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Enhancement of ethanol-induced withdrawal convulsions by blockade of 5-hydroxytryptamine receptors

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Male Swiss-Webster mice were made physically dependent on ethanol using the ethanol vapour inhalation technique. Animals pretreated with methysergide, a known 5-hydroxytryptamine receptor blocking agent, had significantly greater alcohol-induced withdrawal convulsions than saline pretreated controls. These findings suggest that the reduction of 5-HT at receptor sites may result in the augmentation of the withdrawal convulsions.

Goldstein (1973) has recently reported the effects of drugs which modify neurotransmission on the behavioural withdrawal reactions in mice made physically dependent to ethanol. The results of her study indicate that the interference with the brain catecholamine system aggravates the alcohol-induced withdrawal but that 5-hydroxytryptamine does not appear to be directly involved. In support of this, Griffiths, Littleton & Ortiz (1974) have demonstrated that inhibition of catecholamine synthesis potentiates the associated convulsive state, whereas, inhibition of 5-HT synthesis does not appear to affect the convulsive aspects of ethanol withdrawal.

Although our own work (Blum & Wallace, 1974) confirms the finding that α-methyl-*p*-tryosine, a catecholamine synthesis inhibitor, significantly augments the withdrawal convulsion scores induced by ethanol vapour exposure, we do not agree with Goldstein's conclusions of the non-involvement of 5-HT. The purpose of this report is to characterize the effects of 5-HT receptor blockade on ethanol withdrawal reactions in mice.

METHODS AND MATERIALS

Ethanol vapour chamber technique

Male Swiss-Webster mice (18–25 g) were made physically dependent on ethanol using the Goldstein-Pal (1971) technique. Groups of 24 mice were housed in an air-tight chamber and exposed to ethanol vapour by a slow stream of air for 3 days. The animals all received a priming dose of ethanol of 1·67 g kg^{-1} (i.p.) together with 68 mg kg^{-1} of pyrazole, a compound known to inhibit ethanol metabolism (Theorell & Yonetani, 1963), to insure stable blood ethanol concentrations. The mice were removed from the ethanol vapour chamber once a day for 45 min to permit the collection of blood samples and injections of pyrazole and were exposed to a vapour concentration of 21 mg $litre^{-1}$ for 3 days. Then the mice were removed from the vapour chamber 24 h after their last dose of pyrazole, The grading system for assessing the severity of the withdrawal reaction by measuring the resultant convulsions has been described by Goldstein & Pal (1971). Ethanol concentrations in the chamber air and in the blood were determined daily by a modification of the gas chromatographic procedure of Wallace & Dahl (1966), as described in an earlier report from this laboratory (Blum, Hudson & others 1974a).

Drug treatments

To study the effects of the 5-HT system on ethanol-induced withdrawal symptoms in mice, the following drug treatment groups were examined:

(A) Saline-intraperitoneal injection (i.p.). (B) Cerebral spinal fluid (csf)—intracerebral injection (i.c.). (C) 5-Hydroxytryptophan (5-HTP)—200 mg kg^{-1} (i.p.). (D) 5-HT—50 mg kg^{-1} (i.p.). (E) 5-HT—1 μg (i.c.) (F) Methysergide—10 μg (i.c.). (G) *p*-chlorophenylalanine (PCPA)—620 mg kg^{-1} (i.p.).

In this investigation, the 1 μg dose of 5-HT was intracerebrally injected on the 5th and 13th hour post-ethanol withdrawal. Intracerebral injections of 5-HT were accomplished by utilizing the modified technique of Haley & McCormick (1957). Methysergide at 10 μg was similarly injected into the brain of mice.

The artificial csf had the following formula (g $litre^{-1}$) NaCl 8·98, KCl 0·25, $CaCl_2$ 0·14, $MgCl_2$ 0·11, NaH_2PO_4 0·0142, Na_2HPO_4 0·066, urea 0·13,

* Correspondence.

and glucose 0·61. The pH of the solution was adjusted to 7·0 with 0·1 N NaOH.

All other drugs were injected intraperitoneally for 3 consecutive days and then on the fourth day readministered at the 5th and 13th hour after cessation of exposure to ethanol. In the 5-HTP experiments, 100 mg kg^{-1} rather than 200 mg kg^{-1} was injected at the 5th and 13th hour post-ethanol withdrawal. The drugs were administered as the bases in amounts calculated from body weights except for intracerebral injections in which the amount represents the total per animal.

Materials

The 5-hydroxytryptophan HCl (base mol wt = 220·22), the 5-hydroxytryptophan creatinine sulphate (base mol wt = 176·21) and *p*-chlorophenylalanine (base mol wt = 199·6) were purchased from Sigma Chemical Company and the methysergide HCl (base mol wt = 353·45) was kindly supplied by Sandoz Laboratories.

To evaluate the role of alteration of brain content of 5-HT, the tryptophan synthesis inhibitor (Koe & Weissman, 1966) PCPA was used to lower 5-HT content whereas 5-HTP and 5-HT itself were utilized to raise its brain content. The 620 mg kg^{-1} dose of PCPA has been shown elsewhere (Blum, Calhoun & others, 1973b) to reduce brain 5-HT content by approximately 70%.

Analysis of variance showed that there was no significant difference between PCPA and its saline control ($P > 0·05$), 5-HTP and its saline control ($P > 0·05$) and 5-HT and its saline control ($P > 0·05$) and artificial csf.

It is noteworthy that the intracerebral injection of 5-HT resulted in inconsistent data. Thus, it either enhanced or inhibited the withdrawal convulsion response.

To further assess the role of the 5-HT system in ethanol-induced withdrawal convulsions in mice, methysergide, a known 5-HT receptor blocking agent (Burks & Kennedy, 1973), was administered to another group.

Fig. 1 shows that methysergide intensified the withdrawal response. Analysis of variance revealed that there was a significant difference between the methysergide group and its artificial csf control. It is important to note that methysergide by itself at 10 μg did not produce spontaneous convulsions in mice when handled. With this drug the score was higher (258%) than that of its paired artificial csf control at peak difference. Furthermore, the mean

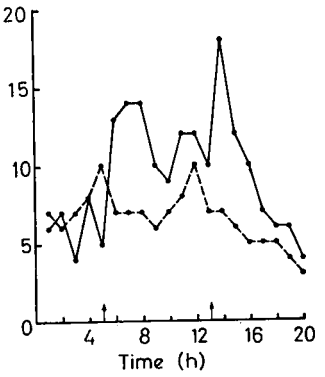

FIG. 1. Effects of methysergide on ethanol-induced withdrawal convulsions in mice. (●——●) Methysergide injected intracerebrally at 10 μg on the 5th and 13th hour during the withdrawal period. (●- - -●) Artificial cerebral spinal fluid (csf) injected intracerebrally (same volume as methysergide) using the same time schedule. Arrows indicate time of injection. Twenty mice were used in each treatment group. y axis–Mean score convulsions.

convulsion score (mean ± s.e.) for csf was 0·66 ± 0·17 whereas for methysergide it was 0·94 ± 0·37 indicating augmentation.

DISCUSSION

The findings that the 5-HT synthesis inhibitor, PCPA the 5-HT precursor amino acid, 5-HTP, and 5-HT itself, had no consistent significant effects on ethanol-induced withdrawal convulsions substantiates the work of Goldstein (1973) and Griffiths & others (1974).

However, one cannot, on the basis of these results, negate the possibility that 5-HT plays a role in the ethanol withdrawal response of mice. Perhaps the concentration of 5-HT which still remained after PCPA treatment was sufficient to maintain its pharmacological activity and for this reason the production of withdrawal convulsions was not altered. Furthermore, one could explain the lack of effect of 5-HTP and 5-HT by the intraperitoneal route because it is known that 5-HTP endogenously applied may not enter functional pools of 5-HT (Aghajanian & Asher, 1971) and 5-HT has difficulty in penetrating the blood-brain barrier (Bertler, Falck & Rosengrin, 1963).

It is interesting to note the apparent course of action of methysergide on the augmentation of these withdrawal convulsions. The subjects in the drug group received an intracerebral injection of methysergide at the 5th and 13th hour after being removed from the ethanol vapour chambers. For the first 5 h,

the drug and control group curves were not significantly different. However, after the 5th hour and an injection of methysergide, the drug group showed significantly different withdrawal convulsion scores from the control group. In addition, after the 13th hour and another injection of methysergide, the drug group again showed significantly higher withdrawal convulsion scores than the control group. This augmentation of ethanol withdrawal convulsions by methysergide dissipated not long after each injection. The implication of these observations is that there is a rather short-term effect of methysergide but only in conjunction with ethanol or its respective metabolites.

Since methysergide, a compound which reduced 5-HT activity, intensified significantly ethanol-induced withdrawal convulsion, then it should follow that increasing brain 5-HT would result in a suppression of ethanol-induced withdrawal convulsions in mice. In fact, other investigators (Lehman, 1967, and Schlesinger, Boggan & Freedman, 1968) have shown that lowering brain 5-HT in rodents increases audiogenic-induced seizures whereas raising this biogenic amine suppresses these induced convulsions.

Comer & Iturrian (1973) reported that convulsions on handling were correlated to susceptibility to sound induced seizure during acute ethanol withdrawal. In support of these findings, we have shown that methysergide, a compound which reduces the pharmacological response of 5-HT in the brain (Blum, Wallace & others, 1974b), significantly intensified ethanol-induced withdrawal convulsions. In support of a 5-HT involvement in ethanol withdrawal Collier, Hammond & Schneider, (1974) have produced evidence that ethanol withdrawal head twitches in mice may be mediated in part by 5-HT.

Previous studies have also implicated the importance of 5-HT in the actions of ethanol. 5-HT and its closely related metabolites have been shown to enhance ethanol-induced sleep time in mice (Feldstein & Kucharski, 1971; Blum, Calhoun & others, 1973a). Most recently, Geller (1973), has shown that lowering of 5-HT by PCPA increased ethanol preference, while raising 5-HT by administration of its precursor, 5-HTP, decreased ethanol preference. Also, in another study, it was found that the β-carboline, 6-methoxy-1-methyl-1, 2, 3, 4-tetrahydro-2-carboline elevates brain 5-HT concentrations and also reduces ethanol preference (Geller, Purdy & Merritt, 1972). However, in other investigations, opposite effects have been reported (Ahtee & Eriksson, 1973; Myers & Martin, 1973).

The result of this investigation further supports the possible role of 5-HT in the actions of ethanol and provides additional impetus to further investigate the possible link between 5-HT and its metabolites and ethanol-induced physical dependence in mice.

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