EFFECTS OF CATECHOLAMINE SYNTHESIS INHIBITION ON ETHANOL-INDUCED WITHDRAWAL SYMPTOMS IN MICE

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α-Methyl-*p*-tyrosine, a catecholamine synthesis inhibitor, was studied to determine its effects against ethanol-induced withdrawal symptoms in mice. Significant ($P < 0.001$) potentiation of the withdrawal convulsion score induced by ethanol vapour exposure for three days was observed in mice. The synergistic effect was not due to alteration of ethanol metabolism. These results indicate that reductions in catecholamines (dopamine and noradrenaline) augment seizure activity induced by subchronic exposure to ethanol.

The recent availability of a model of 'physical dependence' on ethanol in the mouse (Goldstein & Pal, 1971) allows testing of the adrenergic synthesis inhibitor, α-methyl-*p*-tyrosine (α-MPT) on this system. Previous research has suggested a possible relationship between brain monoamines and the neuropharmacological actions of ethanol (Rosenfield, 1960; Smith & Gitlow, 1967; Davis & Walsh, 1970; Feldstein & Kucharski, 1971; Blum, Merritt, Wallace, Owen, Hahn & Geller, 1972; Blum, Calhoun, Merritt & Wallace, 1973a; Blum, Calhoun, Wallace, Merritt & Geller, 1973b; Geller, Purdy & Merritt, 1973). In fact, Blum *et al.* (1972) recently reported that pretreatment with the catecholamine synthesis inhibitor, α-MPT, significantly enhanced ethanol narcosis in mice. It was conjectured that this α-MPT-induced augmentation of ethanol narcosis was due to an alteration of the balance between free 5-hydroxytryptamine (5-HT) and catecholamines. Under these conditions, α-MPT produced a temporary increase in the ratio of 5-HT/catecholamines resulting in 5-HT > catecholamines and the accompanying enhanced ethanol-induced behavioural depression.

A recent report (Griffiths, Littleton & Ortiz, 1973) indicates that chronic administration of ethanol produced an increase in catecholamines of 50% over control values. The withdrawal phase was reported to be modified by administration of the tyrosine hydroxylase inhibitor, α-methyltyrosine methylester. However, no characterization or type of modification was reported. The purpose of this communication is to report on the characterization of ethanol-induced withdrawal symptoms after monoamine synthesis inhibition by α-MPT.

Methods Swiss-Webster mice were made physically dependent on ethanol by the Goldstein & Pal (1971) inhalation technique. Mice were placed in an air-tight chamber and exposed to ethanol vapour for three days after which time they were abruptly withdrawn from the alcohol vapour and dependence was quantitated by measuring the resultant convulsions. 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 (Theorell & Yonetani, 1963) to ensure stable blood ethanol levels. The mice were exposed to a vapour concentration of 21 mg/litre for three days. The mice were removed from the alcohol vapour 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 & Pal (1971).

Blood analysis of alcohol was determined by a modification of the gas chromatographic procedure of Wallace & Dahl (1966).

α-MPT was suspended in 0.5% carboxymethylcellulose and administered at a concentration of 5 mg/ml. Two groups of 12 mice each were given either 0.9% w/v NaCl solution (saline) or 120 mg/kg of α-MPT for three consecutive days during exposure to ethanol vapour. Then on the 4th day, α-MPT was administered at the 5th hour and again at the 13th hour after ethanol withdrawal. α-MPT and saline were administered intraperitoneally and the dose of α-MPT was calculated as the base per kilogram body weight. The data were analysed by Student's *t* test.

Results In this experiment, with air concentrations of 21 mg/litre, the blood ethanol concentration ranged from 1.17 ± 0.13 to 1.42 ± 1.21 mg/ml for three days. To assure reproducibility of results and to be certain that we could simulate the Goldstein (1972) experiment a comparison was made between our findings and those reported by Goldstein (1972). A total of 170 mice were used in our experiments and 66 mice were used by Goldstein (1972). Statistical

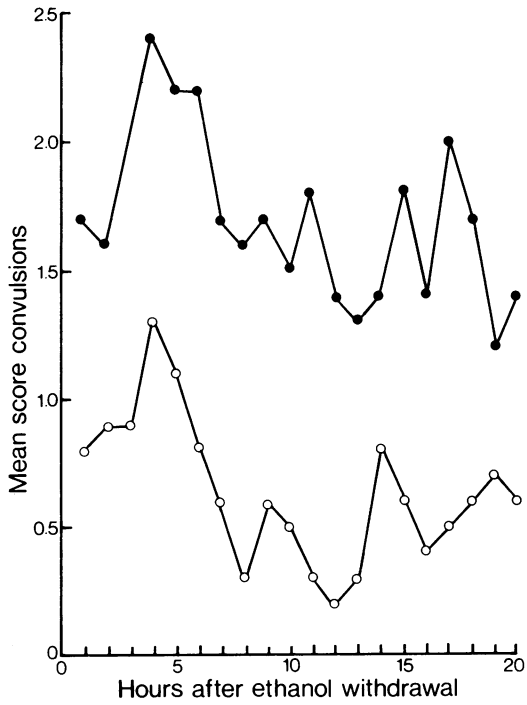

Fig. 1 Effects of α-methyl-*p*-tyrosine (α-MPT) on ethanol-induced withdrawal symptoms in mice. (●), α-MPT injected intraperitoneally at 120 mg/kg for four consecutive days. (○), Saline injected intraperitoneally (same volume as α-MPT) for four consecutive days. At least 10 mice were used in each drug treatment group. The areas under the curve for the α-MPT and saline control curves were 32.4 ± 3.1 and 13.0 ± 2.1, respectively.

analysis of the data reveals no significant difference between our experiments and the studies reported by Goldstein (1972).

Figure 1 illustrates the effects of α-MPT on ethanol-induced withdrawal symptoms in mice in a single paired experiment consisting of a total of 24 mice. α-MPT at a dose of 120 mg/kg injected daily for four days significantly intensified ($P < 0.001$), the withdrawal convulsion scores in mice. With this drug, the scores were higher (85%) than those of its paired saline control. Furthermore, the area under the curve (mean ± s.e.) for saline was 13.0 ± 2.1, whereas for α-MPT it was 32.4 ± 3.1 ($P < 0.001$) also indicating potentiation. The duration of action of α-MPT was at least 20 hours.

Ethanol in the blood after a four-day treatment with saline did not differ significantly from that obtained after α-MPT treatment for four days.

Discussion It has been shown that α-MPT at 80 mg/kg for three days reduces mouse brain dopamine and noradrenaline levels by more than 60% (Blum *et al.*, 1972). In this experiment, we found that α-MPT at 120 mg/kg for four days significantly ($P < 0.001$) enhanced ethanol-induced withdrawal convulsion scores in mice. This finding is not surprising since it has already been shown that drugs which lower the brain levels of 5-HT, dopamine and noradrenaline increase susceptibility to audiogenic seizures in mice (Lehman, 1967; Schlesinger, Boggan & Freedman, 1968). These investigators found that decreasing the brain levels of 5-HT, dopamine and noradrenaline enhances seizure susceptibility, whereas increasing the brain levels of these amines tends to protect against sound-induced convulsions.

Results from this preliminary investigation certainly implicate the involvement of the catecholamines in ethanol-induced withdrawal seizure activity in mice. However, more definitive conclusions regarding ethanol dependence and monoamine metabolism must await further experiments, particularly those concerned with the intracerebral application of dopamine, noradrenaline and 5-HT and/or other putative neurotransmitter substances.

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