Enhancement of the Ambulation-Increasing Effect of Opioid Analgesics by Ethanol in Mice

Hisashi Kuribara, Toshio Asashi and Sakutaro Tadokoro

Division for Behavior Analysis, Behavior Research Institute, Gunma University School of Medicine,
3-39-22 Showa-machi, Maebashi 371, Japan

Received February 16, 1991 Accepted May 7, 1991

ABSTRACT—The interaction between opioid analgesics (morphine and buprenorphine) and central depressants (ethanol, pentobarbital and diazepam) was investigated by means of ambulatory activity in mice. The ambulation-increasing effect of both morphine (10 mg/kg, s.c.) and buprenorphine (1 mg/kg, s.c.) was enhanced by the combined administration of ethanol (0.8 – 3.2 g/kg, p.o.) in a dose-dependent manner. Naloxone (0.1 mg/kg, s.c.) was effective for reducing the enhanced ambulatory activity. The pretreatment with Ca-cyanamide (5 mg/kg, p.o., 30 min before) reduced the enhancement of the ambulation-increasing effect induced by the combined administration of opioid analgesics with ethanol, although it scarcely modified that of morphine and buprenorphine alone. On the other hand, neither pentobarbital (1 – 30 mg/kg, s.c.) nor diazepam (0.25 – 2 mg/kg, s.c.) modified markedly the ambulation-increasing effect of morphine and buprenorphine. The present results suggest that ethanol specifically interacted with opioid analgesics when the mouse's ambulatory activity was used as the indicator.

Ethanol is categorized as a general depressant, and its pharmacological properties are partially similar to sedative hypnotics and benzodiazepine anxiolytics. However, quite different from other drugs, ethanol is almost freely available, and it is commonly consumed in our every day life. Therefore, it is highly probable that ethanol is abused in combination with various kinds of central-acting drugs. For example, combined abuse of ethanol with dependence-producing drugs including opioids has been frequently reported (1, 2). However, there is no systematic study in which the interaction between opioids and ethanol is behaviorally evaluated. In these respects, it is important to study whether ethanol modifies the pharmacological effects of opioids in an animal experiment.

Morphine is a prototypic opioid drug with a potential dependency (3). Hence, the purposes of this study were to evaluate the combined effect of morphine and ethanol by means of ambulatory activity in mice. In additional experiments, the interaction between buprenorphine (4), which possesses an agonistic-antagonistic action on mu- and kappa-receptors with a low dependency (5–8), and ethanol was also evaluated. Furthermore, effects of pentobarbital and diazepam, prototypic sedative hypnotic and benzodiazepine anxiolytic drugs, respectively, on the ambulation-increasing effect of morphine and buprenorphine were also investigated to assess whether ethanol specifically modified the effect of morphine and buprenorphine.
MATERIALS AND METHODS

Animals
Male mice of the dd strain were obtained from the Breeding Colony of Gunma University School of Medicine at 3 weeks of age and had been kept in a controlled room (light period: 6 a.m.–6 p.m., temperature: 23 ± 1°C, and relative humidity: 50 ± 3%) with a free access to food and water. The mice were used in the experiment at the age of 7 weeks and weighed 30–35 g.

Drugs
The drugs used were morphine HCl (Takeda Chem.), buprenorphine HCl (Lepetan Inj., Otsuka Pharm.), ethanol (Kanto Chem.), pentobarbital Na (Nembutal Inj., Abbott Lab.), diazepam (Cercine Inj., Takeda Chem.), naloxone HCl (Sigma Chem.) and Ca-cyanamide (Cyanamide Solution, Yoshitomi Pharm.). Ethanol and the commercial preparation of Ca-cyanamide were diluted by distilled water. Morphine and naloxone were dissolved in physiological saline. The commercial preparation of buprenorphine was diluted by physiological saline. The commercial preparations of pentobarbital and diazepam were diluted by 5% propylene glycol. Ethanol and Ca-cyanamide were administered orally (p.o.), and the other 4 drugs were 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.

Experimental procedures
The ambulatory activity of the mouse was measured by a tilting-type ambulometer (AMB-10; O'Hara & Co.). Ten mice were individually put into activity cages, and the ambulatory activity of each mouse was measured for 3 hr after the drug administration. In each drug test, 10–20 mice were used.

The doses of morphine and buprenorphine were fixed to 10 mg/kg and 1 mg/kg, respectively. These doses have been considered to be optimum for increasing the mouse's ambulatory activity (9).

In the 1st experiment, mice were given either morphine or buprenorphine in combination with ethanol (0: distilled water, 0.8, 1.6, 2.4 and 3.2 g/kg), pentobarbital (0: propylene glycol, 1, 3, 10 and 30 mg/kg), and diazepam (0: propylene glycol, 0.25, 0.5, 1 and 2 mg/kg). This combined administration was conducted simultaneously after an adaptation period of 30 min. The p.o. administration of ethanol and the s.c. administration of pentobarbital and diazepam with s.c. administration of saline (vehicle for morphine and buprenorphine) were also carried out.

In the 2nd experiment, the combined administration of 3 drugs: morphine or buprenorphine, ethanol (2.4 g/kg) and naloxone (0.1 and 0.3 mg/kg) was conducted. The doses of naloxone were taken according to our previous study as optimum doses to inhibit the ambulation-increasing effect of both morphine (10 mg/kg) and buprenorphine (1 mg/kg) (9). These 3 drugs were administered simultaneously after an adaptation period of 30 min.

In the 3rd experiment, modification by Ca-cyanamide (5 mg/kg) of the combined effects of morphine or buprenorphine with ethanol (0: distilled water, 0.8 and 1.6 g/kg) was observed. The dose of Ca-cyanamide was effective for causing the accumulation of acetaldehyde and to significantly suppress the ethanol consumption without eliciting a marked change in the food intake (10). Ca-cyanamide was given 30 min prior, i.e., immediately before the start of the adaptation period, to the combined administration of morphine or buprenorphine with ethanol.

Statistical analysis
The overall ambulatory activity counts for 3 hr were firstly analyzed using ANOVA. In a case of significant variance, Student's t-test and/or the Cochran-Cox test were applied to compare mean values. When P values were equal to or less than 0.05, they were defined to be significantly different.
RESULTS

Figures 1, 2 and 3 show the mean overall ambulatory activity counts for 3 hr after the combined administration of morphine (10 mg/kg) or buprenorphine (1 mg/kg) with ethanol (0.8–3.2 g/kg) (Fig. 1), pentobarbital (1–30 mg/kg) (Fig. 2), and diazepam (0.25–2 mg/kg) (Fig. 3), respectively. Although ethanol alone only slightly and temporarily increased the mouse's ambulatory activity immediately after the administration due to its disinhibitory action, the changes in the overall activity count did not reach a significant level. However, ethanol enhanced the morphine- and buprenorphine-induced increase in the ambulatory activity in a dose-dependent manner. A gross observation revealed that there was no marked change in the ataxia induced by comparatively higher doses of ethanol even after the combined administration. Pentobarbital and diazepam alone did not produce any significant change in the mouse's ambulatory activity. In the combined administration test, pentobarbital, at 3 and 30 mg/kg, significantly but only slightly enhanced the ambulation-increasing effect of morphine and buprenorphine, respectively. The other doses of pentobarbital (1 and 10 mg/kg) and diazepam (0.25–2 mg/kg) did not significantly enhance the ambulation-increasing effect of morphine and buprenorphine, but instead, the higher doses of diazepam reduced the effect of morphine and buprenorphine.

![Fig. 1](image-url)  
Fig. 1. Mean overall ambulatory activity counts for 3 hr after the combined administration of morphine (10 mg/kg, s.c.) or buprenorphine (1 mg/kg, s.c.) with ethanol (0: distilled water, 0.8, 1.6, 2.4 and 3.2 g/kg, p.o.) in mice. Two drugs were administered simultaneously. The solid band in the bottom of each column indicates the activity count after the administration of ethanol (p.o.) with saline (s.c.). *: P < 0.05 vs. each morphine- or buprenorphine alone-treated control value. N = 10–20 in each experiment.
Figure 4 shows the mean overall ambulatory activity counts after the combined administration of 3 drugs: morphine (10 mg/kg, s.c.) or buprenorphine (1 mg/kg, s.c.) with naloxone (0.1 and 0.3 mg/kg, s.c.), ethanol (2.4 g/kg) and naloxone (0.1 and 0.3 mg/kg). In this figure, the activity counts after the combined administration of morphine or buprenorphine with naloxone are also shown. Naloxone reduced the morphine- and buprenorphine-induced increase in the ambulatory activity in a dose-dependent manner. The mean overall ambulatory activity counts after the combined administration of 3 drugs (morphine or buprenorphine, ethanol and naloxone) were almost identical with those after the combined administration of 2 drugs without ethanol.

Figure 5 shows the modification of the combined effects of morphine (10 mg/kg) or buprenorphine (1 mg/kg). The activity counts after the combined administration of morphine or buprenorphine with ethanol and naloxone are also shown. Naloxone reduced the morphine- and buprenorphine-induced increase in the ambulatory activity in a dose-dependent manner. The mean overall ambulatory activity counts after the combined administration of 3 drugs (morphine or buprenorphine, ethanol and naloxone) were almost identical with those after the combined administration of 2 drugs without ethanol.
Fig. 4. Mean overall ambulatory activity counts for 3 hr after the combined administration of 3 drugs: morphine (10 mg/kg, s.c.) or buprenorphine (1 mg/kg, s.c.), ethanol (2.4 g/kg, p.o.) and naloxone (0.1 and 0.3 mg/kg, s.c.). These three drugs were administered simultaneously. *: P < 0.05 vs. each value after administration of morphine or buprenorphine alone. #: P < 0.05 vs. each value without combination or naloxone. N = 10 - 20 in each experiment.

Fig. 5. Effects of Ca-cyanamide (5 mg/kg, p.o.) on the enhancement of mouse's ambulatory activity induced by the combined administration of morphine (10 mg/kg, s.c.) or buprenorphine (1 mg/kg, s.c.) and ethanol (0.8 and 1.6 g/kg, p.o.). Ca-cyanamide was pretreated 30 min prior to the combined administration. *: P < 0.05 vs. each value after the administration of morphine or buprenorphine alone. #: P < 0.05 vs. each value after the combined administration of morphine or buprenorphine with distilled water (dose of ethanol = 0). #: P < 0.05 vs. each value without pretreatment with Ca-cyanamide. N = 10 - 20 in each experiment. □: Without Ca-cyanamide, ☑: Pretreated with Ca-cyanamide.
renorphine (1 mg/kg) with ethanol (0.8 and 1.6 g/kg) by Ca-cyanamide (5 mg/kg). The pretreatment with Ca-cyanamide scarcely modified the ambulation-increasing effect of morphine or buprenorphine alone. However, when the administration of Ca-cyanamide preceded the combined administration of morphine or buprenorphine with ethanol, the ambulatory activity counts were smaller than those without ethanol.

DISCUSSION

The present experiment demonstrated that ethanol, but not pentobarbital and diazepam, dramatically enhanced the ambulation-increasing effect of opioids, morphine and buprenorphine, indicating a specific interaction between ethanol and opioids in terms of ambulatory activity in mice.

It has been considered that the ambulation-increasing effect of opioids appears through an indirect stimulation of dopaminergic systems which is produced by an agonistic action on mu-receptors (11-13). Buprenorphine is considered to possess agonistic-antagonistic actions on mu- and kappa-receptors (4). However, all results obtained were almost the same between morphine and buprenorphine, indicating that the agonistic action on mu-receptors, which is the common action between morphine and buprenorphine, plays the most important role in the interaction between opioids and ethanol.

For the ethanol-induced enhancement of the effects of morphine and buprenorphine, four plausible mechanisms can be considered. The 1st possibility is a direct stimulation of the dopaminergic neurons by ethanol. However, this mechanism may be inappropriate for explaining the present result. Methamphetamine potentially stimulates dopaminergic systems by enhancing the release and inhibiting the reuptake of dopamine (14). We have reported that ethanol, at 0.8-1.6 g/kg, does not enhance the methamphetamine-induced increase in the mouse's ambulatory activity, but rather reduces it at 2.4 g/kg (15). In contrast, both pentobarbital and diazepam dramatically enhance the ambulation-increasing effect of methamphetamine at the doses used in this study (mean overall 3 hr activity counts ranged between 1.5-3 times as high as that in the single administration of methamphetamine; H. Kuribara, unpublished data).

The 2nd possibility is a stimulation of the opioid receptor by salsolinol after administration of ethanol. However, this mechanism may also be inadequate. Salsolinol is synthesized by the conjugation of dopamine with acetaldehyde, the main metabolite of ethanol, and it can bind to opioid receptors (16-18). Ca-cyanamide inhibits acetaldehyde dehydrogenase and increases acetaldehyde accumulation after ethanol administration. If the accumulation of acetaldehyde could yield an increase in the synthesis of salsolinol, the pretreatment with Ca-cyanamide should potentiate the interaction between opioids and ethanol. However, Ca-cyanamide failed to enhance, but rather reduced, the interaction. The details of the mechanism for reduction of the ambulation-increasing effect by Ca-cyanamide are hard to precisely elucidate. However, a prominent accumulation of acetaldehyde was effectively inhibited the general activity of rats (10).

The 3rd possibility is a change in the metabolism of morphine and buprenorphine by ethanol. Although ethanol has been reported to inhibit the metabolism of some drugs (19), there is no evidence that ethanol elicits the retardation of opioid metabolism (20).

The 4th possiblity is an ethanol-induced release of endogenous opioids (21), and these endogenous opioids show additive and/or synergistic interaction with the administered opioids. The ethanol-induced enhancement of the ambulation-increasing effect of morphine and buprenorphine was prevented by naloxone, suggesting that this mechanism is likely to explain the interaction between opioids and ethanol. However, to make a definite conclusion, a further study such as the measurement of endorphine levels is required.

The ethanol-induced enhancement of the
ambulation-increasing effect of opioids is very important from the viewpoint of opioid abuse. This is because the dopaminergic as well as opioid systems are involved in the dependency of opioids (22), and because, although the neuronal pathways are different, the dopaminergic and opioid systems are also related to the opioids-induced increase in the ambulatory activity in mice. In addition, the close interaction between opioids and ethanol suggests a modification of the opioid-induced analgesic effect by a concomitant use of ethanol. These concerns are the targets of our next investigation.

On the other hand, pentobarbital and diazepam elicited no marked enhancement of the ambulation-increasing effect of morphine and buprenorphine. This finding is consistent with the clinical applications such as the administration of these drugs prior to anesthetization. Opioid analgesics have sometimes been used in combination with barbiturates and/or benzodiazepines without any significant problems.

REFERENCES