Selective Suppression of Schedule-Induced Ethanol Drinking by Antialcoholic Drugs in Rats

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Abstract—Effects of disulfiram and calcium cyanamide, antialcoholic drugs, on schedule-induced ethanol drinking as well as on schedule-controlled response (lever-pressing) under a fixed interval 1 min schedule of food reinforcement were investigated in Wistar strain rats. When ethanol solution was available, the schedule-induced ethanol drinking decreased depending on the ethanol concentration (2–8%). However, the dose of ethanol intake during the 1 hr experimental session was at maximum (2.8 g/kg) when 4% ethanol solution was available. Thereafter, 4% ethanol solution was used in the experiment for studying the effects of disulfiram and calcium cyanamide on the schedule-induced ethanol drinking. Disulfiram (100–200 mg/kg, p.o.), pretreated at 1 hr before the start of the experiment, tended to suppress schedule-induced water drinking. However, the same treatment of calcium cyanamide (5–10 mg/kg, p.o.) did not produce a marked change in it. In contrast, disulfiram (100 and 200 mg/kg) and calcium cyanamide (5 and 10 mg/kg) markedly suppressed schedule-induced ethanol drinking without eliciting a marked change in schedule-controlled response. The present results suggest that both disulfiram and calcium cyanamide selectively suppress ethanol drinking in rats.

Disulfiram and calcium cyanamide have been commonly used as antialcoholic drugs. It has been well known that these drugs inhibit aldehyde dehydrogenase and accumulate acetaldehyde in the body after taking alcoholic beverages, producing aversive effects such as warmth, flush, throbbing headache, nausea, vomiting, drowsiness and hangover in humans, i.e., the disulfiram-ethanol or cyanamide-ethanol reaction (1).

In preclinical and clinical evaluation of the combined effects of disulfiram or calcium cyanamide with ethanol, blood pressure, heart rate and respiration rate as well as ethanol and acetaldehyde levels in the blood and/or the brain have been used as the indicators (2–13). However, there are relatively few reports (14–16) which have examined effects of these drugs on ethanol-drinking in rodents. In these experiments, ethanol preferring animals were selectively used for the experimental subjects.

Previously, Falk (17) reported that rats voluntarily drank a relatively large amount of water under an intermittent food delivery schedule. This behavior has been called "schedule-induced drinking" or "adjunctive drinking". Thereafter, many researchers (18–25) applied the schedule-induced drinking to induce voluntary drinking of ethanol solution in rats and to make rats physically dependent on ethanol. However, there have been no reports on the effect of antialcoholic drugs on schedule-induced ethanol drinking. In this experiment, we investigated changes in schedule-induced ethanol drinking developed under a fixed interval 1 min (FI 1) schedule of food reinforcement after administration of disulfiram and calcium.
cyanamide. In addition, the effects of these drugs on the schedule-controlled response (lever-pressing for food reinforcement) were also examined.

Materials and Methods

Animals: The experimental animals used were 10 adult male rats of the Wistar strain which had been provided by the Institute of Experimental Animal Research, Gunma University School of Medicine. They were housed in groups of 3–4 in stainless steel wire mesh cages of 45 (D) × 25 (W) × 20 (H) cm, and they were allowed to freely take solid diet (MF: Oriental Yeast Co., Tokyo) and tap water until the start of the experiment. The breeding room was artificially illuminated by fluorescent lamps on a 12 hr light-dark schedule (lights on at 6:00 and off at 18:00), and the room temperature was regulated to 23±2°C. The rats were employed for the experiments when they were 10 weeks of age and weighed 300–350 g. Prior to the start of conditioning in the FI 1 min food reinforcement situation, they were reduced to 80–85% of their free feeding body weight by food deprivation. Daily food intake was limited to 10–15 g/rat/day throughout the experimental period. However, water was still freely available.

Schedule: Each food-deprived rat was trained to press a lever in an operant chamber of 20 (D) × 25 (W) × 20 (H) cm for food pellets of 90 mg each as the reinforcement. The spherical food pellet was specially made and was hard enough to prevent pulverization at delivery. On the 1st session of the conditioning, each lever-pressing was reinforced (continuous reinforcement schedule: CRF). On the 2nd session and thereafter, the food reinforcement schedule was changed from CRF to FI 1 min. Thus, every lever-pressing which was emitted 1 min after the previous food delivery was reinforced. The other lever-pressings did not affect the food reinforcement schedule, but were recorded. During the conditioning, water was freely available in the operant chamber through a drinking spout (SE TV-15: O’hara & Co. Ltd., Tokyo), and amount of water intake was measured by a drinkometer (LA-1: O’hara & Co. Ltd.). The principle of the device and the method for measurement were previously reported by us (26). Experimental sessions consisted of 1 hr per day and were held every day except Sunday. After 15 sessions of conditioning using the routine procedure (27, 28), drug testing was started. The experiment was carried out between 9:00–17:00.

Drugs: The drugs used were ethanol (Kanto Chemical Co., Tokyo), disulfiram (Antabuse “D” Tablet; Tokyo Tanabe Pharm. Co., Tokyo) and calcium cyanamide (Cyanamide Solution; Yoshitomi Pharm. Co., Osaka). Ethanol was diluted by tap water and was voluntarily drunk by the rats during the experimental sessions. Hereafter, the sessions in which water and ethanol solution were available are defined as the water session and the ethanol session, respectively. Disulfiram was suspended in a 0.5% carboxymethyl cellulose Na suspension, and calcium cyanamide was diluted by distilled water. They were orally given 1 hr before the start of the experiment. Each volume administered was fixed at 1 ml/kg body weight. Ethanol sessions were held once per 2 weeks, and disulfiram or calcium cyanamide was administered once per week. Therefore, effects of these drugs on rats’ behaviors in water and ethanol sessions were monitored every other week. Carboxymethyl cellulose Na suspension or distilled water was given as the control administration.

Statistical analysis: The data obtained were statistically analyzed by Student’s t-test. When P values were equal to or less than 0.05, there was considered to be a significant difference.

Results

After 15 sessions of conditioning, all the rats exhibited a stable schedule-controlled response which was characterized with an accelerating pattern of the response rate during the FI. Eight out of 10 rats exhibited typical schedule-induced water drinking and drank 15–50 ml during a 1 hr session. However, the other 2 rats failed to drink more than 10 ml of water. These rats were excluded from further drug testing.

Figure 1 shows changes in the schedule-controlled response and schedule-induced
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ethanol drinking by means of the overall response rate and the amount of fluid intake, respectively, during 2, 4, 6 and 8% ethanol sessions. Doses of ethanol intake are also shown in this figure. In the 8% ethanol session, the overall response rate increased significantly as compared with that in the water session. The decrease in the amount

Fig. 1. Changes in schedule-controlled response and schedule-induced drinking, expressed in terms of response rate (left panel) and amount of fluid intake (middle panel), respectively, under a fixed interval 1 min schedule of food reinforcement in rats during the 2, 4, 6 and 8% ethanol session. Doses of ethanol intake (right panel) are also shown in this figure. The rat's behaviors were observed for 1 hr. *P<0.05, **P<0.01: Significant difference as compared with the value in the water session (Student's t-test).

Fig. 2. Effects of disulfiram (100 and 200 mg/kg, p.o.) on schedule-controlled response (left panel) and schedule-induced water or ethanol drinking (right panel) under a fixed interval 1 min schedule of food reinforcement in rats. Disulfiram was pretreated at 1 hr before start of the experiment, and the rat's behaviors were observed for 1 hr. Open and hatched columns indicate the results in the water session and the 4% ethanol session, respectively. *P<0.05: Significant difference as compared with the value after administration of carboxymethyl cellulose Na solution (dose=0) in the water session. **P<0.01: Significant difference as compared with the value after administration of carboxymethyl cellulose Na suspension (dose=0) in the 4% ethanol session.
of fluid intake was dependent on the ethanol concentration. However, the dose of ethanol intake was at maximum (2.8 g/kg) in the 4% ethanol session. Therefore, this ethanol concentration was used in the experiment for studying effects of disulfiram and calcium cyanamide on schedule-induced ethanol drinking.

Figure 2 shows the effects of disulfiram (100 and 200 mg/kg, p.o.), pretreated at 1 hr before the start of the experiment, on the schedule-controlled response and schedule-induced water and ethanol drinking by means of the overall response rate and the amount of fluid intake, respectively, during the 1 hr session. In the water sessions, disulfiram tended to suppress the schedule-controlled response, but the change was not significantly different as compared with the value in the water session after administration of carboxymethyl cellulose Na. Disulfiram (200 mg/kg) significantly decreased the amount of water intake. In contrast, disulfiram (100 and 200 mg/kg) did not induce a marked change in the schedule-controlled response in the ethanol sessions, but produced a significant decrease in the amount of ethanol intake. The change was more marked than that observed in the water sessions.

Figure 3 shows the effects of calcium cyanamide (5 and 10 mg/kg, p.o.), pretreated at 1 hr before the start of the experiment, on the schedule-controlled response and schedule-induced water and ethanol drinking. The data are presented in the same way as in Fig. 2. In the water sessions, calcium cyanamide did not produce a marked change in either the schedule-controlled response or schedule-induced water drinking. However, calcium cyanamide significantly suppressed schedule-induced ethanol drinking in a dose-dependent manner without eliciting a marked change in the schedule-controlled response in the ethanol sessions.

Discussion

The present experiment demonstrated that under the Fl 1 min schedule of food reinforcement, rats voluntarily drank ethanol solutions at concentrations of more than 4%, which rats generally refuse to drink under normal breeding conditions (29, 30). The dose of ethanol intake during the 1 hr sessions attained a maximum (2.8 g/kg) in the 4% ethanol session. These results are almost
identical with those reported by many researchers (18–25).

The schedule-controlled response increased in the 8% ethanol sessions. However, this behavioral change is unlikely to be attributable to a pharmacological effect of ethanol. This is because although the dose of ethanol intake was higher in the 4 and 6% ethanol sessions than in the 8% ethanol sessions, no marked change in the schedule-controlled response was found in these sessions. We have observed that 2–3 g/kg p.o. of ethanol, given to rats immediately before the start of the session, scarcely induced changes in either the schedule-controlled response or schedule-induced water drinking (H. Kuribara et al., unpublished data). In addition, Cook and Singer (31) reported a marked increase in the response rate after artificial suppression of the schedule-induced water drinking by taking off the drinking spout. These results suggest that the marked increase in the schedule-controlled response observed in the 8% ethanol session is attributable to suppression of the schedule-induced ethanol drinking due to nonspecific aversive effects of ethanol such as taste, etc.

Disulfiram (200 mg/kg) tended to suppress both the schedule-controlled response and schedule-induced water drinking. These results are probably due to a sedative effect of this drug (1). In contrast, no marked change in either the schedule-controlled response or schedule-induced water drinking was found after administration of calcium cyanamide in the water sessions. It has been reported that calcium cyanamide alone at the doses used clinically scarcely produces an adverse reaction in humans (1). The results observed after administration of disulfiram and calcium cyanamide in the water sessions are fairly consistent with those reported in humans.

Both disulfiram and calcium cyanamide markedly suppressed schedule-induced ethanol drinking without eliciting a marked change in the schedule-controlled response. This result suggests that these drugs strongly affect ethanol drinking behavior. The effect of calcium cyanamide on schedule-induced ethanol drinking was about 20 times as strong as that of disulfiram. Clinically, the daily doses of disulfiram and calcium cyanamide for treatment of alcoholic patients are reported to be 250–500 mg and 50–100 mg, respectively (1). The results obtained from our experiment are fairly consistent with the clinical data. However, it can not be explained only by the present experiment why no marked change in the schedule-controlled response was produced even though marked suppression of schedule-induced ethanol drinking was induced after administration of disulfiram and calcium cyanamide. A further study is required to elucidate the effect of these drugs on the schedule-controlled response in ethanol sessions.

For studying the combined effect of disulfiram or calcium cyanamide with ethanol, physical signs or acetaldehyde levels in the blood and/or the brain have been usually measured as the indicators (2–13). We did not examine them in the present experiment. However, it can be concluded that schedule-induced ethanol drinking in rats is applicable to behavioral studies on the interaction between ethanol and antialcoholic drugs. This is not only because rats voluntarily drink relatively large dose of ethanol within a short period under the experimental situation, but also because the ethanol drinking can be used as the indicator of the effect of antialcoholic drugs.

References


