding properties in animals, not only of those with substantial caloric content like ethanol (Carroll, 1982). For this reason restriction of food intake was maintained throughout the experimental period to ensure stable levels of consumption of the freely available ethanol solution.

Moreover, a slightly sweetened alcoholic solution was used since it appears to be important in order to obtain an oral self-administration of pharmacologically active doses of ethanol (Samson et al., 1988). A high intake of a solution containing only water and ethanol is very difficult to achieve in rats. The addition of a small amount of glucose (3%, w/v) to the ethanol solution affords high levels of ethanol consumption. Although glucose is not regarded as having a 'sweet' taste it does lessen the aversive taste of ethanol and in this way it facilitates the maintenance of high levels of ethanol consumption over prolonged periods of time. Moreover, it has been shown that compounds that decrease intake of a sweet alcoholic solution would be even more potent in decreasing intake of an unsweetened alcoholic solution (Nichols et al., 1992).

We think that, in a free-choice procedure, high levels of ethanol intake and consequently the high ethanol impregnation state of the normal rat, more than the type of ethanol solution or state of food deprivation, could be the most important factor to study. Nevertheless, further studies are required to determine the effect of greater access to food and the effect of solutions with less or no glucose on ethanol consumption and subsequent drug interactions on ethanol intake.

In this study only SKF 38393, a dopamine D_1 partial agonist, significantly reduced both ethanol consumption and preference in a selective manner, i.e., without altering water consumption. Similar results have been reported using genetically selected mice and rats (Dyr et al., 1993; Ng and George, 1994). Likewise, dopamine receptor agonists also have been reported to produce reductions in ethanol drinking in unselected rats (Pfeffer and Samson, 1986; Rassnick et al., 1993b). One interpretation might be that dopamine receptor agonists can substitute for the dopaminergic actions of ethanol, since oral self-administration of ethanol (Weiss et al., 1992) increases extracellular dopamine levels in the nucleus accumbens. The present results indicate that this effect may be mediated largely by dopamine D_1-type actions.

The dopamine D_1 receptor antagonist SCH 23390 had no selective effect on ethanol consumption. Other studies have shown that SCH 23390 decreases ethanol intake in HAD rats (Dyr et al., 1993). While the use of genetically selected rats in the latter study may explain the discrepancy it is also worth noting that doses which reduced ethanol intake in this study also decreased water consumption indicating a non-specific suppression of fluid intake.

The putative selective dopamine D_3 receptor agonist 7-OH-DPAT at the lowest tested dose (0.01 mg/kg) significantly increased both ethanol intake and preference. However, at higher, and possibly less selective, doses (0.03–01 mg/kg) no significant effects were seen. Meert and Clincke (1994) have shown that 7-OH-DPAT at doses ≥ 0.04 mg/kg reduced the preference for ethanol. However, this effect is more consistent when neither ethanol intake nor ethanol preference had achieved maximal and stable levels, while in rats in which a high and stable level of ethanol preference is fully established, 7-OH-DPAT was mainly without effect (Meert and Clincke, 1994). Furthermore, it would seem that dopamine D_3 receptors are largely localized to limbic structures such as the nucleus accumbens, which suggests that the dopamine D_3 receptor may be involved in brain reward pathways and drug abuse (Schwartz et al., 1994; Sokoloff et al., 1990).

However, the increase in ethanol consumption and preference seen in this study with 7-OH-DPAT appears to be in contrast with previous results where systemic administration of dopamine D_2/D_3 receptor agonists such as bromocriptine (Ng and George, 1994; Weiss et al., 1990) and quinpirole (Dyr et al., 1993) have been reported to decrease ethanol consumption in outbred rodents selected for ethanol drinking. This discrepancy may reflect the

opposing effects seen with dopamine receptor agonists in other paradigms such as locomotor activity where low doses of dopamine receptor agonists such as apomorphine or quinpirole are associated with decreases in locomotor behaviour while higher doses induce hyperactivity (Brown et al., 1985). This low-dose effect can be explained either in terms of presynaptically mediated suppression by dopamine D_3 receptors of endogenous dopamine release (Sokoloff et al., 1990) leading to a decrease in locomotor activity or by direct selective stimulation of a postsynaptic inhibitory receptor (Stähle, 1992; Waters et al., 1993). The suppressive effects on ethanol intake of higher doses used in the study by Meert and Clincke (1994) may reflect the high-dose stimulant effects seen in the locomotor studies in addition to the methodological issues outline above.

Raclopride in the dose range used failed to show any selective effect on ethanol consumption. This contrasts with the findings of Samson et al. (1993) who found that intra-accumbens microinjections of raclopride decreased ethanol-reinforced responding in rats. Doses higher than those used in the present study have been shown to cause sedation (Ericson et al., 1991) and thus confound any effect seen on ethanol consumption.

No effects on ethanol consumption were observed following administration of the 5-HT_2/dopamine D_2 receptor antagonist risperidone. It has previously been suggested that risperidone reduces both ethanol consumption and preference in non-selected Wistar rats (Panocka et al., 1993a). However, another study failed to show effects with risperidone in sP rats, except at the highest dose tested (10 mg/kg) (Panocka et al., 1993b), which is higher than the dose required to observe non-specific behavioural effects (Janssen et al., 1988). It is possible therefore that the reductions in ethanol consumption observed in the study by Panocka et al. (1993a) were due to non-specific motor impairment caused by high doses of the drug. In the present study water consumption was significantly reduced indicating a reduction in consummatory behaviour.

Studies that showed a significant effect of SCH 23390 (Dyr et al., 1993), raclopride (Samson et al., 1993) or risperidone (Panocka et al., 1993a) on ethanol intake all used methods or ethanol solutions which induced lower levels of ethanol consumption than achieved in this study. Studies using free access to ethanol can establish levels of intake higher than those using restricted access. It has previously been suggested that baseline levels of ethanol consumption may be an important factor in determining drug effects (Myers and Lankford, 1993; Svensson et al., 1993). Although the current study can be criticized for using glucose-adulterated ethanol solutions in food-deprived rats and thus confounding the motivational factors underlying ethanol consumption, the higher levels of ethanol consumed in the model used in this study may have lessened the possibility of obtaining non-specific effects on ethanol consumption.

In conclusion, ethanol consumption was significantly and selectively reduced by the dopamine D_1 receptor agonist SKF 38393 and significantly and selectively increased by a low dose of the dopamine D_3 receptor agonist 7-OH-DPAT, whereas ethanol consumption was unaffected by dopamine receptor antagonists such as the dopamine D_1 receptor antagonist SCH 23390, the dopamine D_2/D_3 receptor antagonist raclopride and the 5-HT_2/D_2 receptor antagonist risperidone. Thus, it would seem that ethanol intake behaviour could be regulated largely by dopamine receptor activation, suggesting the involvement of the dopaminergic system in ethanol intake and ethanol reinforcement with dopamine D_1 and D_3 receptors playing opposing roles. Blockade of dopamine D_2 receptors had no selective effect on ethanol consumption and preference. However, a highly selective dopamine D_2 receptor agonist would be lacking to complete this hypothesis. Likewise, further studies with 7-OH-DPAT and other dopamine D_3 receptor-specific agents across a wider range of doses may usefully clarify the role of the dopamine D_3 receptor in drug reinforcement.

References

Brown, F., W. Campbell, P.J. Mitchell and K. Randall, 1985, Dopamine autoreceptors and the effects of drugs on locomotion and dopamine synthesis, Br. J. Pharmacol. 84, 853.

Carroll, M.E., 1982, Rapid acquisition of oral phencyclidine self-administration in food-deprived and food-satiated rhesus monkeys, Pharmacol. Biochem. Behav. 17, 341.

Dyr, W., W.J. McBride, L. Lumeng, T.-K. Li and J.M. Murphy, 1993, Effects of D_1 and D_2 dopamine receptor agents on ethanol consumption in the high-alcohol-drinking (HAD) line of rats, Alcohol 10, 207.

Ericson, E., J. Samuelsson and S. Ahlenius, 1991, Photocell measurements of rat motor activity, J. Pharmacol. Methods 25, 111.

Fahlke, C., S. Hansen, J.A. Engel and E. Hard, 1994, Effects of ventral striatal 6-OHDA lesions of amphetamine sensitization on ethanol consumption in the rat, Pharmacol. Biochem. Behav. 47, 345.

George, S.R., T. Fan, G.Y.K. Ng, S.Y. Jung, B.F. O'Dowd and C.A. Naranjo, 1995, Low endogenous dopamine function in brain predisposes to high alcohol preference and consumption: reversal by increasing synaptic dopamine, J. Pharmacol. Exp. Ther. 273, 373.

Hamdi, A. and C. Prasad, 1992, Bidirectional changes in striatal D_2-dopamine receptor density during chronic ethanol intake, Alcohol 9, 133.

Hamdi, A. and C. Prasad, 1993, Bidirectional changes in striatal D_1-dopamine receptor density during chronic ethanol intake, Life Sci. 52, 251.

Hodge, C.W., H.H. Samson and M. Haraguchi, 1992, Microinjections of dopamine agonists in the nucleus accumbens increase ethanol-reinforced responding, Pharmacol. Biochem. Behav. 43, 249.

Hodge, C.W., M. Haraguchi, H. Erickson and H.H. Samson, 1993, Ventral tegmental microinjections of quinpirole decrease ethanol and sucrose-reinforced responding, Alcohol. Clin. Exp. Res. 17, 370.

Imperato, A. and G. Di Chiara, 1986, Preferential stimulation of dopamine release in the nucleus accumbens of freely-moving rats by ethanol, J. Pharmacol. Exp. Ther. 239, 219.

Janssen, P.A.J., C.J.E. Niemegeers, F. Awouters, K.H.L. Schellenkens, A.A.H.P. Megens and T.F. Meert, 1988, Pharmacology of risperidone (R55 766), a new antipsychotic with serotonin-S_2 and dopamine-D_2 antagonistic properties, J. Pharmacol. Exp. Ther. 244, 685.

Koob, G.F., 1992, Drugs of abuse: anatomy, pharmacology and function of reward pathways, Trends Pharmacol. Sci. 13, 177.

Korpi, E.R., J.D. Sinclair and O. Malminen, 1987, Dopamine D-2 receptor binding in striatal membranes of rat lines selected for differences in alcohol-related behaviors, Pharmacol. Toxicol. 61, 94.

Levy, A.D., J.M. Murphy, W.J. McBride, L. Lumeng and T.-K. Li, 1991, Microinjections of sulpiride into the nucleus accumbens increases ethanol drinking in alcohol-preferring (P) rats, Alcohol Alcohol. Suppl. 1, 417.

Lograno, E., F. Matteo, M. Trabucchi, S. Govoni, R. Cagiano, C. Lacomba and V. Cuomo, 1993, Effects of chronic ethanol intake at a low dose on the rat brain dopaminergic system, Alcohol 10, 45.

Meert, T.F. and H.C. Clincke, 1994, 7-OH-DPAT and alcohol consumption, withdrawal and discriminative stimulus properties in rats, Alcohol Alcohol. 29, 489.

Melchior, C.L. and R.D. Myers, 1976, Genetic differences in ethanol drinking on the rat following injection of 6-OHDA, 5,6-DHT or 5,7-DHT into cerebral ventricles, Pharmacol. Biochem. Behav. 5, 63.

Myers, R.D. and M.F. Lankford, 1993, Failure of the 5-HT_2 receptor antagonist, ritanserin, to alter preference for alcohol in drinking rats, Pharmacol. Biochem. Behav. 45, 233.

Nadal, R., M. Pallarés and N. Ferré, 1992, Conditioned place preference for ethanol and individual differences in rats, Personal. Indiv. Diff. 13, 287.

Ng, G.Y.K. and S.R. George, 1994, Dopamine receptor agonist reduces ethanol self-administration in the ethanol-preferring C57BL/6J inbred mouse, Eur. J. Pharmacol. Mol. Pharmacol. Sect. 269, 365.

Nichols, M.L., J.D. Delconte, K.D. Wild, C.L. Hubbell and L.D. Reid, 1992, Rats' intakes of different alcoholic beverages, in: Novel Pharmacological Interventions for Alcoholism, eds. C.A. Naranjo and E.M. Sellers (Springer-Verlag, New York, NY) p. 233.

Pallarés, M., R. Nadal and N. Ferré, 1992, Effects of oral ethanol self-administration on the inhibition of the lever-press response in rats, Pharmacol. Biochem. Behav. 43, 589.

Panocka, I., P. Pompei and M. Massi, 1993a, Suppression of alcohol preference in rats induced by risperidone, a serotonin 5-HT_2 and dopamine D_2 receptor antagonist, Brain Res. Bull. 31, 595.

Panocka, I., R. Ciccocioppo, P. Pompei and M. Massi, 1993b, 5-HT_2 receptor antagonists do not reduce ethanol preference in Sardinian alcohol-preferring (sP) rats, Pharmacol. Biochem. Behav. 46, 857.

Pellegrino, S.M. and M.J. Druse, 1992, The effects of chronic ethanol consumption on the mesolimbic and nigrostriatal dopamine systems, Alcohol. Clin. Exp. Res. 16, 275.

Pfeffer, A.O. and H.H. Samson, 1986, Effects of pimozide on homecage ethanol drinking in the rat: dependence on drinking session length, Drug Alcohol Depend. 17, 47.

Rassnick, S., L. Pulvirenti and G.F. Koob, 1992, Oral ethanol self-administration in rats is reduced by the administration of dopamine and glutamate receptor antagonists into the nucleus accumbens, Psychopharmacology 109, 92.

Rassnick, S., L. Stinus and G.F. Koob, 1993a, The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-administration of ethanol in the rat, Brain Res. 623, 16.

Rassnick, S., L. Pulvirenti and G.F. Koob, 1993b, SDZ-205,152, a novel dopamine receptor agonist, reduces oral ethanol self-administration in rats, Alcohol 10, 127.

Samson, H.H. and R.A. Harris, 1992, Neurobiology of alcohol abuse, Trends Pharmacol. Sci. 13, 206.

Samson, H.H., A.O. Pfeffer and G.A. Tolliver, 1988, Oral ethanol self-administration in rats: models of ethanol-seeking behavior, Alcohol. Clin. Exp. Res. 12, 591.

Samson, H.H., C.W. Hodge, G.A. Tolliver and M. Haraguchi, 1993, Effect of dopamine agonists and antagonists on ethanol-reinforced behavior: the involvement of the nucleus accumbens, Brain Res. Bull. 30, 133.

Schwartz, J.-C., J. Diaz, N. Griffon, D. Levesque, M.-P. Martres and P. Sokoloff, 1994, Multiple dopamine receptors: the D_3 receptor and actions of substances of abuse, in: Toward a Molecular Basis of Alcohol Use and Abuse, eds. B. Jansson, H. Jörnvall, U. Rydberg, L. Terenius and L. Valle (Birkhauser, Basel) p. 81.

Silvestre, J.S., M.F. O'Neill, A.G. Fernández and J.M. Palacios, 1996, Comparison of effects of a range of 5-HT receptors modulator on ethanol consumption and preference in rats, Pharmacol. Biochem. Behav. (in press).

Sokoloff, P., B. Giros, M.-P. Martres, M.-L. Bouthenet and J.-C. Schwartz, 1990, Molecular cloning and characterization of a novel dopamine receptor (D_3) as a target for neuroleptics, Nature 347, 146.

Stähle, L., 1992, Do autoreceptors mediate dopamine agonist-induced yawning and suppression of exploration of exploration, Psychopharmacology 106, 1.

Stefanini, E., M. Frau, B. Garau, F. Fadda and G.L. Gessa, 1992, Alcohol-preferring rats have fewer dopamine D_2 receptors in the limbic system, Alcohol Alcohol. 27, 127.

Svensson, L., C. Fahlke, E. Hard and J.A. Engel, 1993, Involvement of the serotonergic system in ethanol intake in the rat, Alcohol 10, 219.

Waters, N., K. Svensson, S.R. Haadsma-Svensson, M.W. Smith and A. Carlsson, 1993, The dopamine D-3 receptor: a postsynaptic receptor inhibitory on locomotor activity, J. Neural Transm. (Gen. Sect.) 94, 11.

Weiss, F., M. Mitchiner, F.E. Bloom and G.F. Koob, 1990, Free-choice responding for ethanol versus water in alcohol preferring (P) and unselected Wistar rats in differentially modified by naloxone, bromocriptine, and methysergide, Psychopharmacology 101, 178.

Weiss, F., Y.L. Hurd, U. Ungerstedt, A. Markou, P.M. Plotsky and G.F. Koob, 1992, Neurochemical correlates of cocaine and ethanol self-administration, Ann. NY Acad. Sci. 654, 220.

Yoshimoto, K., W.J. McBride, L. Lumeng and T.-K. Li, 1991, Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens, Alcohol 9, 17.