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5-HT_{2A} and 5-HT_{2C}/5-HT_{1B} Receptors Are Differentially Involved in Alcohol Preference and Consummatory Behavior in cAA Rats

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MAUREL, S., J. DE VRY, R. DE BEUN AND R. SCHREIBER. *5-HT_{2A}* and 5-HT_{2C}/5-HT_{1B} receptors are differentially involved in alcohol preference and consummatory behavior in cAA rats. PHARMACOL BIOCHEM BEHAV **62**(1) 89–96, 1999.—The present study aimed to investigate the role of serotonin 5-HT_{2A} and 5-HT_{2C} receptors in the control of alcohol preference and consummatory behavior in alcohol-preferring cAA rats. Effects of the 5-$HT_{2A/2C}$ receptor agonist, DOI, the 5-$HT_{2C/1B}$ receptor agonist, mCPP, the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, and the 5-HT_{2A} receptor antagonist, MDL 100,907, on ethanol (EtOH, 10% v/v) preference and intake, as well as total fluid and food intake were evaluated in a 12-h limited-access two-bottle paradigm. DOI (0.3–3 mg/kg, IP) reduced EtOH intake and preference, but not total fluid or food intake; whereas mCPP (1–10 mg/kg, SC) reduced EtOH, total fluid, and food intake. Therefore, it is concluded that DOI induces a specific and selective antialcohol effect, whereas mCPP rather induces a general suppressive effect on consummatory behavior. Ritanserin (1–10 mg/kg, IP) did not affect EtOH intake and preference, nor total fluid and food consumption. MDL 100,907 (0.1–1 mg/kg, IP) induced only a small reduction of food intake at the highest dose tested. Pretreatment with ritanserin (3 mg/kg, IP) and MDL 100,907 (0.3 mg/kg, IP) blocked the effects of DOI (3 mg/kg, IP), but not those of mCPP (10 mg/kg, IP). It is suggested that activation of 5-HT_{2C} and/or 5-HT_{1B} receptors results in a general decrease of consummatory behavior, whereas activation of 5-HT_{2A} receptors selectively decreases EtOH intake and preference, as assessed in the cAA rat model of alcoholism. © 1998 Elsevier Science Inc.

Animal model of alcoholism Ethanol 5-HT_{1B} receptor 5-HT_{2A} receptor 5-HT_{2C} receptor

SEROTONIN (5-hydroxytryptamine, 5-HT) has been implicated in the mechanisms underlying excessive consumption of alcohol (26,35,46). Low brain 5-HT levels were found to correlate with high ethanol (EtOH) intake (18,37), and cerebrospinal fluid levels of 5-hydroxyindoleacetic acid, the main serotonergic metabolite, are decreased in alcoholic patients (2). Therefore, a dysfunction of the 5-HT system may be causally related to alcohol abuse/dependency, at least in a subgroup of patients (2,39). Hence, drugs that modulate brain serotonergic activity may attenuate alcohol intake and therefore possess potential for the treatment of alcoholism. Indeed, as assessed in animal models of alcoholism, this has been confirmed with a variety of serotonergic compounds (9,34,46). Furthermore, clinical studies—mostly performed with selective 5-HT reuptake inhibitors (SSRIs)—suggest that these compounds are effective in particular subgroups of alcoholic patients (25,40).

However, drugs modulating brain serotonergic activity may also have general effects on consummatory behavior, and therefore, it is not clear whether effects on alcohol intake are selective or a consequence of the anorectic properties of the compound. Thus, a variety of SSRIs have been shown to reduce EtOH intake at doses that also decrease food and palatable liquid consumption, suggesting that their effects on EtOH intake are merely a consequence of a general reduction of consummatory behavior (1,13). The discovery of various 5-HT receptor subtypes led to the expectation that selective interaction with (a) particular subset(s) of receptors may induce a more selective therapeutic effect. Rodent studies suggest that 5-HT_{1A} receptor agonists such as 8-OH-DPAT [8-hydroxy 2-(di-N-propylamino)tetralin] and ipsapirone (8), the preferential 5-HT_{1B} receptor agonist, TFMPP [1-(3-trifluoromethylphenyl) piperazine; (33)], the 5-$HT_{2A/2C}$ receptor agonist, DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; (33)],

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the 5-$HT_{2C/1B}$ receptor agonist, mCPP [m-chlorophenylpiperazine; (3)], and the 5-HT_3 receptor agonist, 2-Me-5-HT [2-methyl-5-HT; (11)] possess alcohol intake-reducing properties with different degrees of selectivity (9). Although some preclinical studies reported anti-alcohol effects for 5-$HT_{2A/2C}$, 5-HT_3, and 5-HT_4 receptor antagonists (24,35,42), this could not be confirmed by others (6,7,9,41), indicating that the preclinical findings with 5-HT receptor antagonists are less robust than those obtained with particular 5-HT receptor agonists. Therefore, it appears that drugs acting as agonists at either 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, or 5-HT_{2C} receptors may be more promising as potential pharmacotherapy of alcoholism than compounds that act as antagonists at these receptors.

In the present study, the role of 5-HT_{2A} and 5-HT_{2C} receptors in the control of EtOH preference and general consummatory behavior was further investigated by comparing the behavioral profiles of agonists and antagonists with a certain degree of selectivity for these receptors in the cAA rat model of alcoholism. These rats derive from the AA rats (see the Method section) and have been selectively bred for a high preference for, and consumption of, 10% v/v EtOH in a 12-h limited-access, two-bottle choice situation. Employing this model, the differential effects of these compounds on alcohol intake and preference, as well as on fluid and food intake can be directly compared with the effects previously obtained with other serotonergic compounds in the model; such as, 5-HT releasers [i.e., fenfluramine; (9)], SSRIs [i.e., fluoxetine; (9,28)], and 5-HT_{1A} receptor agonists [8-OH-DPAT and ipsapirone; (45)], as well as with other drugs that have been claimed to affect EtOH intake (6,7). The compounds tested in the present study included the agonists DOI (pKi values for 5-HT_{2A} and 5-HT_{2C} receptors: 7.3 and 7.8, respectively), and mCPP (6.7 and 7.8), and the antagonists ritanserin (8.8 and 8.9) and MDL 100,907 [9.4 and 6.9; all pKi values from (47)]. Anti-alcohol effects of a compound were considered specific when reductions in both preference for, and intake of, EtOH were obtained, and considered selective when total fluid intake and food consumption were not affected at doses which were found to reduce EtOH intake. To further characterize the receptor subtypes involved in the effects of DOI and mCPP, it was investigated in the two-bottle procedure to what extent the effects of these compounds could be antagonized by pretreatment with ritanserin and MDL 100,907.

METHOD

Animals

Male and female cAA rats from the F69 generation were used, derived from a breeding program running at our institute. The foundation stock of these animals was bred at Alko Laboratories Ltd. (AA rats, Helsinki, Finland; however, to indicate that the present animals were bred in Cologne, they were renamed cAA rats in 1996). For each experimental group, between 8 to 10 animals showing the required baselines of EtOH intake and preference were selected from the available stock of rats (for selection criteria, see Procedure). About half of the animals of each experimental group were female and the other half were male. Because of the limited amount of animals available, individual subjects were repeatedly tested, with a drug washout period of at least 7 days. All animals were between 6 and 12 months of age. Body weight ranged between 180 and 220 g for females, and between 350 and 400 g for males. Throughout the studies, the animals were individually housed in standard Makrolon® type 3 cages, located in the experimental room, under a reversed 12 L:12 D regime (lights off at 1200 h). Ambient temperature and relative humidity were maintained at 22 ± 1°C and at 55 ± 5%, respectively. The animals were deprived of food, water and EtOH from 1200 h to 2400 h in the two-bottle procedure. Experimental protocols and conditions were conform to the local regulations on animal welfare.

Apparatus and Experimental Setting

For each animal, all sessions for voluntary EtOH consumption were conducted in the respective home cage. The Makrolon type 3 cages (37 × 25 × 16 cm) were bedded with sawdust covered by a metal grid, thus preventing the animals from pushing sawdust against the drinking spouts. Two small shafts were fitted on the metal grid to keep the food cup in place near the center of the back wall. The construction of the food cup, a cylindrical metal container (5 cm of heigth, 10 cm diameter) with a removable metal lid containing a round opening (4 cm diameter in the middle, minimized spilling and allowed for measurement of amount of food consumed. Two bottles (of 300 ml content each) were placed next to each other on top of the cage, near the front wall. The drinking spouts (each fitted with double stoppers) protruded about 1 cm into the cage. The distance between the drinking spouts was approximately 15 cm. An in-house constructed automated device, interfaced with an IBM PC, was mounted on top of the cage. This device automatically regulated the animal's access to both the food cup and the two drinking bottles. During the daily 12-h sessions, the animals were offered vitamin-enriched, powdered lab chow (Snniff Spezialdiäten GmbH, Soest, Germany), a 10% v/v EtOH solution in one bottle and plain tap water in the other bottle. The positions of the bottles were interchanged every day to control for position preference. Experimental sessions were conducted during the dark phase of the day/night cycle under red light conditions.

Drugs

The 10% v/v EtOH solution for the drinking sessions was prepared from absolute EtOH (99.8%; Riedel-de Hån AG; Seelze, Germany) and tap water. DOI, ritanserin and mCPP (RBI, Natick, MA) and MDL 100,907 (synthesized by the Chemistry Department of Bayer AG, Wuppertal, Germany) were dissolved in water and a few drops of lactic acid. Compounds were administrered intraperitoneally (IP) or subcutaneously (SC), the application volume being 1 ml/kg.

Procedure

Dose–response experiments with agonists and antagonists: For each dose of a given drug tested, rats were selected from the available pool of experimental animals on the basis of a sufficient baseline maintained over the two sessions preceding the test session (7). The criterion set for an appropriate individual baseline was an absolute EtOH intake of at least 5 g/kg/day together with a relative EtOH intake [preference, calculated as being (absolute EtOH intake)/(Total fluid intake) × 100)] of at least 70%. A maximum of four groups were tested in parallel, always including a control group treated with the corresponding vehicle of the particular drug(s) tested. Different doses of the same drug, and with the same route of application, were tested in parallel as much as possible. The rats received a single injection of a particular dose of a given drug, or its vehicle, immediately before the respective test session. Subsequently, effects on absolute EtOH intake, as well as on EtOH preference, were measured over the complete 12-h test

session and compared to the last 12-h baseline session, preceding this test session. To assess the degree of selectivity of the drug effects on EtOH intake and preference, effects on total fluid and food intake were simultaneously determined. Animals, food cups, water and EtOH bottles were weighed before and after the two baseline sessions and the test session. Rats treated with a high dose of a particular drug were later preferentially treated with a low dose of another drug (or vehicle), and vice versa. The consummatory profiles induced by DOI (0.3, 1, and 3 mg/kg, IP), mCPP (1, 3, and 10 mg/kg, SC), ritanserin (1, 3, and 10 mg/kg, IP), and MDL 100,907 (0.1, 0.3, and 1 mg/kg, IP) were compared.

Antagonism Experiments

In a second series of studies, it was tested whether the anti-alcohol effects produced by DOI (3 mg/kg, IP) and mCPP (10 mg/kg, IP) were blocked by pretreatment with ritanserin (3 mg/kg, IP), or MDL 100,907 (0.3 mg/kg, IP). The procedure was identical to the method described above, with the exception that animals received the antagonist (or vehicle) 30 min before injection of the agonist (or vehicle).

Data Analysis

Results were expressed as percentage of baseline level for each parameter (EtOH intake, EtOH preference, total fluid intake and food intake). Means and SEMs were calculated for all groups. A one-way ANOVA was employed for analysis of dose–response data and a two-way ANOVA [factors agonist, antagonist, and interaction] was employed for the analysis of data obtained from antagonism studies. Following ANOVA, a Tukey post hoc analysis was performed. Antagonism was considered to be complete if 1) the difference between antagonist × agonist- and vehicle × agonist-treated groups was statistically significant, and 2) the difference between antagonist × agonist- and vehicle × vehicle-treated groups was not statistically significant.

RESULTS

Thoughout the experiments, high and stable baselines of both EtOH preference and intake were consistenly obtained for the 32 groups of animals tested. Mean ± 1 SEM of all daily means before treatment for the 32 groups: 83.8 ± 0.8% and 6.4 ± 0.1 g/kg for EtOH preference and intake, respectively. The mean baselines for total fluid and food intake were 55.5 ± 1.1 g/kg and 98.1 ± 2 g/kg, respectively.

Treatment with DOI resulted in a significant reduction of both EtOH preference and intake, $F(3, 35) = 7.13$, $p < 0.001$, and $F(3, 35) = 4.32$, $p < 0.01$, respectively, whereas food intake and fluid intake were not affected (Fig. 1, left panel). Therefore, DOI displays a specific and selective anti-alcohol profile. Post hoc analysis revealed that DOI reduced EtOH preference and intake at 0.3 and 3 mg/kg.

On the other hand, treatment with mCPP resulted in a significant reduction of EtOH intake, as well as food intake and total fluid intake, $F(3, 45) = 11.5$, $p < 0.001$, $F(3, 45) = 3.03$, $p < 0.05$, and $F(3, 45) = 8.16$, $p < 0.001$, respectively. mCPP did not significantly decrease EtOH preference, although a tendency in this direction could be noted, $F(3, 45) = 2.78$, $p < 0.1$; Fig. 1, right panel. The 3 and 10 mg/kg doses of mCPP were effective in reducing EtOH intake, whereas mCPP tended to reduce EtOH preference at a dose of 10 mg/kg ($p < 0.1$). Total fluid and food intake were reduced at the highest dose of

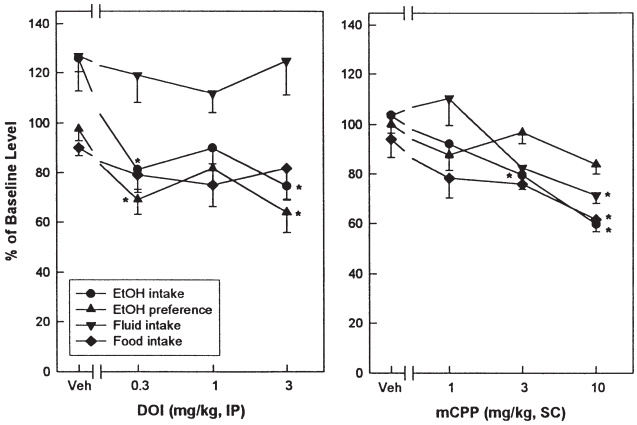

FIG. 1. Effects of DOI and mCPP on EtOH intake, EtOH preference, total fluid intake and food intake. Data are expressed as % of baseline level (mean + SEM). $n = 8$–10 per group (vehicle group $n = 16$ for DOI and $n = 24$ for mCPP). *$p < 0.05$. Absolute values (baseline) for EtOH intake, preference, total fluid and food intake (mean ± SEM) were 6.8 ± 0.2 g/kg, 82.1 ± 1.5%, 105.8 ± 3.3 g/kg, and 54 ± 1.7 g/kg, respectively, for DOI and 6.5 ± 0.2 g/kg, 79.4 ± 1.2%, 106.9 ± 3.2 g/kg, and 57.7 ± 1.9 g/kg, respectively, for mCPP.

mCPP tested. Therefore, the behavioral profile of mCPP is considered to be relatively nonspecific and nonselective.

Ritanserin and MDL 100,907 did not affect EtOH preference or EtOH intake (Fig. 2). Food intake and total fluid intake were generally unaffected, with the exception of MDL 100,907, which reduced food intake, $F(3, 28) = 3.99$, $p < 0.05$, but only at the highest dose tested. Although ANOVA indicated a significant effect of ritanserin on total fluid intake, $F(3, 27) = 2.95$, $p < 0.05$, post hoc analysis did not reveal a significant difference between vehicle and drug treated groups.

In the antagonism studies, treatment with DOI (3 mg/kg) induced again the specific and selective profile obtained in the

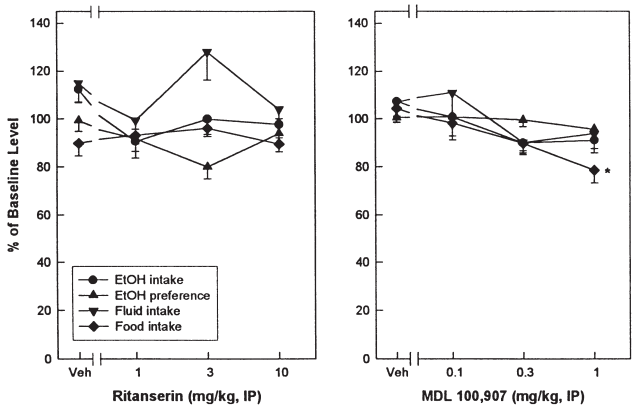

FIG. 2. Effects of ritanserin and MDL 100,907 on EtOH intake, EtOH preference, total fluid intake and food intake. Data are expressed as % of baseline level (mean + SEM). $n = 8$ per group. *$p < 0.05$. Absolute values (baseline) for EtOH intake, preference, total fluid and food intake (mean ± SEM): 6.4 ± 0.2 g/kg, 82.6 ± 1.9%, 98.7 ± 4.2 g/kg, and 59.9 ± 3.2 g/kg, respectively, for ritanserin and 6.9 ± 0.2 g/kg, 87.3 ± 1.7%, 101.5 ± 3.3 g/kg, and 44.2 ± 2.8 g/kg, respectively, for MDL 100,907.

initial dose–response experiment (Figs. 3 and 4). Pretreatment with 3 mg/kg of ritanserin completely blocked the EtOH preference reducing effects of DOI and a tendency to block the DOI-induced reduction of EtOH intake was observed (Fig. 3). A significant effect for the factor interaction was found for EtOH intake and preference, $F(1, 28) = 10.38$, $p < 0.01$, and $F(1, 28) = 26.14$, $p < 0.001$, but not for food intake and total fluid intake. For EtOH preference, post hoc analysis revealed significant differences between the vehicle × vehicle- and the vehicle × DOI-treated groups, as well as between the vehicle × DOI- and ritanserin × DOI-treated groups. Similar effects were obtained for EtOH intake, although the difference between vehicle × DOI and ritanserin × DOI treatment just failed to reach significance ($p < 0.1$). Although the factor antagonist reached significance for the effects of ritanserin on EtOH preference and total fluid intake, $F(1, 28) = 13.56$, $p < 0.001$, and $F(1, 28) = 5.26$, $p < 0.05$, respectively, ritanserin alone had no effects on the latter parameters, as post hoc analysis did not show significant differences between vehicle × vehicle- and ritanserin × vehicle-treated groups. Again, the profile obtained with ritanserin (3 mg/kg) when tested alone is consistent with the profile found in the initial dose–response study.

Pretreatment with 0.3 mg/kg of MDL 100,907 blocked the EtOH intake-reducing effects of DOI (Fig. 4). A significant effect for the factor interaction was found, $F(1, 28) = 8.80$, $p < 0.01$, and post hoc analysis revealed significant differences between the vehicle × vehicle- and the vehicle × DOI-treated groups, as well as between the vehicle × DOI- and MDL 100,907 × DOI-treated groups. For EtOH preference, a significant effect for the factor agonist, $F(1, 28) = 11.3$, $p < 0.01$, and a marked tendency towards an effect for the factor interaction, $F(1, 28) = 3.34$, $p < 0.1$, was found. Post hoc analysis revealed significant differences between the vehicle × vehicle- and the vehicle × DOI-treated groups, whereas the difference between the vehicle × DOI- and MDL 100,907 × DOI-treated group was nearly significant ($p < 0.1$). For total fluid intake, a significant effect for the factor agonist, $F(1, 28) = 4.20$, $p < 0.05$, and a tendency towards an effect for the factor interaction, $F(1, 28) = 3.16$, $p < 0.1$, was found. However, post hoc analysis revealed no difference between groups. For food intake, a tendency towards an effect for the factor interaction, $F(1, 28) = 3.14$, $p < 0.1$, was found. Post hoc analysis revealed again no difference between groups. A similar profile was obtained with MDL 100,907 (0.3 mg/kg) in the initial dose–response experiment and the antagonism study.

Also in the antagonism studies with mCPP, treatment with the agonist (10 mg/kg) replicated the nonspecific and nonselective profile observed in the initial dose–response study; whereas both antagonists again were inactive when tested alone (Figs. 5 and 6).

Pretreatment with 3 mg/kg of ritanserin did not block the mCPP-induced reduction of EtOH intake, food intake, and total fluid intake. The factor agonist reached significance ($p <$

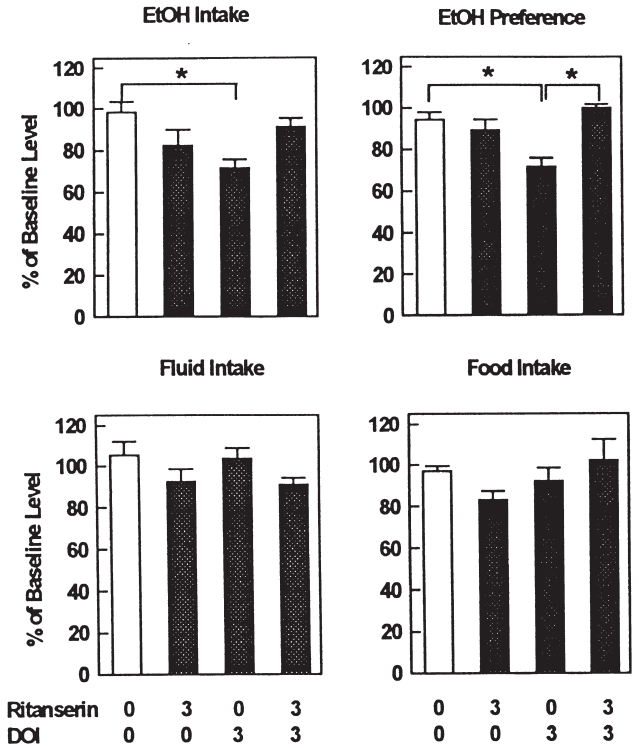

FIG. 3. Effects of combined treatment with ritanserin (3 mg/kg) and DOI (3 mg/kg) on EtOH intake, EtOH preference, total fluid intake and food intake. Data are expressed as % of baseline level (mean + SEM). $n = 8$ per group. *$p < 0.05$. Absolute values (baseline) for EtOH intake, preference, total fluid and food intake (mean ± SEM): 7.8 ± 0.2 g/kg, 86.6 ± 1.3%, 104.1 ± 3.3 g/kg and 71.1 ± 3.8 g/kg, respectively.

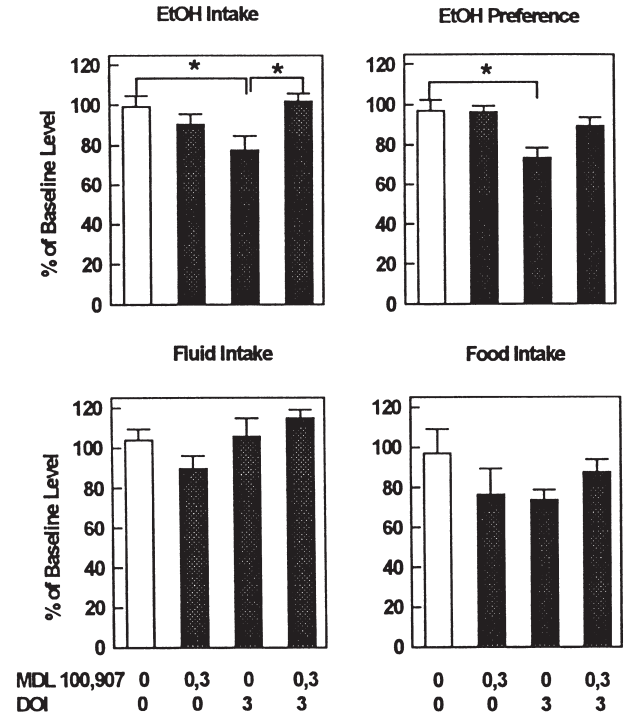

FIG. 4. Effects of combined treatment with MDL 100,907 (0.3 mg/kg) and DOI (3 mg/kg) on EtOH intake, EtOH preference, total fluid intake and food intake. Data are expressed as % of baseline level (mean + SEM). $n = 8$ per group. *$p < 0.05$. Absolute values (baseline) for EtOH intake, preference, total fluid and food intake (mean ± SEM): 5.9 ± 0.2 g/kg, 90.4 ± 1.1%, 82.3 ± 3.5 g/kg, and 58.2 ± 2.4 g/kg, respectively.

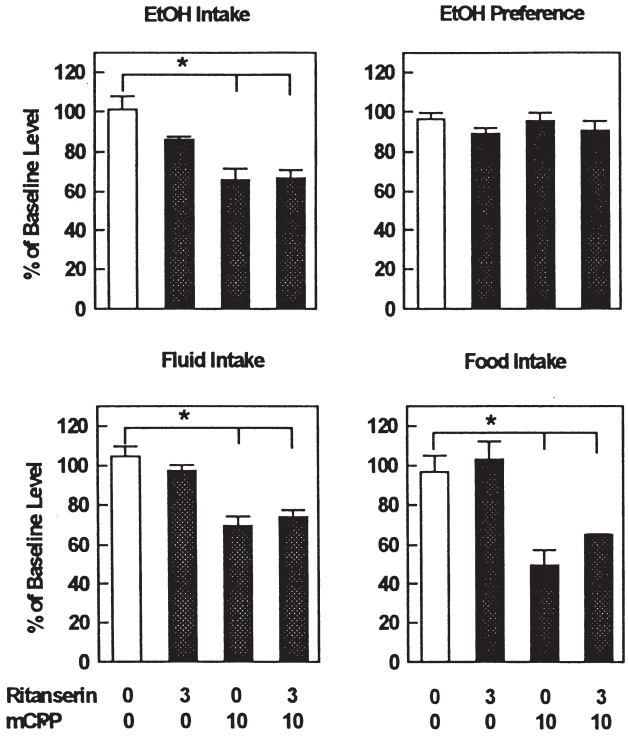

FIG. 5. Effects of combined treatment with ritanserin (3 mg/kg) and mCPP (10 mg/kg) on EtOH intake, EtOH preference, total fluid intake and food intake. Data are expressed as % of baseline level (mean + SEM). $n = 8$ per group. *$p < 0.05$. Absolute values (baseline) for EtOH intake, preference, total fluid and food intake (mean ± SEM): 6.5 ± 0.2 g/kg, 91.1 ± 1.4%, 91.0 ± 3.3 g/kg, and 60.5 ± 2.1 g/kg, respectively.

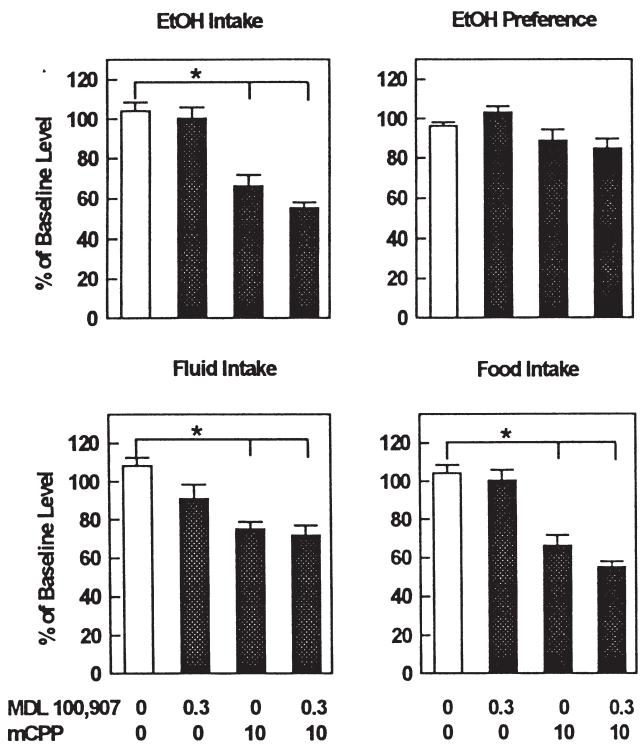

FIG. 6. Effects of combined treatment with MDL 100,907 (0.3 mg/kg) and mCPP (10 mg/kg) on EtOH intake, EtOH preference, total fluid intake and food intake. Data are expressed as % of baseline level (mean + SEM). $n = 8$ per group. *$p < 0.05$. Absolute values (baseline) for EtOH intake, preference, total fluid and food intake (mean ± SEM): 5.9 ± 0.2 g/kg, 89.5 ± 1.1%, 82.4 ± 2.9 g/kg, and 48.3 ± 1.8 g/kg, respectively.

0.001) for EtOH intake, $F(1, 27) = 29.05$, total fluid intake, $F(1, 27) = 48.64$, and food intake, $F(1, 27) = 37.77$. For EtOH intake, total fluid and food intake, post hoc analysis revealed significant differences between the vehicle × vehicle- and vehicle × mCPP-treated groups, as well as between the vehicle × vehicle- and agonist × antagonist-treated groups.

Likewise, pretreatment with 0.3 mg/kg of MDL 100,907 did not block the mCPP-induced reduction of EtOH intake, food intake and total fluid intake. The factor agonist reached significance for EtOH intake, $F(1, 28) = 75.48$, $p < 0.001$, EtOH preference, $F(1, 28) = 9.70$, $p < 0.01$, total fluid intake, $F(1, 28) = 23.25$, $p < 0.001$, and food intake, $F(1, 28) = 5.89$, $p < 0.05$. For EtOH intake, total fluid and food intake, but not EtOH preference, post hoc analysis revealed significant differences between the vehicle × vehicle- and vehicle × mCPP-treated groups, as well as between vehicle × vehicle- and agonist × antagonist-treated groups.

DISCUSSION

In the present series of experiments, the mixed 5-$HT_{2A/2C}$ receptor agonist DOI displayed a specific anti-alcohol effect as it reduced both alcohol intake and preference. The anti-alcohol effect of DOI was considered to be selective as it occurred in the absence of effects on general consummatory behavior. On the other hand, it was found that the 5-$HT_{2C/1B}$ receptor agonist, mCPP, induced a relatively nonspecific and nonselective anti-alcohol effect. This suggests that the antialcohol effects of mCPP are rather due to a general suppressant effect on consummatory behavior. In addition, these results suggest that activaton of 5-HT_{2A} receptors leads to a specific reduction of alcohol consumption; whereas, activation of 5-HT_{2C} and/or 5-HT_{1B} receptors leads to a general decrease in consummatory behavior. The finding that pretreatment with the 5-HT_{2A} receptor antagonist, MDL 100,907, and the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin blocked the alcohol intake-reducing effects of DOI- but not those of mCPP- underscores the proposed role for 5-HT_{2A} receptors in the anti-alcohol effects of DOI and suggests that the general suppressant effect of mCPP on consummatory behavior is not due to stimulation of 5-HT_{2A} receptors.

The finding that DOI induces a specific and selective anti-alcohol effect in alcohol-preferring cAA rats, confirms results obtained previously in this model (9), and extends results obtained in other models for alcoholism, such as alcohol-preferring P rats (33). In the present 12-h limited-access design, DOI did not affect food consumption. However, in another limited-access paradigm not involving EtOH consumption, it has been shown that DOI reduced food intake (23), this effect being depend upon the disruption of the postprandial satiety sequence secondary to motor alterations (44). As opposed to DOI, mCPP induced a decrease of EtOH intake that coincided with a reduction of total fluid and food intake. Interest-

ingly, again in contrast to DOI, mCPP did not produce a major effect on EtOH preference. Such a nonspecific anti-alcohol profile of mCPP has also been reported in other paradigms. Thus, in a limited-access procedure using rats not specifically bred for a high alcohol intake, mCPP induced a decrease of both EtOH and water intake (3); whereas in a continuous access paradigm, EtOH and fluid consumption were decreased during the first hours following administration of the drug (17). It is unclear whether the effects of mCPP on general consummatory behavior as obtained under the present experimental conditions are due to a specific anorectic effect or merely due to general effects on motor behavior [i.e., hypolocomotion; (20)].

The different behavioral profiles induced by DOI and mCPP are most likely related to differential activation of particular 5-HT receptor subtypes. Whereas DOI displays similar affinity for 5-HT_{2A} and 5-HT_{2C} receptors, mCPP possesses, beside its relatively high affinity for 5-HT_{1B} receptors, higher affinity for 5-HT_{2C} receptors than for 5-HT_{2A} receptors (43). Several lines of evidence suggest that the agonist activity of mCPP at 5-HT_{2C} and/or 5-HT_{1B} receptors underlies its reduction of consummatory behavior in cAA rats. Thus, the 5-$HT_{2C/1B}$ receptor antagonist, metergoline, was shown to block the mCPP-induced decrease of water intake in normal rats (3). Furthermore, mCPP-induced hypophagia was completely blocked by metergoline in cAA rats (29) and by the 5-$H7_{2C/2B}$ receptor antagonist, SB 200646, in normal rats (22), but not by ritanserin [(3,21); present results]. The latter findings with SB 200646 and metergoline are in agreement with evidence supporting the involvement of 5-HT_{2C} receptors in the regulation of feeding behavior (5,10,15,16,27,49).

The suggestion that the different behavioral profiles of DOI and mCPP are related to different 5-HT receptor subtypes is further underscored by the antagonism tests with the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, and the 5-HT_{2A} receptor antagonist, MDL 100,907. Thus, it was found that both antagonists blocked the anti-alcohol effects of DOI, but were ineffective against mCPP, supporting the hypothesis that 5-HT_{2A} receptors mediate the alcohol intake- and preference-reducing effects of DOI, but that these receptors are probably not involved in the apparent anti-alcohol effects of mCPP. The involvement of 5-HT_{2C} and/or 5-HT_{1B} receptors in the effects of mCPP on alcohol consumption is further suggested by the findings that metergoline attenuated the alcohol intake-reducing effects of mCPP (29) but not those of DOI (Maurel et al. unpublished). However, it is still unclear whether the latter effects of mCPP are due to stimulation of either 5-HT_{2C} or 5-HT_{1B} receptors or both. Potential involvement of 5-HT_{1B} receptors is at least conceivable, as it was shown that the 5-HT_{1B} receptor agonists, TFMPP (32,33) and CP-94,253 (32), nonselectively reduced alcohol intake, as assessed in P rats and cAA rats, respectively. A role for 5-HT_{1B} receptors in the regulation of EtOH consumption is further supported by the high EtOH intake of transgenic mice lacking 5-HT_{1B} receptors compared to wild-type mice (4).

The present results clearly show that acute treatment with the selective 5-HT_{2A} receptor antagonist, MDL 100,907, and the mixed 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, did not modify EtOH preference and intake of cAA rats. Similar findings were obtained with ritanserin in alcohol-preferring sP rats (41), in cyanamide-treated rats (38), and in Wistar rats showing a preference for 6% EtOH (48). However, the apparent lack of effect of ritanserin contrasts with the results obtained in a nongenetic model of alcoholism (i.e., rats not selectively bred for a high EtOH preference), in which ritanserin was shown to suppress preference for a relatively low EtOH concentration [3% v/v, (35)]. Although ritanserin has been reported to decrease alcohol intake in chronic alcoholics (36), this finding could not be reproduced in a recent clinical trial (19), suggesting that antagonism of 5-$HT_{2A/2C}$ receptors alone is not sufficient to induce a robust decrease of EtOH consumption.

It is very likely that different behavioral mechanisms underly the effects of DOI and mCPP on EtOH consumption. As discussed above, the anti-alcohol effects of mCPP, but not those of DOI, are possibly confounded with nonselective effects on consummatory behavior either due to hypophagia or sedation. However, even in the case that mCPP has intrinsic anti-alcohol effects, different behavioral mechanisms may be involved in the anti-alcohol profiles of both compounds. Thus, mCPP, but not DOI, substituted completely for the discriminative stimulus effects of EtOH in rats (14,31). This finding suggests that the anti-alcohol effects of mCPP, but not those of DOI, may be related to similarities in the discriminative stimulus effects of EtOH and mCPP. In line with this, it has been observed that mCPP induces specific subjective effects in alcoholic patients; the effects being dependent on particular patient characteristics. Thus, following mCPP treatment, the so-called type I alcoholics reported more anger and anxiety, whereas the type II alcoholics reported increased euphoria and greater likelihood of drinking (12). It remains to be evaluated whether the subjective effects of DOI in these patient subgroups differ from the effects induced by mCPP. It was recently found that DOI and mCPP affected operant responding for an orally delivered 10% v/v EtOH solution in normal rats (30). In analogy to the findings obtained in the drug discrimination procedure, the effects of DOI could again be differentiated from those of mCPP. At the same dose ranges effective in the present study, DOI induced a specific and selective reduction of EtOH-reinforced lever pressing. The effect of mCPP was less selective, as its suppression of EtOH-reinforced lever pressing coincided with a general suppressant effect of lever-pressing behavior.

In conclusion, the present findings suggest that activation of 5-HT_{2A} receptors leads to a selective reduction of alcohol intake and preference, whereas activation of 5-HT_{2C} and/or 5-HT_{1B} receptors most likely leads to a general decrease of consummatory behavior. Further experiments using more selective 5-HT_{2C} and 5-HT_{1B} receptor ligands are needed to clarify the exact involvement of the latter 5-HT receptor subtypes in the apparent anti-alcohol effects of mCPP.

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