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Decrease in *d*-Methamphetamine Sensitivity in Mice Due to Ethanol: Apparent Inhibitory and Stimulatory Effects of Ethanol on *d*-Methamphetamine-Induced Locomotor Activity

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KOHDA, H., M. FUNAHASHI, I. SHIKATA AND H. KIMURA. *Decrease in* d-*methamphetamine sensitivity in mice due to ethanol: Apparent inhibitory and stimulatory effects of ethanol on* d-*methamphetamine-induced locomotor activity*. PHARMACOL BIOCHEM BEHAV **25**(5) 1035–1039, 1986.—The locomotor activity of mice was recorded after administration of *d*-methamphetamine-HCl (1.5, 2.5, 5.0 and 7.5 mg/kg body weight) and/or ethanol (0.8 and 1.6 g/kg body weight). Mice injected with lower doses of *d*-methamphetamine (1.5 or 2.5 mg/kg) showed a marked increase in locomotor activity, while in those with higher doses of *d*-methamphetamine (5.0 or 7.5 mg/kg), locomotor activity was not further enhanced, but slightly decreased. Administration of ethanol inhibited the stimulated locomotor activity caused by low doses of *d*-methamphetamine (1.5 or 2.5 mg/kg), while the stimulation of motility after higher doses of *d*-methamphetamine (5.0 or 7.5 mg/kg) was potentiated by administering ethanol. Although apparent inhibition and stimulation of *d*-methamphetamine-induced locomotor activity of mice due to ethanol was observed, it is suggested that mice administered ethanol showed the decreased sensitivity to *d*-methamphetamine by plotting total locomotor activity of mice against doses of *d*-methamphetamine administered. The half maximum effective dose of *d*-methamphetamine for locomotor activity was increased from 1.5 mg/kg to 3.0 mg/kg by concomitant administration of 1.6 g/kg ethanol.

Methamphetamine Ethanol Locomotor activity

SEVERAL studies on the interaction of *d*-amphetamine with central nervous system depressants have been done which define the neurochemical mechanisms of the effect of amphetamine, a central nervous system stimulant [1, 5, 9, 21, 22, 26, 28]. It has been expected that in combined usage, amphetamine and ethanol would be mutually antagonistic with respect to their pharmacologic actions. However, inconsistent results have been reported on the interaction of amphetamine with ethanol, depending on the dosage of each drug, species and strains of experimental animals and methods for measurement of behavior [5, 21, 22, 26, 28]. Spreux-Varoquaux and Simon [26] have reported that hypermotility produced by amphetamine in mice was slightly but significantly suppressed by ethanol, while Todzy *et al.* [28] and Duncan and Cook [5] have shown the potentiation due to ethanol of amphetamine-induced spontaneous motor activity in rats. Amphetamine has also been shown to enhance the ethanol-induced impairment of rotarod performance of rats [21,22]. The reason for inconsistent results is not presently known. We describe here the effect of ethanol on the locomotor activity of mice produced by different doses of methamphetamine, a derivative of amphetamine which shows a similar potency of pharmacologic actions to amphetamine. We observed apparent inhibitory and stimulatory effects of ethanol on locomotor activity induced by low and high doses of methamphetamine, respec-

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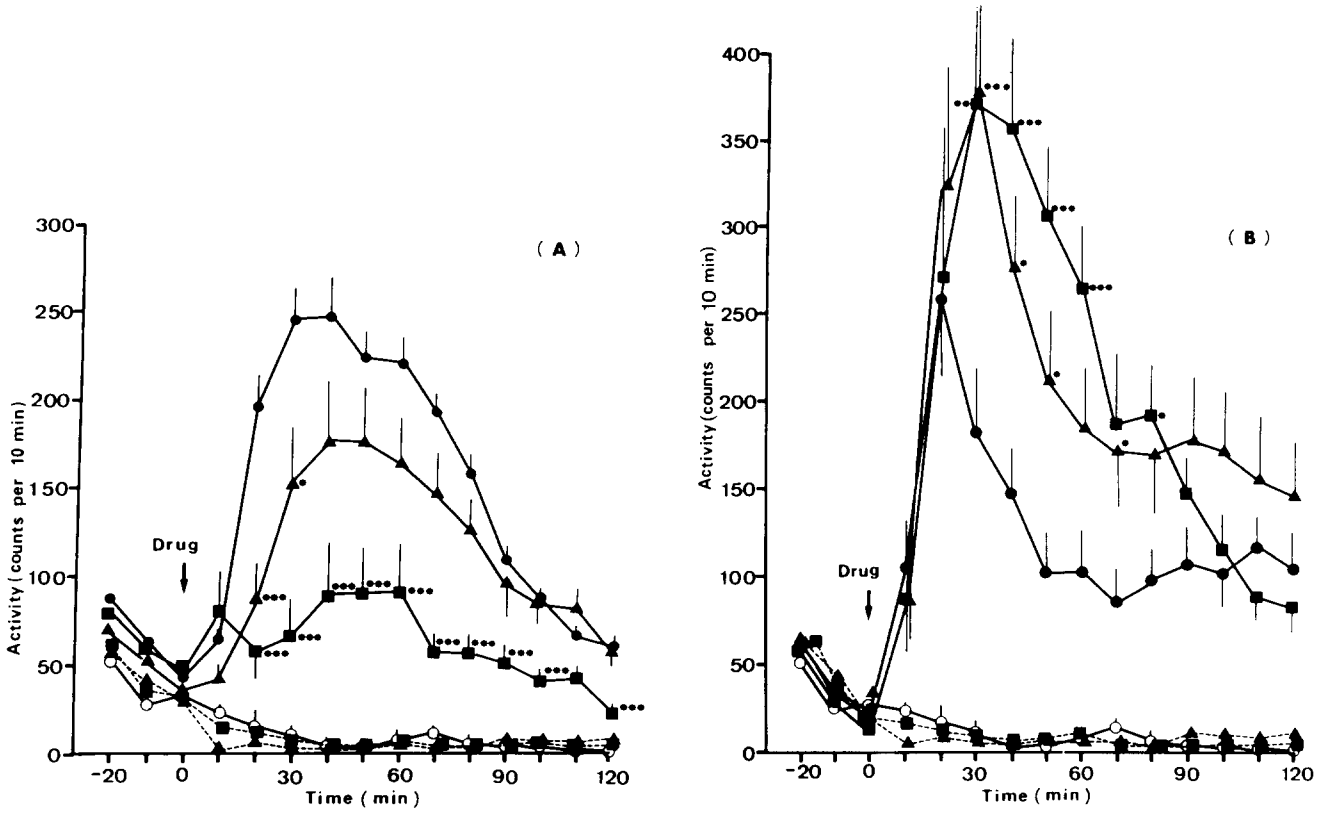

FIG. 1. The effect of ethanol on locomotor activity increased by *d*-methamphetamine. Each point represents mean±SEM of 10 mice. *d*-Methamphetamine-HCl 2.5 mg/kg (13.5 μmoles/kg) (A) or 5.0 mg/kg (27 μmoles/kg) (B) was injected subcutaneously and ethanol intraperitoneally. (●) methamphetamine, (▲—▲) methamphetamine + ethanol 0.8 g/kg, (■—■) methamphetamine + ethanol 1.6 g/kg, (▲— —▲) ethanol 0.8 g/kg, (■— —■) ethanol 1.6 g/kg, (○) saline. Values of $*p<0.05$, $**p<0.02$ and $***p<0.01$ were significantly different from that of methamphetamine alone.

tively, and conclude that this may be due to the decrease in methamphetamine sensitivity due to ethanol.

METHOD

Male *ddY* mice aged 5 weeks (28–32 g) (Shizuoka Laboratory Animal Center) were used. Mice were maintained under controlled conditions of temperature (22±1°C) and light (from 8:00 to 20:00), and had free access to food and tap water. Methamphetamine-HCl (Hiropon: Dainippon Pharm. Co., Osaka) dissolved in saline was administered subcutaneously at a volume of 0.1 ml/10 g body weight. Ethanol was also dissolved in saline, and administered intraperitoneally at a volume of 0.1 ml/10 g body weight. Control animals were injected with an equivalent volume of saline. The dosage of methamphetamine was expressed as a salt. The apparatus used for measurement of locomotor activity was a tilting-type round cage with diameter of 30 cm (ACTY-301, Biomedica Ltd.). The principle of the device has been described elsewhere [8]. Stated briefly, each slight tilt of a Plexiglas activity cage induced by locomotor activity of the mouse is detected by three microswitches attached to the cage. These microswitches activate an electromagnetic counter (Sodeco D1-X-0, Biomedica Ltd.). This apparatus can detect locomotor activity, but cannot detect stereotypy and rearing. Before drug administration, the activity counts were recorded for 30 min in 10 min intervals after placing the mouse in the activity cage. Then, the drugs or saline were administered and the number of counts in every 10 min period was subsequently recorded for 120 min. Stereotyped behavior was observed qualitatively every 10 min during experiments. Experiments were carried out between 10:00 and 15:00 to avoid circadian variation in sensitivity to the effect of methamphetamine [13]. In all experiments, drug-naive mice were used only once in each drug test under a "between-groups" design with ten mice per group. Two-way analysis of variance (ANOVA), followed by Student's *t*-test, was used [17,27]. Generally, $p<0.05$ was regarded as significant.

RESULTS

The effects of methamphetamine alone, ethanol alone and both drugs in combination on spontaneous locomotor activity of mice were recorded over every 10 min period for 120 min. The representative results with 2.5 and 5.0 mg/kg methamphetamine are depicted in Fig. 1. When mice were injected with methamphetamine 2.5 mg/kg, locomotor activity of the mice increased within 10 min, reached a maximum at 30

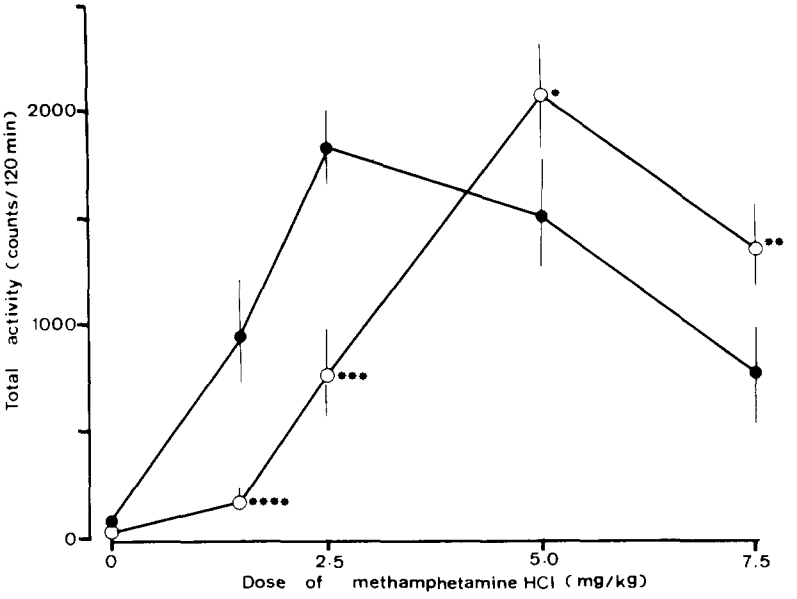

FIG. 2. Mean total locomotor activity counts (±SEM) as a function of dose of *d*-methamphetamine administered with or without 1.6 g/kg ethanol. Experimental conditions were the same as described in the legend to Fig. 1. (●) methamphetamine, (○) methamphetamine + ethanol 1.6 g/kg. Values of $^{*}p<0.05$, $^{**}p<0.025$, $^{***}p<0.01$ and $^{****}p<0.005$ were significantly different from that of methamphetamine alone.

min and decreased progressively thereafter (Fig. 1A). No stereotyped movement was observed with this amount of the drug. Mice injected with saline or ethanol in doses of 0.8 or 1.6 g/kg did not increase their motility [18], although small doses of ethanol are known to stimulate the locomotion of female mice of some strains [17,27]. When ethanol and methamphetamine were administered concomitantly, the locomotor activity stimulated by methamphetamine was significantly reduced and the time required to reach the maximum level of motility was also retarded depending on the dosage of ethanol, but the pattern of increase in locomotion was not affected by ethanol (Fig. 1A). The locomotor activity of mice administered methamphetamine 5.0 mg/kg increased with a peak at 20 min and then decreased rapidly following an increase in stereotypy (Fig. 1B). As stereotypy is incompatible with locomotor activity, activity counts were decreased by doses of methamphetamine of more than 5.0 mg/kg. When ethanol was administered with methamphetamine, ethanol enhanced the increased locomotor activity induced by doses of 5.0 mg/kg of methamphetamine, showing a higher peak activity and a prolonged duration, in contrast to that with doses of 2.5 mg/kg of methamphetamine (Fig. 1B). The stereotypy induced by a high dose of methamphetamine appeared to be suppressed by 1.6 g/kg ethanol, following an increase in locomotor activity. However, in spite of no effect on stereotypy by 0.8 g/kg of ethanol as demonstrated by the same activity pattern as when injected with methamphetamine alone, the locomotor activity induced by methamphetamine was still enhanced by 0.8 g/kg ethanol, suggesting that an increase in locomotion is not due to a decrease in stereotyped behavior by ethanol. The same experiments with methamphetamine in doses of 1.5 and 7.5 mg/kg were carried out and the total activity counts accumulated during 120 min were plotted against the dose of methamphetamine administered with or without ethanol 1.6 g/kg (Fig. 2). Ethanol in doses of 1.6 g/kg alone reduced the accumulated activity slightly in comparison with the activity of control animals (not shown). Injection of methamphetamine in doses of 1.5 and 2.5 mg/kg increased the locomotor activity of mice in a dose dependent manner, while higher doses of methamphetamine (5.0 and 7.5 mg/kg) produced activity counts lower than that by doses of methamphetamine of 2.5 mg/kg, which produced the highest and most highly reproducible locomotor activity without stereotyped activities. A decrease in locomotor activity was associated with an increase in stereotypy such as sniffing, biting, head twitching and circular movement, which competed with spontaneous locomotor activity. When ethanol was administered with methamphetamine, the increased locomotor activity due to low doses of methamphetamine was inhibited and that due to high doses of methamphetamine was potentiated by ethanol (Fig. 2). The half maximal dose of methamphetamine for locomotor activity was 1.5 mg/kg without ethanol and 3.0 mg/kg with a concomitant administration of 1.6 g/kg of ethanol, indicating that the sensitivity of mice to methamphetamine was reduced by ethanol.

DISCUSSION

Since *d*-amphetamine and ethanol are typical stimulant and depressant of the central nervous system, respectively, the interaction of both drugs might be expected to be mutually antagonistic with respect to their pharmacologic actions. Some experiments with a concomitant administration of amphetamine and ethanol showed them to be antagonistic for

sleeping time [28], ethanol's stimulus properties [24] and ethanol-produced activity decrement in a y-maze [14]. Others have reported potentiation of some effects of each drug, such as amphetamine-induced locomotor activity [5,28] and ethanol-produced impairment of rotarod performance [21,22]. However, Spreux-Varoquax and Simon [26] have reported that hyperthermia and hypermotility produced by amphetamine in mice were partly suppressed by ethanol. These different effects and inconsistent results by a combined use of amphetamine and ethanol may be reasonable, since pharmacologic actions of both drugs are enormously complex, eliciting stress reactions that disturb the homeostasis mechanisms for vital functions controlled by the peripheral and central nervous systems. Therefore, different effects and inconsistent results may be observed, depending on the dosage and route of administration of each drug, differences in species, strains and the sex of the animals and the type of behavior examined [9]. In the present study, we examined the effect of low to moderate doses of ethanol on spontaneous locomotor activity induced by relatively low doses of methamphetamine in *ddY* mice. Rather low doses were chosen since the most basic and prominent effect of methamphetamine is the change of locomotor activity and use of higher doses of each drug will produce complex results which are difficult to interpret. We found either antagonistic or potentiating effects of ethanol on methamphetamine-induced locomotor activity, depending on the injected doses of methamphetamine (Figs. 1 and 2). Therefore, we concluded that the sensitivity to methamphetamine with respect to locomotor activity of the mouse is reduced by a concomitant administration of ethanol. By administering a dose 1.6 g/kg of ethanol concomitantly, the half maximal effective dose of methamphetamine for locomotor activity increased two fold compared to that in mice injected with methamphetamine alone (Fig. 2). It has been demonstrated that the metabolism of amphetamine in serum and in the brain is prolonged by coadministration of ethanol [21,28]. However, in this case, the amphetamine-ethanol interaction cannot be explained by ethanol-produced prolongation of amphetamine metabolism, since both antagonistic and synergistic effects on various behavior of rodents were observed [5,9]. Recently, several reports suggest that the mesolimbic dopamine system may play an important role in the locomotor-stimulating effect of amphetamine [11, 12, 20]. Pijnenberg *et al.* [20] have reported that the stimulation of locomotor activity by amphetamine was inhibited by administration of the dopamine antagonist haloperidol into the nucleus accumbens in the rat. Koob *et al.* [12] have described that 6-hydroxydopamine-induced lesions of the mesolimbic neurons virtually abolished the stimulatory effect of amphetamine on locomotor activity in rats. Lyon and Robbins [16] and Segal [25] have postulated a competition between locomotor activity and stereotypy by observing the effect of different doses of amphetamine on behavior in rats. The stimulation of locomotor activity induced by low doses of amphetamine is mediated via mesolimbic dopamine neurons, whereas stereotypy such as sniffing, biting and head twitching, depends on the nigrostriatal pathway [11]. Joyce and Iversen [10] have reported that in rats, stereotypy was reduced after 6-hydroxydopamine-induced lesions of the neostriatum; but locomotor activity increased in a dose-dependent manner at even higher doses of amphetamine. However, it is difficult to explain the interaction of amphetamine and ethanol as due to the change in the release and synthesis of catecholamines by ethanol, because some reports have shown the increase in release and synthesis of catecholamines, while others have shown the decreased metabolism of catecholamines by ethanol [2, 3, 6, 23]. Therefore, we suggest simply that the decrease in methamphetamine sensitivity caused by ethanol may be due to the hypnotic and anesthetic effects of ethanol, resulting in an increase of the threshold of response to methamphetamine at the site(s) of pharmacologic action of methamphetamine. We have to consider the possibility that amphetamine itself may act as a neurotransmitter, since the amphetamine-binding activity has been reported in the central and peripheral nervous systems [7, 15, 19], although it is generally accepted that some of the effects of amphetamine are mediated through release of catecholamines [11, 12, 20, 29].

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