

Experimental Studies on Alcoholism I. Increased in Alcohol Preference by 5,6-Dihydroxytryptamine and Brain Acetylcholine

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Abstract. The effects of an intracisternal injection of 5,6-Dihydroxytryptamine (5,6-DHT; 75 $\mu\text{g}/\text{rat}$) on alcohol preference were studied in rats. Results indicated that alcohol consumption was increased significantly from about the 5th to 11th days after treatment. This 5,6-DHT induced alcohol preference was antagonized by 4-(1-naphthylvinyl) pyridine (NVP) (5 mg/kg, i.p. twice daily), an inhibitor of choline acetyltransferase. The level of brain acetylcholine was also increased significantly ($P < 0.001$) 8 days after 5,6-DHT treatment. It was suggested that the 5,6-DHT induced alcohol preference may be attributed to an increase in central cholinergic activities. The possibility of a modulation of 5-hydroxytryptamine on cholinergic activity was discussed.

Key words: Alcohol Preference — 5,6-Dihydroxytryptamine — Central Cholinergic Activities — Brain Acetylcholine Level — Serotonin Level.

Introduction

Recent studies in the neurochemical correlates of ethanol selection have been focused on monoamine metabolism in the brain. However, the results obtained on alcohol preference are not consistent with regard to the effects of DL-para-chlorophenylalanine (pCPA), an inhibitor of tryptophan hydroxylase. Myers and co-workers (1968, 1969) reported a decrease in the volitional consumption of ethanol in rats after chronic treatment with pCPA whereas no effect was observed after similar treatment with alpha-methyl-para-tyrosine (αMT), an inhibitor of tyrosine hydroxylase. It was further suggested that serotonin (5-HT) rather than catecholamines, may be involved in the selection of ethanol. However Nachman *et al.* (1970) and Geller (1973) were unable to confirm this effect of pCPA. Nachman (1970) contended that a conditioned aversion might be established through the pairing of ethanol or saccharin with the administration

of pCPA or other noxious substances. Geller (1973) found that pCPA increased rather than decreased ethanol selection. Recently Baumgarten *et al.* (1971) reported that 5,6-dihydroxytryptamine (5,6-DHT) produced a prolonged depletion of brain 5-HT but with its effect on brain dopamine (DA) was only of short duration. The availability of 5,6-DHT has provided an useful alternative approach to study the role of 5-HT in alcohol preference. Our recent observations have suggested a possible central cholinergic role in the selection of alcohol (Ho and Kissin, 1974). If these observations are correct then it is reasonable to expect that both serotonergic and cholinergic mechanisms may be operative. Furthermore, the effects of 5,6-DHT on the level of brain acetylcholine (ACh) has not been studied.

In addition, we also wish to test the effect of 4 (1-naphthylvinyl) pyridinium salt (NVP), an inhibitor of choline transferase (ChAc) (Cavallito *et al.*, 1969), on the 5,6-DHT induced changes in ethanol consumptions.

Therefore, the purpose of this study is to further elucidate the role of serotonergic and cholinergic mechanisms in alcohol preference and to explore a possible interaction between these two systems in the central nervous system.

Methods

Adult male Sprague-Dawley rats weighing between 250 to 300 g were used. The animals were individually housed in standard wire mesh cages in a constant temperature room (70° F). Two graduated glass drinking tubes (Richter tube, supplied by Kimax Instrument Co.) were fitted onto the front wall of the cages. One tube was filled with water and the other one with ethanol solution (the alcohol solution was diluted fresh each day from 95% ethanol with tap water on a volume to volume basis to the concentration required). The tubes were randomly rotated each day to prevent the development of a position habit (Myers and Holman, 1966). The preference aversion cut-off concentration was determined for each rat according to the method of Amit *et al.* (1970). Food, water and ethanol were available *ad libitum*. Measurements were taken each day at 10 A.M. for alcohol and water consumptions and body weight. A stable base-line of consumption of each rat was established after at least 4 days. The effects of intracisternal injections of 5,6-DHT on alcohol, water and food consumptions; body weight, body temperature and various gross behavioral changes were observed together with the measurement of brain ACh and 5-HT levels and choline transferase activities. 5,6-DHT (supplied by Regis Chemicals Co.) was dissolved in a chilled solution of 0.1% ascorbic acid in normal saline and made up to a final concentration of 75 µg free base in an injection volume of 20 µl. Intracisternal injection was administered under light ether (diethyl ether, U.S.P.) anaesthesia, according to the technique of Schanberg *et al.* (1967). The control rats were similarly treated with the vehicle solution. The accuracy of the injection procedures was tested by injecting the same volume of Indian ink. Thirty minutes after injection, the ink was found to have penetrated widely in all the ventricular space. In addition, the changes in rectal temperature after 5,6-DHT was also used as an index of successful injections. Rectal temperature was recorded in each rat using a telethermometer inserted at least 5 cm into the rectum at 10, 30, 40 min, 1 and 2 hrs

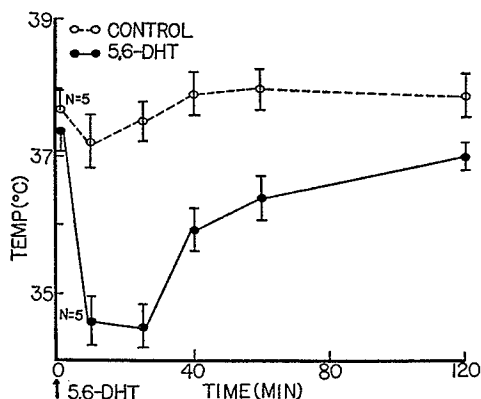


Fig. 1. Graphs showing the effects of 5,6-dihydroxytryptamine (75 μ g/rat) on rectal temperature of rats following an intracisternal injection. Body temperature expressed in $^{\circ}$ C as mean \pm S.E.M. vs time in minutes

after 5,6-DHT treatment. At the end of the treatment period, the rats were killed and the brains were removed over ice.

In a group of 23 rats, the effects of 5,6-DHT on brain ACh, choline transferase (ChAc) activities and 5-HT were estimated on the 8th day after treatment. The brains were dissected into two halves along the mid-line; one half was extracted for ACh and the other half for ChAc determinations.

For ACh extraction, the method described by Hebb (1963) was used. The brains were homogenized in 4 volumes of 10% trichloroacetic acid, centrifuged, washed and the combined supernatant was further extracted with ether to remove the lipids. The last trace of ether was removed by aspiration. ACh was assayed using the guinea-pig ileum preparation in the presence of morphine and diphenylhydramine (Bentley and Shaw, 1952). The response to the extracts was blocked by atropine. ChAc activity was determined using 1-(14 C)-acetylcoenzyme A as substrate according to the method described previously (Ho *et al.*, 1971). In another group of 8 rats, the level of brain 5-HT was determined. 5-HT was extracted in 4 volumes of 0.4 N perchloric acid and n-butanol; assayed fluorometrically as described previously (Ho *et al.*, 1971).

Results

Within the same strain of rats, there were significant individual variations in the preference-aversion cut-off concentrations. In a group of 30 rats tested, the preference-aversion cut-off concentrations ranged from 3 to 11% with the majority of the animals at 5%. Mean daily consumptions of water and alcohol (5%) recorded were 95.0 ± 5.0 ml/kg and 5.0 ± 0.4 ml/kg respectively. Following an intracisternal injection of 75 μ g of 5,6-DHT, there was a marked decrease in rectal temperature which lasted for about 2 hrs (Fig.1). Behaviorally, the rats appeared sedated, lethargic, and there was a loss in body weight within the first 2 days, probably due to a significant decrease in food and water intake

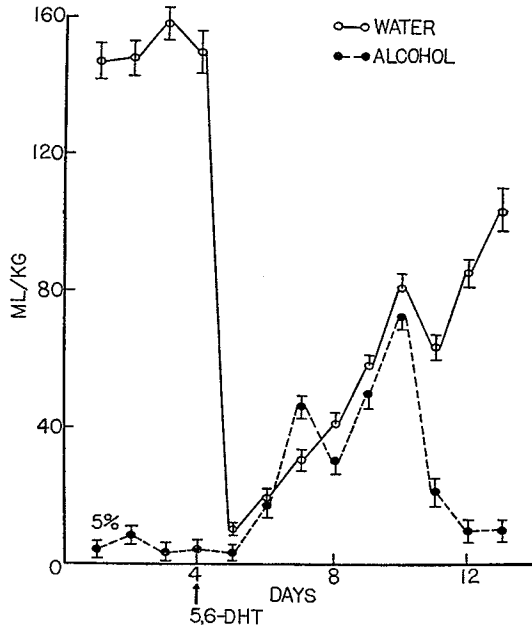


Fig. 2. Effects of 5,6-dihydroxytryptamine ($75 \mu\text{g}/\text{rat}$) on alcohol selection. Alcohol (5% solution) and water consumptions were expressed as ml/kg. Each value was taken from a group of 5 rats and expressed as mean \pm S.E.M. * Significant, $P < 0.05$ to 0.001

($P < 0.05$). Food and water consumptions usually recovered within 3 days after treatment. The animals appeared apprehensive and sensitive to external stimuli such as handling. There appeared to have been no apparent change in urinary output following recovery from 5,6-DHT.

Two groups of rats were given free choice between water and alcohol. In a group of 9 rats, ethanol was presented at 1% below their individual cut-off concentrations on the 4th day after treatment. The selection of ethanol began to increase significantly and this effect lasted for 5 to 6 days. At the same time, there was a reduction in water consumption (Fig. 2). The effects of 5,6-DHT varied with individual rats in the duration of response to increase of ethanol consumption. In the second group of 9 rats, NVP (5 mg/kg, i.p.) was administered every twelve hourly on the 8th and 9th days after 5,6-DHT treatment. At this time, ethanol consumption was found to have increased more than $10\times$ above the base-line level. The selection of ethanol induced by 5,6-DHT was significantly reversed by the treatment with NVP. However, 2 days after the injection of NVP, the animals resumed their increase in the consumption of ethanol (Fig. 3).

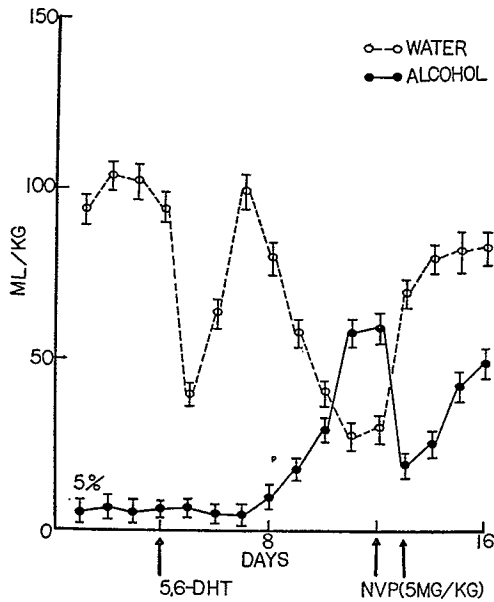


Fig.3. The antagonistic effects of 4-(1-naphthylvinyl) pyridine (NVP) on the 5,6-dihydroxytryptamine ($75 \mu\text{g}/\text{rat}$) induced alcohol preference. Alcohol (5%) and water consumptions were recorded daily from a group of 9 rats. Values were expressed in ml/kg as Mean \pm S.E.M. Drugs were given on the days indicated by (\uparrow)

Table 1. The effects of 5,6-DHT on ACh and 5-HT levels in rat brains

Days of 5,6-DHT treatment	ACh $\mu\text{g}/\text{g}$ wet wt.	5-HT $\mu\text{g}/\text{g}$ wet wt.
0	0.20 ± 0.02 ($N = 12$)	0.52 ± 0.01 ($N = 4$)
8	* 0.38 ± 0.05 ($N = 6$)	* 0.30 ± 0.02 ($N = 4$)

* $P < 0.001$

Table 1 showed the results obtained on the levels of brain ACh and 5-HT after treatment with 5,6-DHT. The level of ACh was found to increase most significantly ($P < 0.001$). Similarly, the mean brain level of 5-HT showed a significant reduction by 42% compared with the control. On the other hand, there was no significant change in the level of ChAc. In order to examine the specificity of the 5,6-DHT induced changes on ethanol selection both quinine ($4 \times 10^{-4}\%$) and saccharin (0.005 M)

solutions were also used in addition to ethanol. Results obtained showed that in a group of 8 rats pre-treated with 5,6-DHT, there was no significant change in the selection of quinine solution vs. water before and after treatment. Similarly, in another group of 8 rats there was a decrease in the selection of saccharin solution vs. water, whereas there was an increase in the selection of alcohol saccharin mixture vs saccharin solution.

Discussion

The results obtained clearly showed that the selection of alcohol and the brain ACh level increased significantly 8 days after treatment with 5, 6-DHT. The level of 5-HT was reduced significantly at this time. Our findings on the 5-HT level appeared to agree with the observations by Baumgarten *et al.* (1971, 1972) that the prolonged depletion of 5-HT produced by 5,6-DHT was due to a specific degeneration primarily in the indolamine-containing axons and axon terminals. The increase in the level of brain ACh but not in the ChAc activity was interesting in that it correlates well with the increase in ethanol selection. Furthermore, this increase induced by 5,6-DHT was antagonized by NVP. Although NVP is known to inhibit ChAc *in vitro* and *in vivo* (Crispin-Smith *et al.*, 1967; Goldberg *et al.*, 1971), the dose used in this study caused only a slight inhibition of brain ChAc. Thus, the possibility that NVP produces inhibitory effects on the alcohol metabolizing enzymes, such as alcohol dehydrogenase and aldehyde dehydrogenase, causing a "disulfiram-like" response should not be excluded. Work is now in progress to study these effects. Nevertheless, NVP (5 mg/kg i.p.) given twice a day produced a significant reduction in the brain level of ACh in C₅₇B1/6J mice and a significant reduction in ethanol consumption (unpublished data). The fact that 5,6-DHT produced no significant increase in the selection of either quinine or saccharin solution alone indicated that the response to alcohol selection was relatively specific. Like other neurotoxic agents, 5,6-DHT may act as a noxious agent causing behavioral aberrations to which alcohol may give some relief. Since both pCPA and 5,6-DHT produce depletion of brain 5-HT, our findings on the increase in alcohol selection appeared to be in good agreement with the observation by Geller (1973) who reported pCPA increased alcohol preference.

The evidence presented in this study is suggestive of a possible interaction between the central cholinergic and serotonergic systems which may be responsible for the increase in the selection of alcohol.

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