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Effects of DM-9384, a Pyrrolidone Derivative, on Alcohol- and Chlordiazepoxide-Induced Amnesia in Mice

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NABESHIMA, T., K. TOHYAMA AND T. KAMEYAMA. *Effects of DM-9384, a pyrrolidone derivative, on alcohol- and chlordiazepoxide-induced amnesia in mice.* PHARMACOL BIOCHEM BEHAV **36**(2) 233–236, 1990.—The effects of N-(2,6-dimethyl-phenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide (DM-9384), a new pyrrolidone derivative, were investigated on ethanol- and chlordiazepoxide (CDP)-induced amnesia animal model using the passive avoidance task in comparison with aniracetam, another pyrrolidone derivative. Pretraining administration of DM-9384 attenuated ethanol- and CDP-induced amnesia, whereas aniracetam failed to do so. The effects of DM-9384 on CDP-induced amnesia were antagonized by bicuculline, a $GABA_A$ receptor antagonist, but not by scopolamine, a muscarinic acetylcholine receptor antagonist and flumazenil, a benzodiazepine receptor antagonist. These results suggest that DM-9384 attenuates CDP-induced amnesia by interacting with the GABAergic neuronal system.

N-(2,6-dimethyl-phenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide (DM-9384) Ethanol Chlordiazepoxide Amnesia GABA Benzodiazepines $GABA_A$ receptors Bicuculline

ALCOHOL is the most widely abused drug in the world. Acute and chronic alcohol consumption is widely assumed to disrupt the memory process in both humans and laboratory animals. Impairment of retention is observed in mice and rats when the drug is given before a training session (1). Memory impairment has also been reported after prolonged ethanol consumption in mice between training and retention test sessions (10). In addition, light alcohol intoxication produces impairment of storage of the short-term memory process in humans (17). Ethanol has been reported to interact with the GABA-benzodiazepine receptor-chloride ionophore 'supramolecular complex': It increases the apparent affinity for benzodiazepines of both membrane and solubilized preparations (5, 8, 29). In addition, ethanol-induced amnesia is antagonized by the benzodiazepine inverse agonist, Ro 15-4513 (23). A number of reports have emerged documenting anterograde amnesia following intravenous administration of diazepam as a premedicant in anaesthetic practice soon after its introduction in 1963 (4,6). Subsequent work showed that the amnesia could be produced by other benzodiazepines as well (16). While amnesia for events occurring prior to surgery may be considered a beneficial side-effect, the same effect in an outpatient population taking benzodiazepines is highly undesirable (16). In addition, there is an animal report concerning the effects of benzodiazepines on the memory process (28).

We have reported that N-(2,6-dimethyl-phenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide (DM-9384), a cyclic derivative of GABA, attenuates GABA antagonist-induced amnesia (18,22) by interacting with a portion of $GABA_A$ receptors directly and/or indirectly (22). It also improves cycloheximide-, scopolamine- and hemicholinium-3-induced amnesia (21,26) in the passive avoidance task of both mice and rats. Furthermore, repeated administration of DM-9384 ameliorates basal forebrain lesion-induced amnesia in multiple T-maze and passive avoidance tasks (25) and increases activity of choline acetyltransferase (15). Furthermore, aniracetam, another cyclic derivative of GABA, improves cycloheximide-, scopolamine- and electroconvulsive shock-induced amnesia in the passive avoidance task (7,27). This drug attenuates acetylcholine (ACh) decrease induced by scopolamine in the hippocampus (27). In the present study, we attempted to investigate 1) whether DM-9384 attenuates ethanol- and chlordiazepoxide (CDP)-induced amnesia in comparison with aniracetam and 2) whether its attenuating effects on the CDP-induced amnesia are antagonized by benzodiazepine, GABA and ACh antagonists.

METHOD

Subject and Drugs

Male mice of the ddY strain (Niphon SLC, Shizuoka), weighing 30–35 g, were used. The animals were kept in a regulated

environment (23 ± 1°C, 50 ± 5% humidity), with a 12-hr light/12-hr dark cycle (8 a.m. and 8 p.m.) and were given food and water ad lib. DM-9384 [N-(2,6-dimethyl-phenyl)-2-(2-oxo-1-pyrrolidinyl) acetamide] (Daiichi Pharmaceutical Co. Ltd., Tokyo) and aniracetam (synthesized and supplied by Daiichi Pharmaceutical Co. Ltd., Tokyo) were used. DM-9384 was dissolved in saline, and aniracetam was suspended in distilled water which contained a few drops of Tween 80. Both drugs were administered PO 15 min before training. Chlordiazepoxide (CDP; Hoffmann-La Roche, Basel) was dissolved in 0.9% saline and administered subcutaneously (SC) 20 min before training. Ethanol (Katayama Chemical, Tokyo) was diluted with 0.9% saline and administered PO 10 min before training. Bicuculline (Sigma, St. Louis, MO) was dissolved in 0.1 N HCl and neutralized by 0.1 N NaOH. Scopolamine hydrobromide (Katayama Chemical, Tokyo) was dissolved in 0.9% saline. The specific benzodiazepine antagonist, flumazenil [ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-(1,5a)(1,4)-benzodiazepine-3-carboxylate, Ro 15-1788; Hoffmann-La Roche] was suspended in distilled water with a few drops of Tween 80. Bicuculline, scopolamine and flumazenil were administered IP immediately after training. A dose of antagonist, that completely reversed GABAergic, AChergic and benzodiazepine neuronal functions, but failed to affect retention, was employed according to previous experiments. There is noneffect of drugs used on shock sensitivity at the doses used.

Apparatus

The passive avoidance apparatus consisted of a Plexiglas rectangular inner box (30 × 30 × 40 cm high) with a grid floor and a semi sound-proof wooden outer box (35 × 35 × 90 cm high) with a 15 W illumination lamp on it. The grid floor consisted of 30 parallel steel rods (0.3 cm in a diameter) set 1.0 cm apart. In the center of the grid floor, a wooden platform (4 × 4 × 4 cm) was set (14). Intermittent electric shocks (ES; 1 Hz, 0.5 sec, 60 V DC) were delivered to the grid floor by an isolated stimulator (Nihon Koden, Tokyo). Inasmuch as the resistance, when an animal was placed in a test cage, varied between 100 and 250 KΩ, the mouse received an electric footshock in the range of 0.24 to 0.6 mA.

Passive Avoidance Procedures

Training. Each mouse was placed gently on the wooden platform set in the center of the grid floor. When the mouse stepped down from the platform and placed all its paws on the grid floor, intermittent electric shocks were delivered. The step-down latency was measured.

Retention test. Twenty-four hour posttraining, each mouse was placed on the platform again, and the step-down latency was measured up to maximum cut-off time of 5 min.

Statistical Analysis

Data were expressed in terms of medians and interquartile ranges and analyzed using a Kruskal-Wallis nonparametric one-way analysis of variance; further statistical analyses for individual groups were done with a two-tailed Mann-Whitney U-test. The criterion for statistical significance was $p<0.05$ in all statistical evaluations.

RESULTS

Effects of DM-9384 and Aniracetam on Ethanol-Induced Amnesia

As shown in Fig. 1, vehicle-treated animals displayed prolonged step-down latency during the retention test. Ethanol (2 g/kg, PO) significantly shortened the step-down latency when administered 10 min before training. DM-9384 attenuated ethanol-induced amnesia, $H(3) = 17.55$, $p<0.01$, although DM-9384 itself failed to change the step-down latency (data not shown). DM-9384 (5 and 15 mg/kg, PO) significantly prolonged the shortened step-down latency induced by ethanol. The dose-response curve of DM-9384 was bell-shaped. Another pyrrolidone derivative, aniracetam (5 and 15 mg/kg, PO), failed to prolong the shortened step-down latency, $H(2) = 4.75$, $p>0.05$.

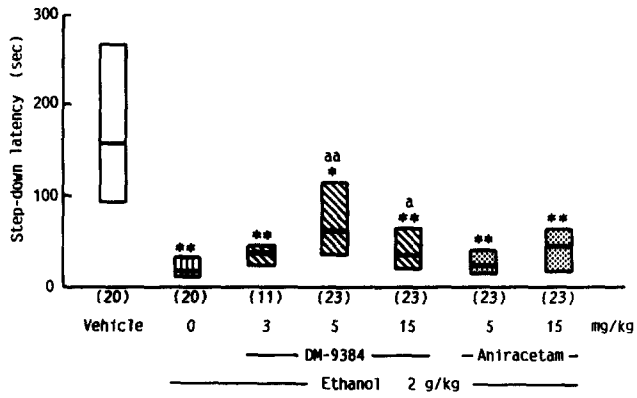

FIG. 1. Effect of DM-9384 and aniracetam on the ethanol-induced impairment of passive avoidance response in mice. Mice were given ethanol (2 g/kg), DM-9384 (3, 5 and 15 mg/kg) and aniracetam (5 and 15 mg/kg) 10, 15 and 15 min before training, respectively. The retention test was performed 24 hr after training. Each value represents the median and interquartile ranges. The figure in parentheses shows the number of animals. $*p<0.05$, $**p<0.01$ vs. vehicle-treated group. $^{a}p<0.05$, $^{aa}p<0.01$ vs. ethanol-treated group.

Effects of DM-9384 and Aniracetam on CDP-Induced Amnesia

As shown in Fig. 2, CDP (10 mg/kg, SC) significantly

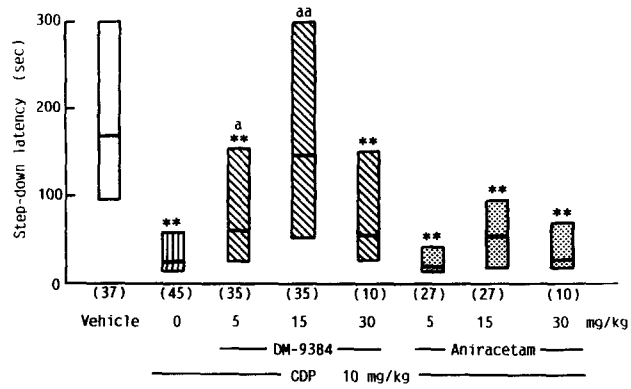

FIG. 2. Effect of DM-9384 and aniracetam on the CDP-induced impairment of passive avoidance response in mice. Mice were given CDP (10 mg/kg), DM-9384 (5, 15 and 30 mg/kg) and aniracetam (5, 15 and 30 mg/kg) 20, 15 and 15 min before training, respectively. The retention test was performed 24 hr after training. Each value represents the median and interquartile ranges. The figure in parentheses shows the number of animals. $**p<0.01$, vs. vehicle-treated group. $^{a}p<0.05$, $^{aa}p<0.01$ vs. CDP-treated group.

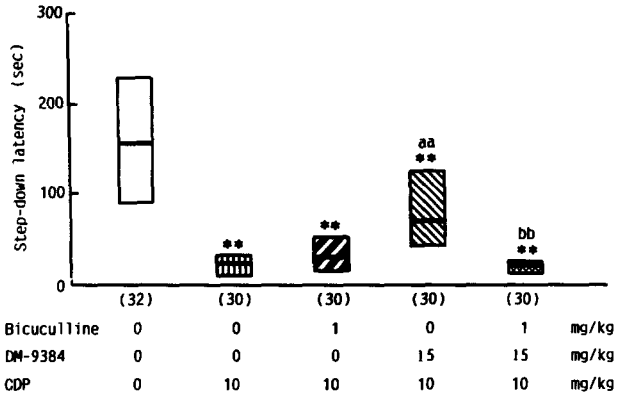

FIG. 3. Effects of bicuculline on the antiamnesic effects of DM-9384 on the CDP-induced amnesia. Mice were given CDP (10 mg/kg), DM-9384 (15 mg/kg) and bicuculline (1 mg/kg) 20 and 15 min before and immediately after training, respectively. The retention test was performed 24 hr after training. Each value represents the median and interquartile ranges. The figure in parentheses shows the number of animals. $^{*}p<0.05$, $^{**}p<0.01$ vs. vehicle-treated group. $^{aa}p<0.01$ vs. CDP-treated group. $^{bb}p<0.01$ vs. (CDP + DM-9384)-treated group.

impaired the passive avoidance response, as there was significant reduction in the step-down latency, when it was given 20 min before training. DM-9384, $H(3)=20.04$, $p<0.01$, but not aniracetam, $H(3)=4.43$, $p>0.05$, attenuated CDP-induced amnesia. DM-9384 (5 and 15 mg/kg, PO) significantly prolonged the shortened step-down latency induced by CDP.

Effects of GABA, ACh and Benzodiazepine Antagonists on the Antiamnesic Effects of DM-9384

We attempted to investigate whether the antiamnesic effects of DM-9384 are mediated via GABA-benzodiazepine receptors and/or muscarinic ACh receptors. As shown in Fig. 3, DM-9384 (15 mg/kg) attenuated the CDP (10 mg/kg)-induced amnesia. This effect was antagonized by bicuculline (1 mg/kg, IP), although bicuculline itself did not change the step-down latency in vehicle- or CDP-treated animals. On the contrary, scopolamine (1 mg/kg) and flumazenil (10 mg/kg) failed to antagonize the antiamnesic effects of DM-9384 (Table 1).

DISCUSSION

As described in the Introduction, alcohol and benzodiazepines produce amnesia in humans and animals. Present results confirmed those previously reported by numerous authors: Ethanol and CDP produced amnesia in the passive avoidance task. It has been reported that ethanol and CDP may interact indirectly and directly, respectively, with a benzodiazepine-GABA-ionophore complex to produce the amnesia (see Introduction). DM-9384, a new pyrrolidone derivative, attenuates GABA antagonist-induced amnesia (18,22) presumably by interacting with a portion of $GABA_A$ receptors directly and/or indirectly, since it displaces [^{3}H]muscimol binding to $GABA_A$ receptors and its antiamnesic effects are antagonized by bicuculline, a $GABA_A$ receptor antagonist (22). Therefore, we investigated DM-9384's effects on ethanol- and CDP-induced amnesia.

With regard to the involvement of GABA in memory there are various reports. The activation of GABA neurons was reported by some to have improved consolidation of memory (12, 13, 19, 20). Picrotoxin, a blocker of GABA neurotransmission, facilitated the acquisition of maze learning and other learning tasks (2, 9, 11). Thiebot (28) showed that muscimol, administered before training, induced an amnesic-like effect in one-trial avoidance of water drinking or lever-pressing for food, as benzodiazepines do, whereas the GABA-mimetic drug progabide had no effect (3). Thus, it is unclear whether the benzodiazepine-induced impairment of memory is related to the GABAergic neuronal systems.

DM-9384 attenuated both ethanol- and CDP-induced amnesia

TABLE 1

EFFECTS OF FLUMAZENIL AND SCOPOLAMINE ON DM-9384-INDUCED RECOVERY FROM CDP-INDUCED IMPAIRMENT OF PASSIVE AVOIDANCE RESPONSE IN MICE

Drugs	Step-Down Latency (sec, median and interquartile range)	N
Experiment 1		
Vehicle + vehicle + vehicle	299.0 (101.5–300.0)	17
CDP + vehicle + vehicle	28.0 (11.5–42.5)†	17
CDP + vehicle + scopolamine	37.0 (15.5–64.5)†	20
CDP + DM-9384 + vehicle	86.0 (46.5–182.0)*§	20
CDP + DM-9384 + scopolamine	89.6 (24.0–96.0)†‡	20
Experiment 2		
Vehicle + vehicle + vehicle	204.0 (107.0–271.0)	20
CDP + vehicle + vehicle	25.0 (14.0–46.0)†	20
CDP + vehicle + flumazenil	36.0 (23.0–75.0)†	20
CDP + DM-9384 + vehicle	125.0 (65.0–223.5)§	20
CDP + DM-9384 + flumazenil	89.0 (56.5–103.5)†§	21

CDP (10 mg/kg), DM-9384 (15 mg/kg) and scopolamine (1 mg/kg) or flumazenil (10 mg/kg) were administered 20 and 15 min before and immediately after training, respectively.

*$p<0.05$, †$p<0.01$ vs. vehicle-treated group, ‡$p<0.05$, §$p<0.01$ vs. CDP-treated group.

in mice, but aniracetam, another pyrrolidone derivative which does not interact with $GABA_A$ receptors (22), failed to do so. To investigate the mechanism of action of DM-9384 in CDP-induced amnesia, antagonists of GABA, ACh and benzodiazepine receptors were administered in combination with DM-9384, since DM-9384 attenuates GABA and ACh antagonist-induced amnesia (18,21) and CDP-induced amnesia is mediated via benzodiazepine receptors (24). The effects of DM-9384 on CDP-induced amnesia were antagonized by bicuculline, a $GABA_A$ receptor antagonist, but not by scopolamine, a muscarinic ACh receptor antagonist or flumazenil, a benzodiazepine receptor antagonist. These results suggest that DM-9384 attenuates CDP-induced amnesia by interacting with $GABA_A$ receptors, but not with muscarinic ACh and benzodiazepine receptors. As described above, aniracetam does not interact with $GABA_A$ receptors, therefore, this drug could not attenuate the CDP-induced amnesia. Posttraining administration of muscimol has been reported to improve the impairment of passive avoidance response induced by cycloheximide, scopolamine, and GABA antagonists (19,20). n-Dipropylacetate, a GABA-synthesis stimulator, also produces an improvement in consolidation (12). Furthermore, GABA antagonists, picrotoxin and bicuculline, and a GABA-synthesis inhibitor, 3-mercaptopropionic acid, impair the passive avoidance response when they are administered immediately posttraining (19). These findings and present results suggest that GABA improves memory consolidation and that DM-9384 interacts with a portion of GABA receptors directly and/or indirectly.

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