Caffeine Does Not Effectively Ameliorate, but Rather May Worsen the Ethanol Intoxication When Assessed by Discrete Avoidance in Mice

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ABSTRACT—Ethanol disrupted the discrete lever-press and shuttle avoidances in mice at doses over 1.6 and 2.4 g/kg, p.o., respectively, eliciting a dose-dependent decrease in the % of avoidance with no significant change or slight increase in the response rate. Caffeine increased the response rate of both the avoidances at the doses of 1-30 mg/kg, p.o., but disrupted the avoidance at 100 mg/kg. Caffeine (10 mg/kg) reduced the decreased % of avoidance by ethanol (1.6 and 2.4 g/kg) with a significant increase in the response rate. In contrast, the % of avoidance was significantly lower after the combined administration of ethanol (3.2 g/kg) with caffeine than after ethanol (3.2 g/kg) alone. Unlike ethanol, diazepam (2 mg/kg, s.c.) and pentobarbital (10 mg/kg, s.c.) significantly decreased both the response rate and the % of avoidance. Caffeine (10 mg/kg) ameliorated the decreased response rate and the % of avoidance produced by diazepam and pentobarbital. The present results suggest that caffeine does not effectively ameliorate, but rather may worsen the ethanol intoxication.

Keywords: Caffeine, Ethanol, Discrete avoidance (mouse), Behavior disruption, Intoxication

Ethanol is classified as a general depressant, and its behavioral pharmacological properties are similar to those of sedative hypnotics and benzodiazepine anxiolytics (1). It has been reported that the adenosine system is involved in the CNS depressant action of ethanol (2, 3). In contrast, it is well-known that the CNS stimulant action of methylxanthines is mainly produced through adenosine antagonism (4-9). Dar et al. (10) demonstrated that ethanol-induced sleep and motor incoordination were attenuated by theophylline, a methylxanthine with the antagonistic action at adenosine receptors. Although many people frequently drink coffee for amelioration of ethanol intoxication, there have been few systematic studies in which the interactions of ethanol with caffeine, the main CNS active compound of the methylxanthine derivative in coffee, have been evaluated (11). Thus, it is important to study whether ethanol intoxication can be effectively ameliorated by caffeine.

The purposes of this study were to evaluate the combined effect of ethanol with caffeine on discrete lever-press and shuttle avoidance in mice. In additional experiments, the combined effects of caffeine with diazepam and pentobarbital, prototypic drugs of sedative hypnotic type and benzodiazepine anxiolytic type, respectively, were also investigated to demonstrate the characteristics of the interaction of ethanol with caffeine.

MATERIALS AND METHODS

Animals
Male mice of the ddY strain (Japan Laboratory Animals) were purchased at 6 weeks of age, and they had been kept in a controlled room (light period: 06:00-18:00, temperature: 23 ± 1°C, and relative humidity: 50 ± 3%) with a free access to food (MF, Oriental Yeast) and water. The mice were used in the experiment at the age of 7 weeks and weighed 27-30 g.

Apparatus and procedure
The apparatus used in the avoidance tests were the experimental chambers for lever-press avoidance (GT-8310, O'Hara & Co.) and shuttle avoidance (GT-8450,
in mice and avoidance-controlling and data-recording units (De CARES GT-M5 and TIDP-10, respectively; O'Hara & Co.). The temporal parameters of the discrete avoidance schedule were an intertrial interval of 25 sec and a warning duration of 5 sec. An electric foot shock of 100 V, 0.3 mA, 50 Hz AC was presented to the mouse through the floor grid of the chamber as the unconditioned stimulus for 0.3 sec when it failed to respond during the presence of the warning signal. The indices of the avoidance response were the response rate (frequency of lever-presses or shuttles) and the % of avoidance (number of avoidance responses/number of avoidance trials).

The discrete avoidance task is appropriate for evaluation of behavioral excitation and depression, as well as behavioral disruption such as disability to correctly respond to an external signal, of the animals through the analysis of the response rate and the % of avoidance. This is because the mice were required to press the lever during the warning period of 5 sec in each trial and because the lever-presses in the intertrial interval reflected an ineffective response for shock avoidance.

**Drugs and administration schedules**

The drugs used were caffeine (Kanto Chem.), ethanol (Kanto Chem.), diazepam (Cercine Inj., Takeda Chem.) and pentobarbital Na (Nembutal Inj., Abbott Lab.). Caffeine was dissolved in distilled water and ethanol was diluted by distilled water and administered per orally (p.o.). The commercial preparations of diazepam and pentobarbital were diluted by 5% propylene glycol and administered subcutaneously (s.c.). The concentration of each drug solution was adjusted so that each volume administered was fixed at 0.1 ml/10 g body weight of the mouse. In the combined administration tests, the dose of caffeine was fixed to 10 mg/kg, because the dose might be optimum for facilitating the avoidance response without eliciting a marked change in the % of avoidance (see Results).

The drug administrations, both the single and combined, were conducted immediately before the start of the avoidance test, and thereafter the avoidance response of each mouse was observed for 1 hr in which 120 trials were held at intervals of 30 sec. The drug-testings were conducted at intervals of 3-4 days; and the days before, distilled water alone (p.o.) or distilled water (p.o.) + propylene glycol (s.c.) was administered as the control or baseline test. Two groups of 10 mice each were used. The effects of the single administration of ethanol and caffeine or of the combined administration of them were evaluated using the 1st group of mice. The interactions of diazepam and pentobarbital with caffeine were evaluated using the 2nd group of mice. The drug tests were carried out in the following order: In each drug test, the doses of drug were changed from the lower dose to the higher one in 5 mice, and the reverse order in the other 5 mice. All the experiments were held between 9:00-14:00.

**Statistical analysis**

The overall response rate and the % of avoidance during the 1-hr tests were first analyzed by ANOVA. In a case of significant variance, comparisons between individual data were conducted by the paired t-test. When P values were equal to or less than 0.05, the two values were considered to be significantly different.

**RESULTS**

**Lever-press avoidance**

As shown in Fig. 1, ethanol, at over 1.6 g/kg, significantly decreased the % of avoidance. However, the response rate was significantly increased by 3.2 g/kg of ethanol. Caffeine significantly increased the response rate at 3-30 mg/kg but not at 100 mg/kg, and it significantly decreased the % of avoidance at 100 mg/kg.
Figure 2 shows the dose-effect relationships for the combined administration of ethanol with caffeine (10 mg/kg) (hatched columns). The response rate after the combined administration of ethanol (0.8 and 1.6 g/kg) with caffeine, and the % of avoidance after ethanol (1.6 g/kg) with caffeine were significantly higher than those after the corresponding doses of ethanol alone (stippled columns). Moreover, the response rate after the combined administration of ethanol (2.4 g/kg) with caffeine was significantly higher than that after caffeine alone (meshed column). The combined administration of ethanol and caffeine tended, but not significantly, to produce recovery in the % of avoidance. In contrast, the % of avoidance after the combined administration of ethanol (3.2 g/kg) with caffeine was significantly lower than those after ethanol and caffeine alone.

Figure 3 shows the combined effects of diazepam (2 mg/kg) and pentobarbital (10 mg/kg) with caffeine (10 mg/kg). Diazepam and pentobarbital significantly decreased the % of avoidance and tended to decrease the response rate. Caffeine significantly ameliorated the decreased % of avoidance induced by pentobarbital. The response rate after the combined administration of pentobarbital with caffeine was significantly higher than that after pentobarbital alone, but the value was almost the same as that after caffeine alone. Caffeine did not change the diazepam-induced decrease in the % of avoidance.

Figure 2. Combined effects of ethanol (0.8, 1.6, 2.4 and 3.2 g/kg, p.o.) with caffeine (10 mg/kg, p.o.) on the discrete lever-press avoidance in mice. Two drugs were simultaneously administered immediately before the start of the avoidance test. *: Significantly different from the values after the administration of the same dose of ethanol alone (P < 0.05). #: Significantly different from the caffeine (CAF)-alone treated value (P < 0.05). N = 10.

Figure 3. Combined effects of diazepam (2 mg/kg, s.c.) or pentobarbital (10 mg/kg, s.c.) with caffeine (10 mg/kg, p.o.) on the discrete lever-press avoidance in mice. Two drugs were simultaneously administered immediately before the start of the avoidance test. Significantly different from: *, the value after the administration of diazepam or pentobarbital with distilled water (W: p.o.); #, from the value after caffeine (CAF) with propylene glycol (PG: s.c.) (P < 0.05); and $\ddagger$, from the value after water with propylene glycol (P < 0.05). N = 10.
Shuttle avoidance

As shown in Fig. 4, ethanol, at over 2.4 g/kg, significantly decreased the % of avoidance. The response rate was significantly increased by 3.2 g/kg of ethanol. Caffeine significantly increased the response rate at the dose range of 3 – 30 mg/kg, but significantly decreased both the response rate and the % of avoidance at 100 mg/kg.

Figure 5 shows the dose-effect relationships for the combined administration of ethanol with caffeine (10 mg/kg) (hatched columns). The response rates and the % of avoidances after the combined administration of ethanol (0.8 – 2.4 g/kg) with caffeine were significantly higher than those after the corresponding doses of ethanol alone (stippled columns). Moreover, the response rates after the combined administration of ethanol (1.6 and 2.4 g/kg) with caffeine were significantly higher than that after caffeine alone (meshed column). In contrast, the % of avoidance after the combined administration of ethanol (3.2 g/kg) with caffeine was significantly lower than that after ethanol alone.

Figure 6 shows the combined effects of diazepam (2 mg/kg) and pentobarbital (10 mg/kg) with caffeine (10 mg/kg). Diazepam significantly decreased the response
rate. Pentobarbital significantly decreased both the response rate and the % of avoidance. Caffeine ameliorated the decreased response rate and % of avoidance by diazepam and pentobarbital, and the values after the combined administrations were almost the same with those after the administration of caffeine with propylene glycol.

DISCUSSION

The single administration test in this study confirmed the CNS depressant and stimulant effects of ethanol and caffeine, respectively. Ethanol decreased the % of avoidance in a dose-dependent manner under both the avoidance situations, whereas, caffeine increased the response rate at comparatively lower doses. The decrease in the % of avoidance after the administration of higher doses of caffeine might reflect a behavioral disruption induced by the toxic action at the over-dose. It has been reported that the adenosine system is involved in the CNS depressant action of ethanol (2, 3) and the CNS stimulant action of caffeine (4–9). Because of these neurochemical reports, before conducting the combined administration of ethanol with caffeine, an antagonistic interaction of these drugs through the adenosine systems was expected.

In the present experiment, the combined administration of ethanol and caffeine showed that there was an intimate interaction between the two drugs. However, the results obtained in this study were much different from the expected ones. Although the decreased % of avoidance by ethanol (1.6 or 2.4 g/kg) was reduced by caffeine, the response rate was significantly higher than that after the single administration of either ethanol or caffeine. This result suggests that the enhancement of the activity yielded an increase in the response rate and non-specifically produced the recovery from the decreased % of avoidance. Such a consideration may be supported by another one of our experiments in which a prominent enhancement of the mouse’s ambulatory activity was produced by the combined administration of ethanol with caffeine (H. Kuribara, unpublished data). In these respects, it is proposed that the light ethanol intoxication is not effectively ameliorated by caffeine (i.e., coffee consumption), but may rather involve a behavioral hyper-excitation produced after the combined consumption of ethanol and caffeine.

Moreover, the highest dose of ethanol, 3.2 g/kg, markedly decreased the % of avoidance with an increase in the response rate. At this dose, the mice show a significant increase in the general activity with an ataxia, reflecting the disinhibitory action of ethanol. After the combined administration of ethanol (3.2 g/kg) with caffeine, the % of avoidance was significantly lower than that after the administration of ethanol alone. This result indicates that caffeine can not ameliorate the heavy ethanol intoxication, but rather it worsens the ethanol-induced behavioral disruption.

On the other hand, both diazepam and pentobarbital decreased the response rate and/or the % of avoidance. This result is consistent with our previous data (11). Such disruption of the avoidance response might reflect the sedative action of these drugs. Unlike the case of ethanol, the diazepam- and pentobarbital-induced decreases in the response rate and the % of avoidance were effectively ameliorated by caffeine. The ambulation-increasing effect of caffeine in mice was reduced by diazepam and pentobarbital (H. Kuribara, unpublished data). These results on diazepam and pentobarbital show characteristics different from those of ethanol by means of both the discrete avoidance and the ambulatory activity in mice, although these 3 drugs have similar behavioral (1) and psychopharmacological effects (12). Diazepam and pentobarbital act on the GABA/benzodiazepine/picrotoxin receptor complex. Ethanol is also considered to have action on the receptor complex (13, 14). It is also reported that some benz-
odiazepines affect the adenosine system (15). The antagonistic interaction of caffeine with diazepam and with pentobarbital is consistent with such neurochemical data. However, these systems are insufficient for elucidation of the interaction of ethanol with caffeine demonstrated in this study.

There are some reports that methylxanthines including caffeine enhance catecholamine release (16, 17) and catecholamine turnover (18), stimulate postsynaptic dopamine receptors (19–22), and increase the sensitivity of catecholamine receptors (23). We have some data which indicate a role of the opioid system in the interaction of ethanol and methylxanthines (H. Kuribara et al., unpublished data). It is therefore probable that dopaminergic, opioid and other systems are involved in the interaction of ethanol and caffeine.

Although further studies are required to elucidate the differential interactions of ethanol, diazepam and pentobarbital with caffeine, the present results clearly suggest that caffeine is inappropriate for amelioration of ethanol intoxication which has been commonly carried out by many people for the following two reasons: 1) The combination of a low dose of ethanol and caffeine may produce hyper-excitation, and 2) the ethanol intoxication induced by a high dose may be enhanced by caffeine.

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