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Comparison of deramciclane to benzodiazepine agonists in behavioural activity of mice and in alcohol drinking of alcohol-preferring rats

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Abstract

Interactions between alcohol and traditional benzodiazepine anxiolytics hamper the treatment of alcoholism-related anxiety disorders. Serotonin 5-HT_2 receptor antagonists, such as deramciclane, are anxiolytic, and considering their pharmacological profile, they might benefit alcoholics with comorbid anxiety. We studied the effects of acute deramciclane (1, 3 and 10 mg/kg ip) on alcohol drinking of alcohol-preferring AA rats drinking 10% (vol/vol) ethanol solution in a 4-h limited-access paradigm. Thereafter, a 5-day repeated-treatment experiment was carried out, under corresponding test design, with deramciclane (3 mg/kg ip) as a test drug and midazolam (1 mg/kg ip) as a benzodiazepine reference compound. Deramciclane had no effect on alcohol consumption in either acute or repeated dosing study. Midazolam increased ethanol drinking, as expected, when administered on successive days. A modified functional observational battery (FOB) procedure was applied to study neurological, behavioural and autonomic effects induced by deramciclane (1–30 mg/kg po) and diazepam (1–30 mg/kg po) in mice at 30 min, 2 h and 4 h after dosing. Deramciclane had a mild dopamine D_2 receptor antagonism-like effect at the highest dose. The effects of diazepam were predictable, myorelaxation-induced motor impairment and anxiolysis-related hyperlocomotion in a novel environment being the characteristic features at the two highest doses. Deramciclane appears to be a safe and well-tolerated drug and we suggest that it might be useful in the treatment of anxiety in alcoholics.

Keywords: Selected rat lines; Alcohol preference; Anxiolytic agents; Functional observational battery procedure

1. Introduction

Benzodiazepine class drugs are extensively used in the pharmacotherapy of anxiety disorders throughout the world (Gorman, 2003; Shader and Greenblatt, 1993). Their well-established anxiolytic properties are entailed by an activation of GABA system, which is the principal inhibitory neurotransmitter system in brain (McKernan and Whiting, 1996). However, the clinical utility of benzodiazepines is limited in distressed patients with current or prior history of alcoholism (Malcolm, 2003). This is evidently due to several pharmacodynamic interactions between the substances when they are used together. Benzodiazepines have been shown to stimulate ethanol drinking in experimental animals (Chester and Cunningham, 2002; Söderpalm and Hansen, 1998; Wegelius et al., 1994), and clinical experience indicates that the treatment of alcohol-related anxiety with benzodiazepines is associated with potentially hazardous consequences, including worsening of substance abuse and fatal intoxication (Koski et al., 2002; Malcolm, 2003).

Serotonin 5-HT_2 receptors have been identified as mediators of anxiolytic-like drug effects (Schreiber et al., 1998; Wood et al., 2001); consequently, they are regarded as potential target molecules for the discovery of novel anxiolytics (Kent et al., 2002). Most preclinical studies have demonstrated that compounds reducing serotonin neurotransmission produce anxiolytic effect in animal models (Briley et al., 1990; Chopin and Briley, 1987; Griebel et al., 1997; Kennett, 1992). Conversely, 5-HT tone-potentiating substances have been shown to induce aversive behaviour in animals (Chopin and Briley, 1987; Gibson et al., 1994; Iversen, 1984). Although conflicting results have been reported (Griebel et al., 1997; Nic Dhonnchadha et al., 2003), the preclinical data available support the idea linking increased 5-HT function with anxiety. In disagreement with this conclusion is the

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finding that selective serotonin reuptake inhibitors (SSRIs) relieve anxiety in humans (Gorman, 2003; Kent et al., 1998; Vaswani et al., 2003). The SSRIs increase extracellular serotonin content in synapse (Kent et al., 1998). However, the importance of changes in 5-HT turnover in the pathogenesis of anxiety has been established (Handley, 1995), and the pharmacological manipulation of serotoninergic systems provides a basis for the development of novel drugs to treat either acute or chronic anxiety disorders (Kent et al., 2002).

Deramciclane is an anxiolytic drug candidate that pharmacologically behaves as an antagonist at 5-HT_{2A} receptors and as an antagonist/inverse agonist at 5-HT_{2C} receptors (Pälvimäki et al., 1998). It alleviates anxiety-like behaviour in rats at doses (0.7–10 mg/kg) (Gacsályi et al., 1997) that have been demonstrated to significantly occupy these receptors in vivo (Pälvimäki et al., 1998). Preliminary clinical evaluation (Phase I) showed that deramciclane is safe and well tolerated at the dose ranges of 3–50 mg orally (Kanerva et al., 1999), and in a multicenter clinical trial (Phase II), deramciclane had a dose-related effect (10–60 mg/day) in patients with generalized anxiety disorder (Naukkarinen, 2001). Currently, deramciclane is employed in a Phase III multicenter clinical trial.

The purpose of the present study was to test the effects of deramciclane on ethanol intake in an animal model of alcoholism (AA rats; Eriksson, 1968) at doses with anxiolytic activity in rats. The effects were compared with those induced by the benzodiazepine agonist midazolam, which previously has been shown to increase ethanol drinking in the AA rats (Wegelius et al., 1994). A comprehensive behavioural test battery was performed in mice pretreated with either deramciclane or diazepam, a prototype benzodiazepine, to gather information on the behavioural responses evoked by the drugs from different classes of anxiolytics and to evaluate the safety profile of deramciclane.

2. Materials and methods

2.1. Experimental animals and housing conditions

Alcohol-preferring AA rats were obtained from the Department of Mental Health and Alcohol Research, National Public Health Institute, Helsinki, Finland. Rats were 8 months old at the start of the experiment and weighed between 319 and 468 g. They were individually housed in polycarbonate Macrolon III (Scanbur, Denmark) cages that were located in an experiment room at an ambient temperature of 21 ± 3 °C under 12-h dark/light cycle. Clean cages with aspen chip bedding were changed twice a week. All training and experimental procedures were done between 9 a.m. and 5 p.m. The Institutional Animal Use and Care Committee of the University of Turku approved all experimental procedures for rat experiments.

NMRI male mice about 6 weeks old at arrival (mean weight more than 25 g when tested) were purchased from B&K Universal (Box 6023, Vibyvägen 72, 19206 Sollentuna, Sweden). Animals were housed in solid-bottomed Macrolon Type III cages, 10 animals per cage, at an ambient temperature of 22 ± 2 °C under 12-h dark/light cycle (lights on 6 a.m.–6 p.m.), according to the rules of the European Convention and the National Research Council, USA. Mice were habituated to the new laboratory environment for at least 1 week before the start of the experimentation. Softwood granulated aspen bedding (Beekay Bedding, GLP bedding, B&K Universal, Nittedal, Norway) was used. Standard certified dry pelleted rat and mouse feed RM1 SQC, supplied by Special Diets Services (P.O. Box 705, Witham, Essex CM8 3AD, England, UK), and tap water were available for rats and mice ad libitum in this study except as noted below. The mouse experiments were performed in the laboratories of Orion Pharma (Turku, Finland) in adherence to good laboratory practice (GLP) regulations.

2.2. Ethanol-drinking paradigm

Rats were well acclimatised to the testing conditions before experiment initiation. During this period, animals were trained to drink alcohol in a limited-access paradigm (Ingman et al., 2003b; Sinclair et al., 1992). First, rats had forced alcohol drinking for 6 days, 10% ethanol (by volume) in tap water being the only fluid available. Thereafter, rats were allowed free access to both water and alcohol bottles over the following 6 weeks, after which ethanol preference was constantly more than 50% in proportion to total fluid consumed. The limited access was completed by gradually restricting alcohol availability to 4 h per day three times a week, i.e., on every second working day. During the four access hours, the volume of alcohol solution consumed by rats was recorded (rounded off to the nearest 0.2 ml) at 1 and 4 h after the presentation of alcohol-containing Richter tubes. Rats and water bottles were weighed immediately before ethanol-drinking sessions. Water consumption during the 4-h ethanol exposure was negligible and therefore 24-h water consumption was measured (g).

2.3. Ethanol-drinking experiments

2.3.1. Acute treatment

Animals were weighed and given vehicle injections intraperitoneally 30 min before limited-access drinking session preceding the experiment with drugs. Three treatment groups ($n = 7$ in each group) were formed according to equal 4-h baseline ethanol drinking. On the experimental day, rats were weighed, treated with 1, 3 or 10 mg/kg ip of deramciclane and presented with ethanol-containing tubes 30 min after the drug injections. Ethanol intakes were determined 1 h after the drinking initiation and at the end of the 4-h session.

2.3.2. Repeated treatment

Following a washout period of 18 days, rats experienced intraperitoneal vehicle injections and baseline drinking

session as described above. Animals were then assigned to receive vehicle intraperitoneally, midazolam (1 mg/kg ip) or deramciclane (3 mg/kg ip) treatment ($n = 7$ in each group), average basal 4-h ethanol consumption again being equal in these groups. Thereafter, rats were weighed daily, individual drug doses were determined and corresponding drugs were administered over five consecutive days. Limited-access drinking was initiated 30 min after dosing and ethanol drinking was monitored at the 1- and 4-h time points.

2.4. Functional observational battery (FOB) procedure

The procedure applied here is a modification of published procedures but is essentially in line with the methods described by Irwin (1968) and Rogers et al. (1997). The person responsible for behaviour testing was well trained and experienced in observation and rating of rodent behaviour and was kept blind to the dose groups. Ten mice were studied per day. In the afternoon preceding the experiment, the mice were separated into two clean Macrolon III cages, identified and placed on a shelf apart from other mice; the mice were provided with water ad libitum but not food. On the experimental day, the mice were weighed and moved into the study room. The experiment was initiated by giving the mice oral drug doses [vehicle, diazepam (1–30 mg/kg) or deramciclane (1–30 mg/kg)] by gavage at 7-min intervals and FOB evaluation was performed 30 min, 2 h and 4 h after dosing. Blind assessment of each animal began with observation of undisturbed behaviour in a transparent cylindrical viewing jar (11 cm in diameter). The behaviours observed and scored in each phase are summarized in Table 1. Thereafter, the mouse was transferred to an open arena (55 × 33 cm) that consisted of 15 even-sized squares (11 × 11 cm) for testing of transfer arousal and observation

Table 1
Behaviours recorded on the FOB procedure and the criteria for scoring

(1) In viewing jar	(2) In arena		(3) In tail–neck grip
Body position and activity in undisturbed situation	Transfer arousal	Visual placing	Skin colour
0 Lies on its side	0 Coma, practically no movements	0 Reaction to lowered grid when it touches head	0 normal
1 Lies flat	1 Moves a little but has great difficulties	1 Reaction occurs at 1–3 mm above the grid	1 Abnormal
2 Lies flat but tries to move a little	2 Moves clearly slower than normal mice	2 Reaction occurs >3 mm above the grid (normal)	Lacrimation
3 Stands without any activity	3 Slightly slower, not clearly alert	Trunk curling	0 Normal
4 Stands and has some occasional activity	4 Alert	0–1 No or yes	1 Abnormal
5 Is active almost continuously and may move	5 Alert and moves lively	Limb grasping	Provoked biting
6 Moves and is active all the time	6 Very excited	0–1 No or yes	0 No biting
7 Very active	Locomotor activity	Grip strength	1 Active
8 Extremely active and also jumping may occur	Number of squares entered by all feet during the first 30 s in the open arena. Data presented in Fig. 1	0 Not able to resist manipulation	Heart rate
Piloerection	Gait	1 Very weak	0 Lowered
0 None	0 Normal	2 Slightly weakened	1 Normal
1 Marked	1 Slightly abnormal	3 Normal	2 Elevated
Respiration	2 Clearly abnormal or do not walk	4 Strengthened	Limb tone
0–2 Irregular gasping to hyperventilation	Pelvic elevation	Body tone	0 All limbs completely flaccid
Tremor	0 Flattened, pelvis touches floor	0 Flaccid	1 Limbs somewhat flaccid
0–2 None to continuous	1 Lowered	1 Normal	2 Normal tone
Urine and feces	2 Normal, approximately 3 mm	2 Firm	3 Limbs rigid
0 None	3 Elevated more than 3 mm	Ear reflex	Abdominal tone
1 Some but considered normal	Tail position	0 No ear retraction in response to light air blow	0 Flaccid
2 Abnormal	0 Flattened	1 Observable retraction	1 Normal
	1 Horizontal	2 Very clear/exaggerated retraction	2 Resistant
	2 Elevated	Corneal reflex	Body temperature (measured with rectal probe)
	Startle reaction	0–2 None to exaggerated retraction	Data shown in Fig. 2
	0–3 None to strong reaction	Toe pinch reflex (possible analgesic effect)	
	Palpebral closure	0–4 No reaction to hypersensitive reaction	
	0–4 Eyes wide open to eyes closed	Wire maneuver: animal suspended from horizontal wire by forelimbs and released	
	Touch escape	0 Animal immediately and skillfully bend rear paws on the wire	
	0–3 No response to extremely vigorous	1 Bending occurs during 1–3 s after release	
	Reaction to handling	2 Delayed bending (>3 s) without dropping	
	0 Struggling when suspended from tail	3 Dropping after hanging for at least 2 s	
	1 Struggling when kept in tail–neck grip	4 Not able to hang or dropping almost immediately	
	2 Struggling when kept lying on cage floor	Contact righting reflex in a rotating plastic tube	
	3 Struggling when suspended from rear limb	0–2 Quick turn to no turn	
	4 No reaction to mentioned manipulations		

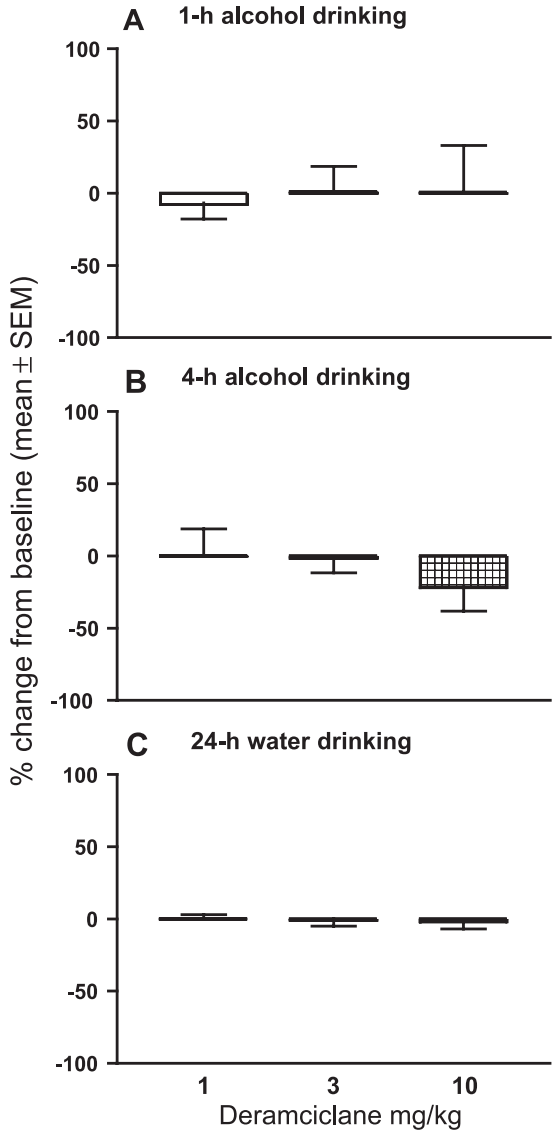

Fig. 1. Effects of acute deramciclane (1, 3 and 10 mg/kg) on 1-h (A) and 4-h (B) limited access alcohol drinking. Animals were intraperitoneally injected with deramciclane 30 min before commencing the limited access drinking session. Changes in 24-h water consumption are illustrated in Panel C. Data are presented as mean percentage change (± S.E.M.) from the baseline levels ($n = 7$ in each group).

of normal behaviour. In the third and final phase, the mouse was restrained in a supine position using a tail–neck grip to evaluate autonomic functions.

2.5. Drugs

EtOH solution for limited-access oral ingestion was prepared from 99.5% ethyl alcohol (Primalco, Rajamäki, Finland) by diluting it with tap water for a concentration of 10% (vol/vol). Deramciclane fumarate [(1*R*,2*S*,4*R*)-(−)-dimethylaminethoxy-2-phenyl-1,7,7-trimethylbicyclo[2.2.1] heptane 2-(*E*)-butenodioate] was supplied by EGIS Pharmaceuticals, Budapest, Hungary. It was dissolved in 0.4% carboxyl methylcellulose (Sigma). Two benzodiazepine class reference compounds were used in this study. Midazolam maleate (Dormicum; 1 mg/ml, Roche, Switzerland) was administered as such and diazepam (Stesolid Novum; 5 mg/ml, Dumex-Alpharma, Denmark) was diluted with 10% intralipid. All intraperitoneal injections were administered in a volume of 1 ml/kg.

2.6. Data processing and statistics

Data from the ethanol-drinking experiments were evaluated with analysis of variance (ANOVA) for repeated

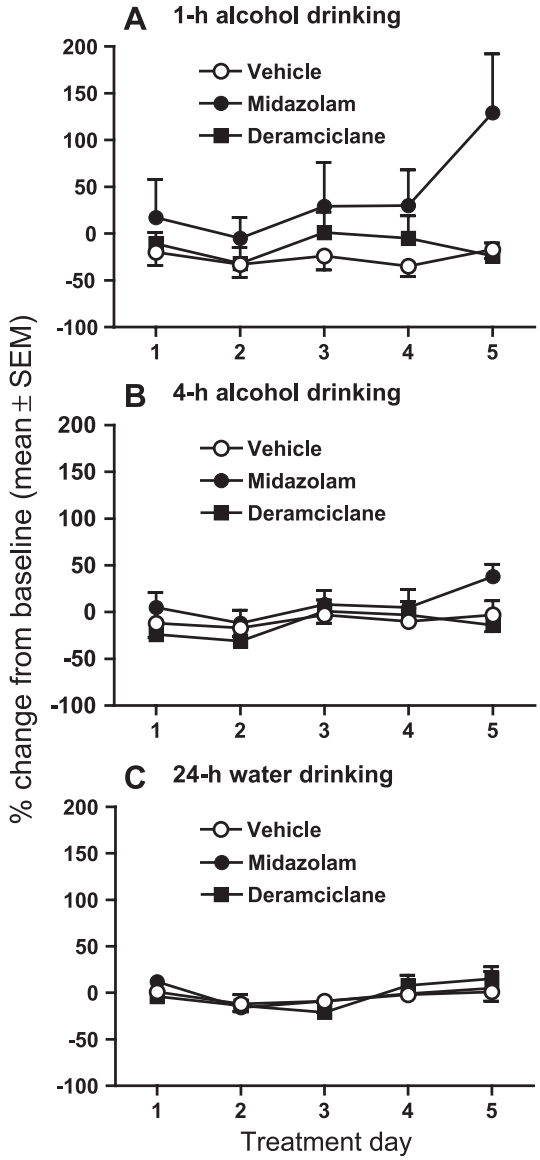

Fig. 2. Limited access 1-h (A) and 4-h (B) alcohol drinking in AA rats pretreated with vehicle, deramciclane (3.0 mg/kg) and midazolam (1.0 mg/kg). Treatments were administered intraperitoneally 30 min prior to the limited access drinking session over five consecutive days. Twenty-four-hour water intakes (C) were measured throughout the experiment. Data are expressed as percent of baseline intakes (mean ± S.E.M.), $n = 7$ in each group.

measures. In the acute treatment study, the main effects were treatment, session (baseline, test) and time (1 or 4 h), the latter two effects being repeated variables. In the analysis of the repeated-treatment data, the main effects were treatment, day (1–5) and time (1 or 4 h), day and time being repeated variables. A P value less than .05 was considered statistically significant. When possible, pairwise comparisons were made using contrast analysis with Bonferroni adjustment (SAS software version 6.12, SAS Institute, Gary, NC). Raw data from the FOB study sheets were transferred to computer data sheets and were analyzed by the statistical software package SPSS for Windows 11.1 (SPSS, Chicago, IL). The FOB data with continuous variables (locomotor activity counts and body temperature) were analyzed with one-way ANOVA followed by Dunnett's t test. Categorized ratings were evaluated by a chi-square or Fischer's nonparametric test (when applicable).

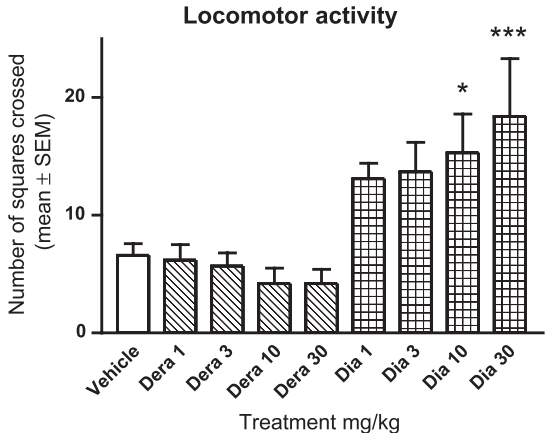

Fig. 3. Number of squares entered by mice with all four feet during the first 30 s in the open arena 2 h after oral dosing with vehicle (n=20), deramciclane (1–30 mg/kg) or diazepam (1–30 mg/kg). Each bar represents the mean of number of squares entered ± S.E.M. (n=10 in each group receiving drug treatments). Significant difference relative to vehicle treatment is denoted by *(P<.05) and ***(P<.001), determined by one-way ANOVA and Dunnett's test.

3. Results

3.1. Effects of acute deramciclane on ethanol and water intake

The mean ethanol consumption on the baseline day was 0.76 ± 0.07 g/kg after 1-h drinking and 1.44 ± 0.11 g/kg (S.E.M., n=21) at the end of the drinking session. Basal 24-h water consumption was 18.6 ± 1.3 ml (S.E.M., n=21). Deramciclane treatment (1, 3 and 10 mg/kg) did not produce significant effects on 1- or 4-h ethanol drinking [F(1,72)=1.42, P=.24], nor did it affect 24-h water intake [F(1,18)=0.55, P=.47] as illustrated in Fig. 1.

3.2. Effects of repeatedly administered deramciclane and midazolam on ethanol and water drinking

Basal 1- and 4-h alcohol intakes in the drinking session preceding the experiment were 0.94 ± 0.10 and 1.43 ± 0.11 g/kg (S.E.M., n=21), respectively. Average 24-h water intake after vehicle-pretreatment was 20.7 ± 1.3 ml (S.E.M., n=21). Data on the effects of administration over 5 days of vehicle, midazolam (1 mg/kg) and deramciclane (3 mg/kg) are presented in Fig. 2. ANOVA revealed significant effect for treatment [F(2,177)=21.78, P<.001], test day [F(4,177)=2.83, P=.026] and Treatment × Time interaction [F(2,177)=3.35, P=.037]. The midazolam-induced effect appears to increase across the treatment days and statistical analysis confirmed the visual impression by yielding a tendency to a significant Treatment × Test Day interaction [F(8,177)=1.78, P=.084]. Pairwise comparisons indicated that the midazolam-treated rats consumed significantly more ethanol than either the vehicle-(P<.001) or deramciclane (P<.001)-treated animals. Water intake (24 h) was not significantly modulated by the treatments [F(2,87)=2.93,

Table 2
Comparison of the effects of deramciclane and diazepam on behaviours observed in the FOB 2 h after drug administration

Observed behavior in the FOB battery	Number of animals exhibiting the behaviour/total number of animals in group							
	Vehicle	Deramciclane dose (mg/kg)				Diazepam dose (mg/kg)			
		1	3	10	30	1	3	10	30
Body posture: lying when undisturbed	2/20 (10%)	0/10	2/10	1/10	7/10**	0/10	1/10	7/10**	10/10***
Abnormal pelvic height when walking	0/20 (0%)	1/10	1/10	1/10	1/10	2/10	3/10*	4/10*	6/10**
Flattened tail when walking	0/20 (0%)	2/10	0/10	0/10	0/10	0/10	1/10	5/10**	8/10***
Decreased grip strength	2/20 (10%)	2/10	0/10	0/10	0/10	1/10	4/10	5/10*	8/10***
Decreased body tone	0/20 (0%)	0/10	0/10	0/10	0/10	0/10	1/10	4/10**	8/10***
Exaggerated ear retraction reflex	0/20 (0%)	0/10	0/10	0/10	0/10	0/10	4/10**	2/10	8/10***
Unable to hang on wire	2/20 (10%)	0/10	0/10	0/10	2/10	0/10	1/10	6/10*	8/10**
Decreased limb tone	3/20 (15%)	2/10	1/10	2/10	3/10	2/10	3/10	8/10**	10/10***
Decreased abdominal tone	2/20 (10%)	0/10	1/10	2/10	0/10	0/10	2/10	7/10**	8/10***

* Significant difference from vehicle treatment (P<.05) evaluated by the chi-square test or Fischer's nonparametric test.
** Significant difference from vehicle treatment (P<.01) evaluated by the chi-square test or Fischer's nonparametric test.
*** Significant difference from vehicle treatment (P<.001) evaluated by the chi-square test or Fischer's nonparametric test.

P=.059], but there was a significant effect for treatment days [$F(4,87) = 4.48$, P=.002] that was due to a slight decrease in water consumption concerning animals in all the groups after the second and third treatments.

3.3. Effects of deramciclane and diazepam on behaviours recorded in the FOB assessment

The FOB evaluation was made three times for each mouse at the time points of 30 min, 2 h and 4 h after drug administration. Both deramciclane and diazepam clearly induced the most prominent effects in comparison with vehicle treatment at the 2-h time point; the main findings are summarized in Table 2. After 5-min habituation in the viewing jar, discernible flattened body posture was observed in the animals pretreated with deramciclane [30 mg/kg (P=.008)] and diazepam [10 mg/kg (P=.001), 30 mg/kg (P<.001)]. Upon the animals' arrival in the open arena, stimulated motor activity was recorded in the animals treated with diazepam (10 and 30 mg/kg; Fig. 3). When walking, the diazepam-treated mice had lowered pelvic height [3 mg/kg (P=.030), 10 mg/kg (P=.010), 30 mg/kg (P=.002)] and flattened tail [10 mg/kg (P=.002), 30 mg/kg (P<.001)] compared to the vehicle-treated animals. Furthermore, manual estimation revealed decreased grip strength [10 mg/kg (P=.026), 30 mg/kg (P<.001)] and body tone [10 mg/kg (P=.008), 30 mg/kg (P<.001)] in the diazepam-treated mice when compared with the control animals. In the ear retraction reflex test, the diazepam-treated animals showed greater activity [3 mg/kg (P=.008), 30 mg/kg (P<.001)] than the vehicle-treated mice. In the wire-maneuver test, the diazepam-treated mice displayed worse performance [10 mg/kg (P=.026), 30 mg/kg (P=.003)] than the vehicle-treated animals. Examination of the limbs (by manipulation) and the abdomen (by palpation) showed that the limbs and abdomen were more flaccid in the diazepam-treated animals [10 mg/kg (P=.001), 30 mg/kg (P<.001) and 10 mg/kg (P=.002), 30 mg/kg (P<.001), respectively] than in the control animals. Additionally, diazepam (10 and 30 mg/kg) caused a significant decrease in body temperature, whereas deramciclane did not affect it (Fig. 4).

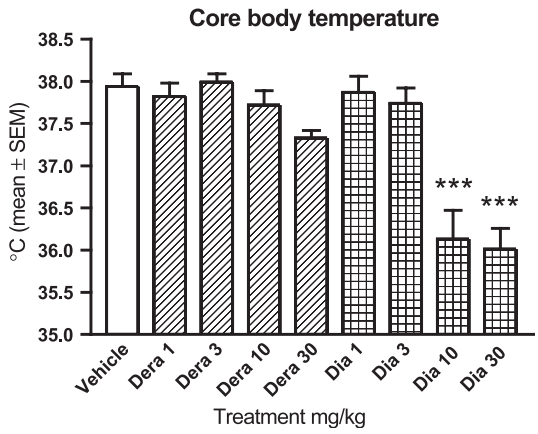

Fig. 4. Effects of deramciclane (1–30 mg/kg) and diazepam (1–30 mg/kg) on core body temperature of mouse 2 h after dosing. Temperature was measured using a rectal probe (inserted 2.5 cm inside the anal sphincter) and a digital thermometer. Data are cited as means ± S.E.M. (n = 10 in each group, except 20 for vehicle group). Asterisks (***) indicate a statistically significant difference (P<.001) from vehicle group, analyzed with one-way ANOVA and Dunnett's test.

4. Discussion

The present study demonstrates that deramciclane does not affect ethanol drinking in alcohol-preferring AA rats. Acutely administered deramciclane did not change limited-access ethanol drinking in comparison with baseline levels. Thereafter, deramciclane had no effect on alcohol drinking in a 5-day repeated-treatment regimen, whereas the positive control, midazolam, increased ethanol consumption. The results predict that deramciclane may have substantial advantage over traditional benzodiazepine anxiolytics in the management of anxiety disorders in alcoholic patients.

The extensive and partly conflicting literature concerning the effects of serotoninergic ligands on alcohol-drinking behaviour suggests that drugs increasing serotonin neurotransmission suppress ethanol drinking in experimental animals (McBride and Li, 1998; Overstreet et al., 1994). Particularly, SSRIs (Wilcox and McMillen, 1998) and 5-HT_{1A} receptor agonists (McKenzie-Quirk and Miczek, 2003) have been effective in decreasing ethanol intake, but citalopram failed to reduce alcohol drinking in the AA rats (Wegelius et al., 1994). The pharmacological modulation of 5-HT_2 receptors in alcohol-drinking rodents has produced variable results. Antagonists at 5-HT_2 receptors have been shown effective (Lin and Hubbard, 1994; Meert, 1994), ineffective (Myers and Lankford, 1993; Panocka et al., 1993) and effective only at doses not selective for ethanol drinking (Overstreet et al., 1997). Acutely administered risperidone has failed to alter ethanol consumption in the AA rats at doses presumably selective for 5-HT_2 receptors (Ingman et al., 2003a). Importantly, to our knowledge, no report on increased ethanol consumption by 5-HT_2 receptor antagonists has been published to date.

The increase in ethanol consumption during repeated midazolam administration is in agreement with earlier results because midazolam (1 mg/kg) has significantly stimulated ethanol intake of the AA rats in a limited-access paradigm (Wegelius et al., 1994). Furthermore, midazolam (5 mg/kg) has increased ethanol consumption and preference without affecting water intake in Wistar rats (Söderpalm and Hansen, 1998) and another $GABA_A$ receptor agonist, THIP, has stimulated alcohol drinking in Long–Evans rats while total fluid intake remained unchanged (Boyle et al., 1993). There is a body of evidence suggesting that benzodiazepines increase feeding and drinking in animals, presumably at least partially, by enhancing the palat-

ability of naturally rewarding ingestions (Berridge and Pecina, 1995), although this effect appears to require significantly higher drug doses than the one that produced increased ethanol drinking (Berridge and Pecina, 1995). Another explanation given for increased alcohol drinking suggests that benzodiazepine agonists and ethanol interact at $GABA_A$ receptors contributing to enhanced GABAergic activity (Grobin et al., 1998; Korpi, 1994) that in turn leads to the altered motivational effects of alcohol and stimulates drinking (Chester and Cunningham, 2002).

The behavioural screening began with observation of body position in an undisturbed situation that can reflect drug effects in several levels of neuromuscular and behavioural control. Diazepam elicited a marked effect at doses that additionally decreased body, abdominal and limb tone, and thus it is apparent that lying posture under diazepam effect was due to muscle weakness. In addition, deramciclane caused abnormal lying posture at the highest dose, which can be attributed to dopamine D_2 receptor blockade, known to occur at high doses (>20 mg/kg; Gacsályi et al., 1997) resulting in deramciclane plasma concentrations up to 200 ng/ml 2 h after oral dosing (unpublished data). Animals treated with diazepam (10 and 30 mg/kg) had flattened pelvic height and reduced tail elevation when walking. These abnormal observations usually appear together and are presumably due to diazepam-induced muscle flaccidity (Irwin, 1968). Impaired performance in wire-maneuver test was observed in animals pretreated with either of the highest diazepam dose. This trial tests for subjects' balance, coordination and (rear) limb strength. Taking into account the other behavioural measures discussed here, plausible explanation for poor performance is muscle weakness related to high diazepam doses (10 and 30 mg/kg) in these mice. Consistently, decreased forelimb grip strength was scored in animals treated with diazepam (10 and 30 mg/kg).

It is well known that diazepam can have a stimulant effect in behavioural test situations (Zbinden and Randall, 1967) and in motor activity measurements in experimental animals (Marriott and Smith, 1972; Söderpalm et al., 1991). We observed an exaggerated ear retraction reflex in mice treated with 3 or 30 mg/kg of diazepam, although no significant effect was seen at 10 mg/kg in comparison with vehicle-treated animals. A clear diazepam-produced stimulation was also seen in locomotor activity test for the two highest diazepam doses, which significantly increased the number of squares entered by the mice in open arena during the first 30 s. Although hyperlocomotion is principally implicated with relatively low diazepam doses (Söderpalm et al., 1991), and higher sedative doses have reduced locomotor activity (Gardner and Piper, 1982; Söderpalm et al., 1991), our results are not necessarily conflicting with the earlier ones. Locomotor activity during the 30-s observation session in novel environment is partly regulated by anxiety-related behavioural responses (Choleris et al., 2001), and it can be hypothesized that diazepam-induced anxiolytic-like effect increases exploratory behaviour and walk in the open arena (Belzung et al., 2001; Choleris et al., 2001). If so, it appears that deramciclane lacks anxiolytic potency under corresponding conditions. Otherwise, the data regarding locomotor activity test support the earlier results (Gacsályi et al., 1997) and confirm that deramciclane doses we used in alcohol-drinking experiments do not produce dopamine D_2 receptor-mediated behavioural responses but antagonize 5-HT_2 receptors.

It has been shown that diazepam decreases body temperature in mice (Zarrindast and Dibayan, 1989) and $GABA_A$ receptor has been proposed to be the site of drug action (Zarrindast and Dibayan, 1989). However, acute exposure to various toxic substances leads rather uniformly to hypothermia in laboratory rodents, which refers to possibility for a common protective thermoregulatory response in the state of drug intoxication (Gordon, 1991; Gordon et al., 1988). The results from the core body temperature measurements indicate a significant fall after diazepam treatment that is in agreement with the findings discussed above, but deramciclane was without effect.

In sum, deramciclane appears to be a safe and well-tolerated drug that has no influence on alcohol drinking in an animal model of alcoholism. The findings render deramciclane a promising pharmacotherapeutic for patients suffering from anxiety complicated with substance abuse.

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References

Belzung C, Le Guisquet AM, Barreau S, Calatayud F. An investigation of the mechanisms responsible for acute fluoxetine-induced anxiogenic-like effects in mice. Behav Pharmacol 2001;12:151–62.

Berridge KC, Pecina S. Benzodiazepines, appetite, and taste palatability. Neurosci Biobehav Rev 1995;19:121–31.

Boyle AE, Segal R, Smith BR, Amit Z. Bidirectional effects of GABAergic agonists and antagonists on maintenance of voluntary ethanol intake in rats. Pharmacol Biochem Behav 1993;46:179–82.

Briley M, Chopin P, Moret C. Effect of serotonergic lesion on "anxious" behaviour measured in the elevated plus-maze test in the rat. Psychopharmacology (Berl.) 1990;101:187–9.

Chester JA, Cunningham CL. GABA(A) receptor modulation of the rewarding and aversive effects of ethanol. Alcohol 2002;26:131–43.

Choleris E, Thomas AW, Kavaliers M, Prato FS. A detailed ethological analysis of the mouse open field test: effects of diazepam, chlordiazepoxide and an extremely low frequency pulsed magnetic field. Neurosci Biobehav Rev 2001;25:235–60.

Chopin P, Briley MU. Animal models of anxiety: the effect of compounds that modify 5-HT neurotransmission. Trends Pharmacol Sci 1987;8:383–8.

Eriksson K. Genetic selection for voluntary alcohol consumption in the albino rat. Science 1968;159:739–41.

Gacsályi I, Schmidt É, Gyertyán I, Vasar E, Lang A, Haapalinna A, et al. Receptor binding profile and anxiolytic-type activity of deramciclane (EGIS-3886) in animal models. Drug Dev Res 1997;40:333–48.

Gardner CR, Piper DC. Effects of agents which enhance GABA-mediated neurotransmission on licking conflict in rats and exploration in mice. Eur J Pharmacol 1982;83:25–33.

Gibson EL, Barnfield AM, Curzon G. Evidence that mCPP-induced anxiety in the plus-maze is mediated by postsynaptic 5-HT_{2C} receptors but not by sympathomimetic effects. Neuropharmacology 1994;33:457–65.

Gordon CJ. Toxic-induced hypothermia and hypomtabolism: do they increase uncertainty in the extrapolation of toxicological data from experimental animals to humans? Neurosci Biobehav Rev 1991;15: 95–8.

Gordon CJ, Mohler FS, Watkinson WP, Rezvani AH. Temperature regulation in laboratory mammals following acute toxic insult. Toxicology 1988;53:161–78.

Gorman JM. Treating generalized anxiety disorder. J Clin Psychiatry 2003;64:24–9.

Griebel G, Perrault G, Sanger DJ. A comparative study of the effects of selective and non-selective 5-HT_2 receptor subtype antagonists in rat and mouse models of anxiety. Neuropharmacology 1997;36:793–802.

Grobin AC, Matthews DB, Devaud LL, Morrow AL. The role of GABA(A) receptors in the acute and chronic effects of ethanol. Psychopharmacology (Berl.) 1998;139:2–19.

Handley SL. 5-Hydroxytryptamine pathways in anxiety and its treatment. Pharmacol Ther 1995;66:103–48.

Ingman K, Honkanen A, Hyytiä P, Huttunen MO, Korpi ER. Risperidone reduces limited access alcohol drinking in alcohol-preferring rats. Eur J Pharmacol 2003a;468:121–7.

Ingman K, Salvadori S, Lazarus L, Korpi ER, Honkanen A. Selective δ-opioid receptor antagonist *N*,*N*$(CH_3)_2$-Dmt-Tic-OH does not reduce ethanol intake in alcohol-preferring AA rats. Addict Biol 2003b;173–9 (June).

Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 1968;13:222–57.

Iversen SD. 5-HT and anxiety. Neuropharmacology 1984;23:1553–60.

Kanerva H, Kilkku O, Helminen A, Rouru J, Scheinin M, Huupponen R, et al. Pharmacokinetics and safety of deramciclane during multiple oral dosing. Int J Clin Pharmacol Ther 1999;37:589–97.

Kennett GA. 5-HT_{1C} receptor antagonists have anxiolytic-like actions in the rat social interaction model. Psychopharmacology (Berl.) 1992;107: 379–84.

Kent JM, Coplan JD, Gorman JM. Clinical utility of the selective serotonin reuptake inhibitors in the spectrum of anxiety. Biol Psychiatry 1998; 44:812–24.

Kent JM, Mathew SJ, Gorman JM. Molecular targets in the treatment of anxiety. Biol Psychiatry 2002;52:1008–30.

Korpi ER. Role of $GABA_A$ receptors in the actions of alcohol and in alcoholism: recent advances. Alcohol Alcohol 1994;29:115–29.

Koski A, Ojanperä I, Vuori E. Alcohol and benzodiazepines in fatal poisonings. Alcohol Clin Exp Res 2002;26:956–9.

Lin N, Hubbard JI. The increased ethanol preference in rats induced by choice, darkness, or drugs is reduced by ritanserin. Brain Res Bull 1994; 33:633–8.

Malcolm RJ. GABA systems, benzodiazepines, and substance dependence. J Clin Psychiatry 2003;64:36–40.

Marriott AS, Smith EF. An analysis of drug effects in mice exposed to a simple novel environment. Psychopharmacologia 1972;24:397–406.

McBride WJ, Li TK. Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. Crit Rev Neurobiol 1998;12: 339–69.

McKenzie-Quirk SD, Miczek KA. 5-HT(1A) agonists: alcohol drinking in rats and squirrel monkeys. Psychopharmacology (Berl.) 2003;167: 145–52.

McKernan RM, Whiting PJ. Which $GABA_A$-receptor subtypes really occur in the brain? Trends Neurosci 1996;19:139–43.

Meert TF. Ritanserin and alcohol abuse and dependence. Alcohol Alcohol, Suppl 1994;2:523–30.

Myers RD, Lankford MF. Failure of the 5-HT_2 receptor antagonist, ritanserin, to alter preference for alcohol in drinking rats. Pharmacol Biochem Behav 1993;45:233–7.

Naukkarinen H. Deramciclane in the treatment of patients with generalised anxiety disorder: a randomised, double-blind, placebo-controlled, dose-finding study. Eur Neuropsychopharmacol 2001;11:S301–2.

Nic Dhonnchadha BA, Bourin M, Hascoet M. Anxiolytic-like effects of 5-HT_2 ligands on three mouse models of anxiety. Behav Brain Res 2003; 140:203–14.

Overstreet DH, Rezvani AH, Pucilowski O, Janowsky DS. 5-HT receptors: implications for the neuropharmacology of alcohol and alcoholism. Alcohol Alcohol., Suppl 1994;2:205–10.

Overstreet DH, McArthur RA, Rezvani AH, Post C. Selective inhibition of alcohol intake in diverse alcohol-preferring rat strains by the 5-HT_{2A} antagonists amperozide and FG 5974. Alcohol Clin Exp Res 1997;21: 1448–54.

Pälvimäki EP, Majasuo H, Kuoppamäki M, Mannistö PT, Syvälahti E, Hietala J. Deramciclane, a putative anxiolytic drug, is a serotonin 5-HT_{2C} receptor inverse agonist but fails to induce 5-HT_{2C} receptor down-regulation. Psychopharmacology (Berl.) 1998;136:99–104.

Panocka I, Ciccocioppo R, Pompei P, Massi M. 5-HT_2 receptor antagonists do not reduce ethanol preference in Sardinian alcohol-preferring (sP) rats. Pharmacol Biochem Behav 1993;46:853–6.

Rogers DC, Fisher EM, Brown SD, Peters J, Hunter AJ, Martin JE. Behavioral and functional analysis of mouse phenotype: SHIRPA, a proposed protocol for comprehensive phenotype assessment. Mamm Genome 1997;8:711–3.

Schreiber R, Melon C, De Vry J. The role of 5-HT receptor subtypes in the anxiolytic effects of selective serotonin reuptake inhibitors in the rat ultrasonic vocalization test. Psychopharmacology (Berl.) 1998;135: 383–91.

Shader RI, Greenblatt DJ. Use of benzodiazepines in anxiety disorders. N Engl J Med 1993;328:1398–405.

Sinclair JD, Hyytiä P, Nurmi M. The limited access paradigm: description of one method. Alcohol 1992;9:441–4.

Söderpalm AH, Hansen S. Benzodiazepines enhance the consumption and palatability of alcohol in the rat. Psychopharmacology (Berl.) 1998;137:215–22.

Söderpalm B, Svensson L, Hulthe P, Johannessen K, Engel JA. Evidence for a role for dopamine in the diazepam locomotor stimulating effect. Psychopharmacology (Berl.) 1991;104:97–102.

Vaswani M, Linda FK, Ramesh S. Role of selective serotonin reuptake inhibitors in psychiatric disorders: a comprehensive review. Prog Neuro-Psychopharmacol Biol Psychiatry 2003;27:85–102.

Wegelius K, Honkanen A, Korpi ER. Benzodiazepine receptor ligands modulate ethanol drinking in alcohol-preferring rats. Eur J Pharmacol 1994;263:141–7.

Wilcox RE, McMillen BA. The rational use of drugs as therapeutic agents for the treatment of the alcoholisms. Alcohol 1998;15:161–77.

Wood MD, Reavill C, Trail B, Wilson A, Stean T, Kennett GA, et al. SB-243213; a selective 5-HT_{2C} receptor inverse agonist with improved anxiolytic profile: lack of tolerance and withdrawal anxiety. Neuropharmacology 2001;41:186–99.

Zarrindast MR, Dibayan M. Involvement of $GABA_A$ receptor sites in diazepam hypothermia. Gen Pharmacol 1989;20:855–9.

Zbinden G, Randall LO. Pharmacology of benzodiazepines: laboratory and clinical correlations. Adv Pharmacol 1967;5:213–91.