Suppression of Ethanol Withdrawal by Dopamine

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Summary. An ethanol-inhalation technique was used to determine a potential relationship between dopamine and central nervous effects produced by alcohol. Both L-DOPA and intracranially injected dopamine resulted in attenuation of ethanol-induced withdrawal convulsion scores, whereas, haloperidol, a known dopaminergic blocker was found to significantly increase convulsion scores.

Previous research has suggested a possible relationship between brain monoamines and the neuropharmacological actions of ethanol[2–9]. In particular, brain dopamine has been implicated in the soporific[10] as well as the stimulatory actions of ethanol[11]. Although Goldstein[12] has reported on the effects of drugs which modify neurotransmission on ethanol withdrawal reactions, little or no information is known with regard to the possible role of dopamine on the ethanol dependence phenomena. Here we provide evidence to implicate dopamine as a suppressor of ethanol withdrawal convulsions.

Swiss Webster mice were made physically dependent on ethanol by the Goldstein and Pal[13] inhalation technique. Mice were placed in an air-tight chamber and exposed to ethanol vapor for 3 days, after which time they were abruptly withdrawn and measured for ethanol dependence. Dependence was defined in terms of resultant convulsion scores. During the initial 3 days, the mice were removed once a day for a 45 min period to permit the collection of blood samples and injection of 68 mg/kg of pyrazole, a compound known to inhibit ethanol metabolism[14] and stabilize blood ethanol levels. The mice were exposed to a vapor concentration of 21 mg/l for these 3 days, and were then removed from the alcohol vapor chamber 24 h after their last dose of pyrazole. The grading system for assessing the severity of the withdrawal reaction has been described by Goldstein and Pal[13]. The mice are observed for approximately 20 h, with scores recorded hourly. In this abstinence syndrome, the most useful sign is the convulsion elicited by holding the mouse up by the tail. The mouse arches his back, tightens his facial muscles into an abnormal grimace, and jerks or twirls violently. Each mouse is given a score from 0 to 4 according to severity of convulsion elicited by handling. The more severe response, as characterized by spontaneous tonic-clonic convulsion upon being gently lifted by the tail, is assigned a score of 4, if the mouse continues to convulse when replaced in the cage. A score of 3 is given if the mouse convulses immediately when picked up and does not convulse when placed back into the cage. A score of 2 is assigned when the mouse convulses after he is gently giggled. A score of 1 is given when the mouse is gently turned 180°. A score of 0 is when there is no convulsions following all the above manipulations.

The method of Haley and McCormick[15] was used for intracerebral injections (i.c.) of drugs into conscious mice. The landmarks used for locating the site of injection were strictly adhered to and we noted the same behavioral effects: quietness for 1 min followed by normal activity after sham injections and artificial cerebral spinal fluid (CSF). A 27 guage needle was used in the injection procedure. Separate groups of mice were each

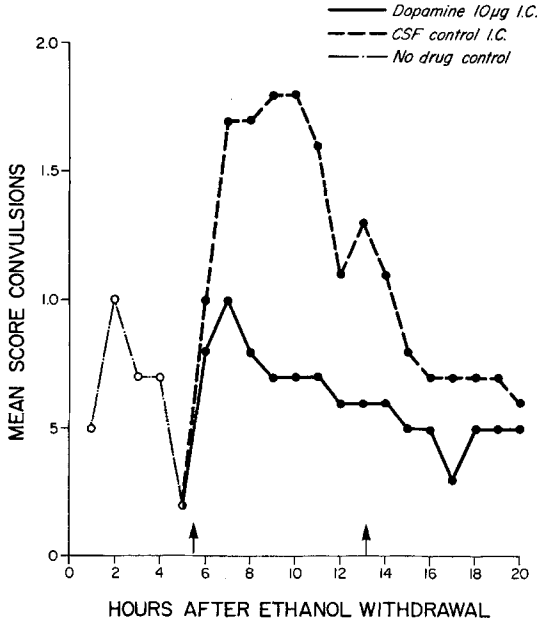

Fig. 1. Effects of dopamine on ethanol-induced symptoms in mice. ●—●, Dopamine injected intracerebrally (i.c.) at 10 µg per mouse immediately after the 5th and 13th h post-alcohol withdrawal. ●- - -●, Cerebral spinal fluid (CSF) injected intracerebrally at equivalent volume to the dopamine injection at the 5th h post-alcohol withdrawal. At least 10 mice were used in each drug treatment group. The mean convulsion score (mean ISE) for SCF was 1.25 ± 0.09 whereas for dopamine it was 0.66 ± 0.06 ($p < 0.05$).

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given one of the following drug treatments: 1. L-DOPA (i.p.) at 620 mg/kg daily for 3 days during ethanol vapor exposure and then on the 4th day after the 5th and 13th h post-ethanol withdrawal. Controls were given CSF according to the same regimen. 2. Dopamine (i.c.) at 10 μg after the 5th and 13th post-ethanol withdrawal. Controls were given saline according to the same regimen. Blood analysis of alcohol was determined by a modification of the gas chromatographic procedure of WALLACE and DAHL[16].

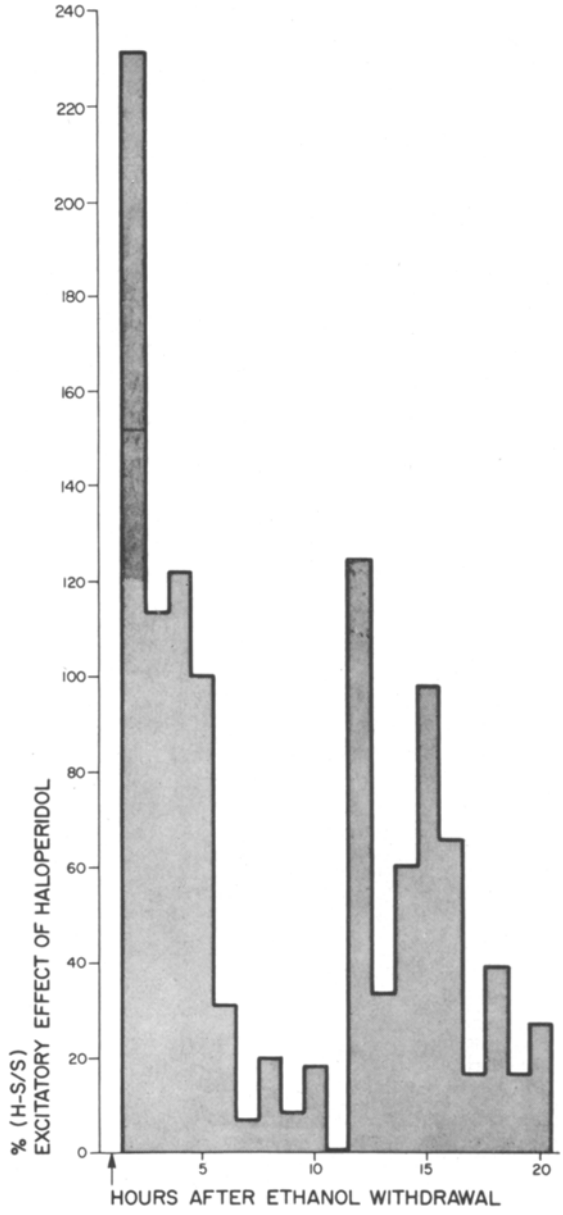

Fig. 2. Effects of haloperidol on ethanol-induced withdrawal convulsions in mice. The fraction H – S/S represents the amount of intensification of ethanol-induced withdrawal convulsions. The ordinate as represented as excitatory effect of Haladol indicates percent excitation or intensification of the response studied. The abscissa represents hours after ethanol withdrawal. Haloperidol or saline was injected i.p. after the first hour post-ethanol withdrawal. Saline was injected at equivalent volume to 2 mg/kg of haloperidol-induced intensification of ethanol withdrawal was significant at least at the ($p < 0.05$).

Dopamine Hydrochloride was dissolved in artificial cerebral spinal fluid and injected intracerebrally (i.c.) at a volume of 10 μl according to the method of HALEY and MCCORMICK[15]. The formula for the artificial CSF is as follows: NaCl 8.98, KCl 0.25 g/1 g, $CaCl_2$ 0.14 g/l, $MgCl_2$ 0.11 g/l, NaH_2PO_4 0.014 g/l, $HaHPO_4$ 0.066 g/l, Urea 0.13 g/l and glucose 0.61 g/l.

The pH of the solution was adjusted to 7.0 with 0.1 *N* NaOH. The dose of dopamine was calculated as the base rather than the salt form. L-DOPA was dissolved in saline and haloperidol was supplied in liquid form. Comparable doses of saline or CSF were administered to the control groups.

L-DOPA at a dose of 620 mg/kg injected daily for 4 days significantly depressed the withdrawal convulsion scores in mice. With L-DOPA, the mean convulsion score (Mean ± SE) was 1.39 ± 0.08 and for saline, it was 2.27 ± 0.08 ($p < 0.001$). The duration of action was at least 20 h.

Figure 1 shows that dopamine at a dose of 10 μg administered into the brain of mice undergoing withdrawal from alcohol significantly depressed the withdrawal reaction for the first post-injection period (hours 6–13). CSF control scores were higher (by 157%) than those of the dopamine treated at peak difference (Figure 1).

The mean convulsion score, over a 15 h period, for CSF was 1.25 ± 0.09, whereas for dopamine it was 0.66 ± 0.06, ($p < 0.01$), showing marked inhibition of ethanol withdrawal convulsions in mice. After the second injection, little difference could be seen between the CSF and the dopamine-treated animals. This finding can probably be attributed to the fact that neither of the two groups were withdrawing at a significant rate 13 h after removal from the chamber. It is useful to note that in doing experiments of this type there is variability in the type of response elicited by a group of mice. This is why it is very important to do paired experiments so that the true drug effect could be determined rather than utilizing a group of experimental controls as a standard. In fact, in this experiment the convulsions dropped dramatically in all the mice just prior to drug injections. This occurs in some animals but is not consistant enough to be considered a charateristic sign of this syndrome. In fact, in another study we have fully characterized this syndrome and have shown that the withdrawal convulsions continuously rise and reaches a peak at about the 11th h following withdrawal from alcohol, and then falls precipitously thereafter[16].

Haloperidol, which has been found to be an important central dopamine receptor blocker[17] at doses as low as 2 mg/kg, was found in this study to be effective at both 2 mg/kg and 10 mg/kg in potentiating withdrawal convulsions. At both dose levels, differences were especially dramatic during the first 6 post-injection hours ($p < 0.05$). Figure 2 emphasizes the excitatory effect of haloperidol in terms of a ratio

$$\frac{H-S}{S}\left(\frac{\text{haloperidol convulsion} - \text{saline convulsion}}{\text{saline convulsion}}\right).$$

According to this ratio, the range of haloperidol-induced excitation attained a maximum effect of 231% at the 2 mg/kg dose. Haloperidol at 1 mg/kg, slightly increased withdrawal convulsions.

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L-DOPA reduced the ethanol-induced withdrawal convulsions in mice. This suppression effect was also observed with an intracerebral injection of dopamine in mice undergoing withdrawal from ethanol vapor. Haloperidol, a drug reported to block dopamine receptors in the corpus striatum[18], significantly enhanced ethanol withdrawal convulsions in mice. We suggest that blocking dopamine receptors would result in augmentation, whereas increasing dopaminergic activity would result in inhibition of ethanol-induced withdrawal convulsions in mice.

Of interest, small doses of ethanol have been shown to induce behavioral stimulation in many species, including man[19,20]. This stimulation has been found to be suppressed by dopamine-agonists.

CARLSSON[11] suggests that the inhibitory effects of dopamine-agonists on the ethanol-induced stimulation of locomotor activity may be mediated by activation of presynaptic inhibitory receptors. Other work in our laboratory has shown that raising brain dopamine levels augments ethanol-induced depression[10].

SEEMAN and LEE[21,22] have shown that ethanol can induce a release of dopamine, from neurons via a calcium-propagated coupling mechanism between the impulse and the neurosecretion of dopamine. In this regard Ross et al.[23] has shown a calcium depleting effect for ethanol and a dopamine-derived tetrahydroisoquinoline (TIQ) alkaloid. Work in our laboratory[24] has shown that TIQ alkaloids intensify ethanol-withdrawal reactions and SHEPPARD and BURGHARDT[25] found that TIQ derivatives had dopamine receptor blocking activity. Ethanol administration at the same dose levels as were used for TIQ can significantly block alcohol withdrawal convulsions in mice[24]. This blocking effect of ethanol on withdrawal convulsions may be due to its effect on releasing dopamine[22].

We suggest that drugs which increase functional activity of dopamine by increasing its release, preventing its breakdown or increasing its synthesis would retard ethanol withdrawal convulsions, whereas drugs which induce lower dopaminergic activity by blocking dopamine receptors, enhancing its breakdown or inhibiting its synthesis would intensify the ethanol convulsion syndrome. Experiments are now in progress to test this hypothesis.

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Acute Toxicity of Dimethylnitrosamine in the Presence of Inhibitors of DMN Demethylase

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Summary. The LD_{50} of DMN was determined in groups of mice in the presence of inhibitors of DMN demethylase. Piperonyl butoxide, dibutylnitrosamine and nitrososarcosine had no effect on the acute toxicity of DMN. Diethylnitrosamine and DMN were markedly synergistic. All mice treated with 100 mg/kg diethylnitrosamine and 10.7 mg/kg DMN died. These results suggest that DMN demethylase may not be involved in the acute toxicity of DMN.

Dimethylnitrosamine (DMN) is a potent carcinogen in all species tested[2]. Generally this carcinogenic action has been related to enzymatic activation (DMN demethylase) and methylation of biological macromolecules[2]. Inhibition of this enzyme activity generally results in a suppression of the acute toxicity[3-6], most frequently measured as inhibition of protein synthesis. For example, aminoacetonitrile[3] and cysteine[4] inhibit DMN demethylase and also suppress DMN mediated inhibition of liver protein synthesis. More recently, nitrososarcosine[5], diethylnitrosamine[5], dibutylnitrosamine[5] and piperonyl butoxide[6] have been shown to inhibit DMN demethylase activity and suppress the inhibition of liver protein synthesis by DMN.

However, the clearest indication of acute toxicity is mortality, due to the unequivocal nature of the response. DMN is highly toxic with a murine LD_{50} of 19 mg/kg. We report here an absence of effect by nitrososarcosine, dibutylnitrosamine and piperonyl butoxide on DMN LD_{50} and a marked synergy between diethylnitrosamine and dimethylnitrosamine.

Male Swiss albino mice weighing between 20 and 25 g were maintained on Purina chow and water ad libitum. All injections were i.p. Dibutylnitrosamine and piperonyl butoxide were dissolved in corn oil and all other chemicals were administered in aqueous solutions. Nitrosarcosine was neutralized with 10 *N* NaOH prior to injection.

Seven logarithmically spaced dose levels of DMN were used in each of these studies. 7 animals were used in each group. LD_{50}'s were calculated by the probit method of Litchfield and Wilcoxon[7]. Each experimental point was fed into a computer and reduced to probits. The computer performed regression analyses and the LD_{50} and its confidence limits and the slope and its confidence limits were calculated. These experiments were repeated on separate occasions with the results all identical.

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