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The Effect of 5,7-Dihydroxytryptamine Treatment on the Response to Ethanol in Mice

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MELCHIOR, C L AND B TABAKOFF *The effect of 5,7-dihydroxytryptamine treatment on the response to ethanol in mice* PHARMACOL BIOCHEM BEHAV 24(4) 955–961, 1986 —In order to assess the role of the serotonergic system in the development of tolerance to ethanol in the mouse, serotonin neurons in the CNS were lesioned with an intracerebroventricular injection of the neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) Mice injected with 5,7-DHT responded to an acute dose of ethanol with a longer sleep time and greater fall in body temperature than CSF-treated mice The increased response to acute administration of ethanol was accompanied by higher circulating levels of ethanol in mice pretreated with 5,7-DHT When mice were fed an ethanol-containing liquid diet for five days, a higher mortality rate was observed in the 5,7-DHT group compared to the CSF pretreated group of mice When the groups of mice were tested for tolerance 24 hours after withdrawal, the 5,7-DHT group was less tolerant than the CSF group Therefore, damage to the serotonin neurons results in altered ethanol disposition, altered initial sensitivity to ethanol, and an inhibition in the development of tolerance in the mouse

Ethanol tolerance Serotonin Mouse

ATTEMPTS to determine the involvement of particular neurochemical systems in the development of tolerance to ethanol have focused on the serotonergic and noradrenergic systems. Several studies, in which rats were used as experimental animals, have shown that the serotonergic system plays an important role in the development of tolerance to ethanol. Depletion of brain serotonin levels produced by drug treatments or electrolytic lesions of the median raphe nucleus retarded the development of tolerance, whereas enhancement of serotonin levels with tryptophan accelerated the rate of development of tolerance [5, 8–10, 13–16]. Depletion of norepinephrine alone had little effect, but depletion of both norepinephrine and serotonin was more effective than the depletion of serotonin alone in inhibiting the development of tolerance [16] On the other hand, previous studies from our laboratories, using mice instead of rats as the experimental subjects, have shown that depletion of central noradrenergic levels with an intraventricular injection of 6-hydroxydopamine (6-OHDA) blocks the development of tolerance.

Two types of chronic tolerance have been described [7, 17, 29], which we have referred to as environment-dependent and environment-independent tolerance [29]. Environment-dependent tolerance is a learned phenomenon, whereas environment-independent tolerance can be exhibited in the absence of any cues previously associated with the administration of the drug.

Both types of tolerance to ethanol in the mouse can be blocked by treatment of the animals with 6-OHDA [22,30]. In examining the effect of serotonin depletion with the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT), it was found that this treatment accelerated the rate of development of environment-dependent tolerance [22]. While these results are not consistent with those found using the rat, it is possible that the type of tolerance examined, as well as the species of experimental subject used, may be critical in determining the effect of a neurochemical manipulation. Therefore, the present studies were designed to assess the importance of the serotonergic system in environment-independent tolerance in the mouse.

METHOD

Male C57B1/6 mice, weighing 22–25 g, were maintained in a 12-hour light/dark cycle at an ambient temperature of 23±1°C. All mice were injected intraventricularly (ICV)

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TABLE 1
BRAIN SEROTONIN LEVELS AFTER 5,7-DHT

μg 5,7-DHT Injected	N	nM/g 5-HT	Percent of Control
0	8	5 44 ± 0 14*	—
12 5	6	3 03 ± 0 13†	55 7
25 0	7	1 85 ± 0 12†	34 0
50 0	8	0 29 ± 0 04†	5 3

*Values represent mean ± S E M
†$p<0$ 01 compared to all other groups, Newman-Keuls test

under ether anesthesia with a 10 μl volume of solution as previously described [30]. 5,7-DHT-treated mice received a solution containing 5,7-dihydroxytryptamine creatinine sulfate (5,7-DHT) in artificial cerebrospinal fluid (CSF) [23] Ascorbic acid (0 2 mg/ml) was added to prevent oxidation of the 5,7-DHT. The control group of mice received the carrier solution (i e , CSF plus ascorbic acid) All mice were given 25 mg/kg of desmethylimipramine (DMI) 45–60 minutes before the ICV injection to protect the noradrenergic neurons from the neurotoxic action of 5,7-DHT

Measurement of Brain Serotonin Levels

One set of animals was sacrificed two weeks after the injection of CSF or 12 5, 25.0 or 50 μg (free base) of 5,7-DHT. Following decapitation, the brains were removed and analyzed for serotonin content by high pressure liquid chromatography [26]

Measurement of Body Weight and Fluid Intake

Since serotonin depletion has been reported to influence food and fluid consumption [1,], and ethanol metabolism can be influenced by alterations in nutritional status and body water content [7, 18, 19], the effect of 5,7-DHT on body weight and fluid intake was assessed Mice were individually housed and given food and water ad lib. Water was available from an inverted, 15 ml graduated cylinder and fluid intake was measured daily The mice were weighed every other day. Baseline values were determined for four days. On the fifth day, mice were intraventricularly injected with CSF or 12 5, 25 0 or 50 0 μg of 5,7-DHT All mice were given DMI 45–60 minutes before the ICV injection, as described above Body weight and fluid intake were then measured for 17 days after injection This time period encompasses the duration of the chronic ethanol experiments (see below) The data were subjected to repeated measures analysis of variance followed by Dunnett's *t*-test

Measures of Behavioral Effects of Ethanol and Barbiturates

Rectal temperature was measured by a telethermometer attached to a rectal probe inserted 2.5 cm into the colon of the mouse After allowing 30 seconds for equilibration, core body temperature was recorded Body temperature was again measured at 30 min after the injection of 3 5 g/kg ethanol, 100 mg/kg sodium barbital, or 50 mg/kg sodium pentobarbital

The elapsed time between loss and regain of the righting

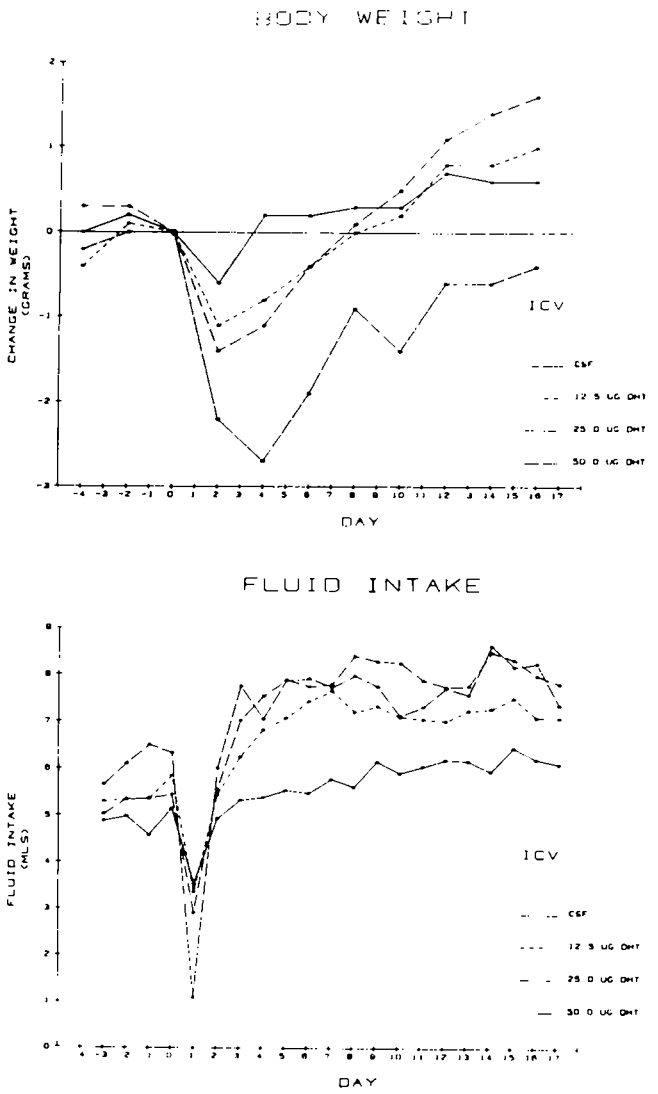

FIG 1 Body weight (top) and fluid intake (bottom) measured after an ICV injection of CSF or 12 5, 25 0 or 50 0 μg of 5,7-DHT on Day 0 N=8 per group S E M s are less than 0 8 grams and 0 8 mls

reflex (sleep-time) was also measured Cross-tolerance between ethanol and sedative barbiturates is well known, barbital was selected for comparative purposes because it is eliminated with only a small amount being metabolized [11], while ethanol is nearly totally converted *in vivo* to carbon dioxide and water CSF- and 12 5 μg 5,7-DHT-treated mice were used Student's *t*-tests were employed to evaluate the difference between the responses of the two groups to each drug

Measurement of Brain Ethanol Levels

At one week after the injection of CSF or 12 5 μg 5,7-DHT, mice were injected intraperitoneally with 3 5 g/kg ethanol. At 10, 30 or 60 minutes after injection of ethanol, animals were decapitated and the brain removed for the

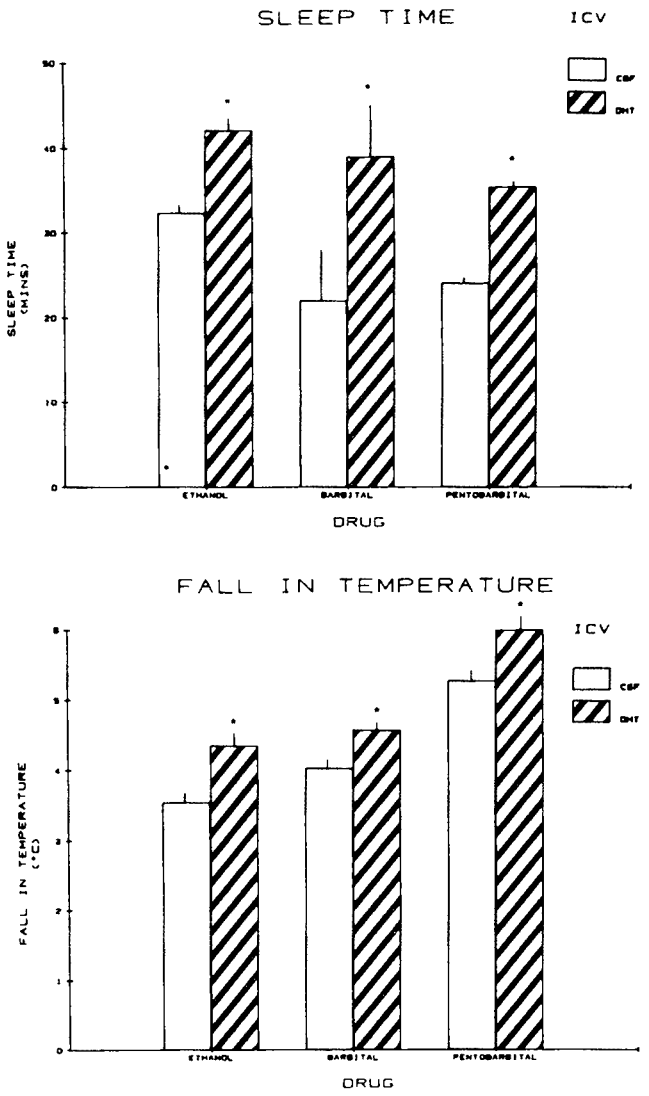

FIG 2 Sleep-time and change in temperature was measured after an injection of 3 5 g/kg ethanol, 100 mg/kg barbital, or 50 mg/kg pentobarbital in mice injected ICV one week earlier with CSF or 12 5 μg 5,7-DHT The data are means±S E M s N=20–30 per group *$p<0$ 01, compared to CSF, Student's t-test

analysis of ethanol levels. Ethanol levels were determined by a head space gas chromatographic technique [28]. The times of sacrifice were selected to represent the approximate times of peak brain and blood levels of ethanol [20,28], and to encompass the times of behavioral measurements

Ethanol Disposition

Blood ethanol levels were determined in mice injected with CSF or 12 5 μg 5,7-DHT. A 20 μl sample of blood was obtained from the tail at 45-minute intervals between 90 and 270 minutes after the intraperitoneal injection of 3 5 g/kg ethanol. The samples were tested for ethanol levels by gas chromatography. Rate of ethanol elimination, rate of decay from blood, and volume of distribution were calculated for each animal, then averaged within groups. Significant differences were determined with Student's t-tests

Chronic Ethanol Administration

One week after the ICV injections, mice were individually caged and placed on a control, Carnation Slender liquid diet for 24 hours [24]. After 24 hours, half of the animals which had been injected with 12.5 μg 5,7-DHT and half of those injected with CSF were given a 7 percent v/v ethanol-containing liquid diet, while the other half of the animals were pair-fed on the isocaloric control liquid diet, as has previously been described [24]. After five days of ethanol consumption, the ethanol-consuming mice were again given the control liquid diet. Statistically significant differences in mortality rate between the 5,7-DHT and CSF groups during exposure to the diet and during withdrawal were determined with χ^2 tests.

Tolerance was tested at 24 hours after withdrawal, when behavioral signs of alcohol withdrawal (i.e., hyperexcitability and hypothermia [24]) were no longer apparent Tolerance was assessed by measuring the sleep-time and fall in body temperature following an IP injection of 3.5 g/kg ethanol in the 5,7-DHT- and CSF-treated mice exposed to either the ethanol or control diet. Statistical significance was determined by analysis of variance, followed by Newman-Keuls tests

Response to ICV Administration of Ethanol

Mice were implanted with cannulae [6] so that ethanol could be administered ICV Using this route of administration, a particular amount of ethanol can be delivered directly to the brain, thus eliminating the contribution of any peripheral alterations of ethanol disposition to the response observed.

Three days after the cannulae were implanted, the animals were injected ICV with CSF or 12.5 μg of 5,7-DHT in a 10 μl volume, with the appropriate DMI pretreatment. One set of animals was tested for its response to ICV ethanol one week after treatment with CSF or 5,7-DHT. Another group of animals started the liquid diet procedure at one week after the ICV injections and was tested for tolerance at 24 hours after withdrawal by injecting ethanol ICV.

For testing, a 10 μl volume of 20% ethanol [24], prepared with artificial CSF [23] was slowly injected into the unrestrained mouse Body temperature was measured before and five minutes after injection, since pilot studies showed that five minutes was the time of maximal temperature change with this procedure CSF alone did not affect body temperature

RESULTS

Serotonin Depletion

As shown in Table 1, a dose-dependent depletion of whole brain serotonin levels was produced by treatment of animals with increasing doses of 5,7-DHT. The 12.5 μg dose of 5,7-DHT reduced serotonin levels to approximately 50% of control Norepinephrine and dopamine levels are unaffected by injection of these doses of 5,7-DHT to animals treated with DMI [22]

Body Weight and Fluid Intake

Mice injected with 5,7-DHT demonstrated dose-dependent changes in body weight, $F(3,21)=4.539$, $p<0.05$. As shown in Fig. 1, the initial fall in body weight was followed by a rapid increase. However, the group injected with

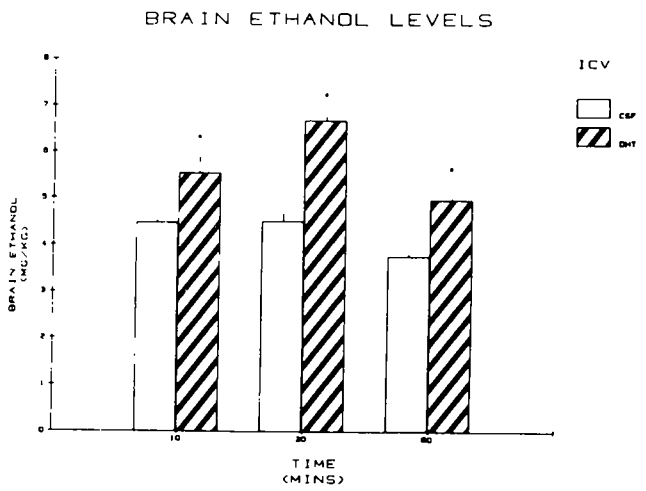

FIG 3 Ethanol levels in the brain were measured at 10, 30 and 60 minutes after the injection of 3 5 g/kg ethanol in mice injected ICV one week earlier with CSF or 12 5 μg 5,7-DHT The data are means±S E M s N=10–18 per group *$p<0$ 01, compared to CSF, Student's t-test

TABLE 2
ETHANOL DISPOSITION

	CSF (N=9)	5,7-DHT (N=7)
Ethanol elimination* mg/kg/hr	521 1 ± 15 0†	436 7 ± 15 8‡
Decay from blood mg/dl/hr	74 1 ± 1 8	81 6 ± 1 9‡
Volume of distribution g/l	0 719 ± 0 016	0 536 ± 0 018‡

*R=B × [A − (C × W)], where R is the rate of ethanol elimination, B is the rate of decay of blood ethanol concentration, A is the dose of ethanol administered, C is the apparent concentration of ethanol at zero time (obtained by extrapolating the line for blood ethanol decay to the y axis), and W is the body weight [18, 32, 33] Volume of distribution = A − (C × W)
†Mean ± SEM
‡$p<0$ 01, t-test

12.5 μg of 5,7-DHT was not significantly different from the CSF group

Dose-dependent alterations in fluid intake were also observed, $F(3,21)=5$ 251, $p<0.01$ Figure 1 shows that all groups reduced their fluid intake during the first 24 hours after injection Thereafter, the CSF-treated mice returned to baseline levels while all the 5,7-DHT-treated mice proceeded to a significantly higher level of intake.

The 12 5 μg dose of 5,7-DHT was selected for use in examining the response to ethanol based on the data above Since a comparison to previous studies with norepinephrine depletion was intended, a reduction of serotonin of approximately 50 percent was appropriate The 12 5 μg dose also had the advantage of not significantly affecting body weight

Behavioral Effects of Ethanol and Barbiturates

As shown in Fig 2, mice treated with 5,7-DHT had a greater fall in body temperature in response to ethanol, barbital, and pentobarbital than did mice given CSF The sleep-time response to each of these compounds was significantly greater for the 5,7-DHT-treated mice than the CSF group For mice given ethanol, the blood alcohol levels at regain of righting reflex were significantly greater ($t=22$ 971, $p<0$ 01) for the 5,7-DHT group, 536 6±14 3 mg/dl (mean±SEM, N=10), than the CSF group, 360 7±5.8 mg/dl (N=6)

Ethanol Levels

As seen in Fig 3, the brain levels of ethanol were higher at all time points in mice treated with 12 5 μg 5,7-DHT than those treated with CSF Higher ethanol levels in the 5,7-DHT-treated mice were reflected in the significant differences in the various dispositional parameters calculated according to the Widmark [32] equation (Table 2), i e , slower rate of elimination, faster rate of disappearance from blood, and smaller volume of distribution

Chronic Ethanol

Daily consumption of the ethanol-containing liquid diet was approximately 14 ml (range=7–16 ml) and did not differ significantly between the CSF- and 5,7-DHT-treated mice Tail blood ethanol levels obtained at 8–9 00 a m on the day prior to withdrawal were as follows CSF=215 3±31 9 mg/dl (mean±SEM, N=18) and 5,7-DHT=409 6±30 1 mg/dl (N=20, $t=4$ 426, $p<0$ 01) On the morning of withdrawal, blood ethanol levels were as follows CSF=375 8±34 9 mg/dl (N=17) and 5,7-DHT=360 2±41 1 mg/dl (N=19) Samples were obtained from different animals on the two days

As shown in Fig 4, compared to the CSF-treated animals significantly fewer ($\chi^2=7$ 046, $p<0$ 01) of those mice injected with 5,7-DHT survived being fed the ethanol-containing liquid diet (5,7-DHT 46/62 vs CSF 43/45) Of the mice surviving to the time of withdrawal, significantly less ($\chi^2=21$ 036, $p<0$ 01) of the 5,7-DHT- than CSF-treated mice survived withdrawal (18/46 vs 38/43) This may be attributed to a greater amount of convulsive activity [24] as well as a more severe hypothermia (data not shown) in the 5,7-DHT group

When tested for tolerance with an IP injection of 3 5 g/kg ethanol, both the 5,7-DHT- and CSF-treated mice exposed to the ethanol-containing liquid diet demonstrated tolerance on both the sleep-time, $F(3,110)=19$ 541, $p<0$ 01, and temperature, $F(3,110)=8$ 349, $p<0$ 01, measures compared to mice given the control diet (Fig 5) However, on both measures, the CSF-treated mice were more tolerant than the 5,7-DHT-treated mice ($p<0$ 01, Newman-Keuls test)

As with the acute experiments, in the mice given the control diet, the 5,7-DHT-treated animals were significantly more affected by ethanol than the CSF-treated mice Therefore, the data were also analyzed by calculating the response of each animal exposed to the ethanol diet as a percent of the mean response of the appropriate (i e , 5,7-DHT- or CSF-treated) group exposed to the control diet Following an arcsine transformation to make the data appropriate for

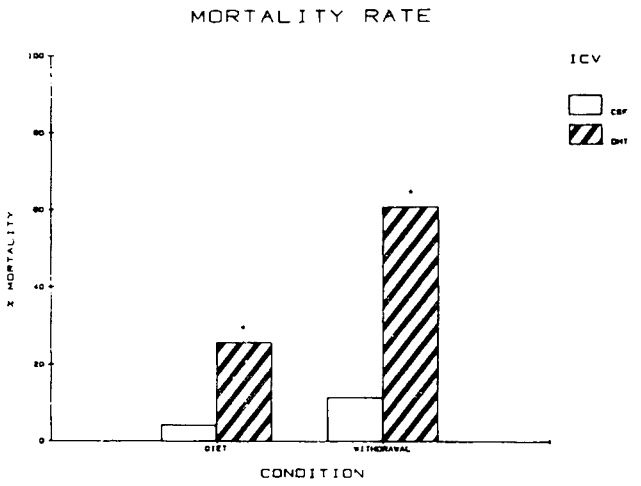

FIG 4 The percentage of mice surviving the five day ethanol diet treatment and subsequent withdrawal period was determined *$p<0.01$, compared to CSF, χ^2

TABLE 3
FALL IN TEMPERATURE AFTER ICV ETHANOL

	CSF	5,7-DHT
Control Diet	1 77 ± 0 07 (6)*	2 40 ± 0 09 (10)†
Ethanol Diet	0 73 ± 0 10 (9)‡	2 74 ± 0 06 (14)†
Ad libitum Chow	1.98 ± 0 10 (8)	2 94 ± 0 06 (10)†

*Mean ± SEM, °C The number in parenthesis is the number of animals per group

†$p<0.01$, Newman-Keuls, compared to CSF group of the same diet

‡$p<0.01$, Newman-Keuls, compared to all other groups

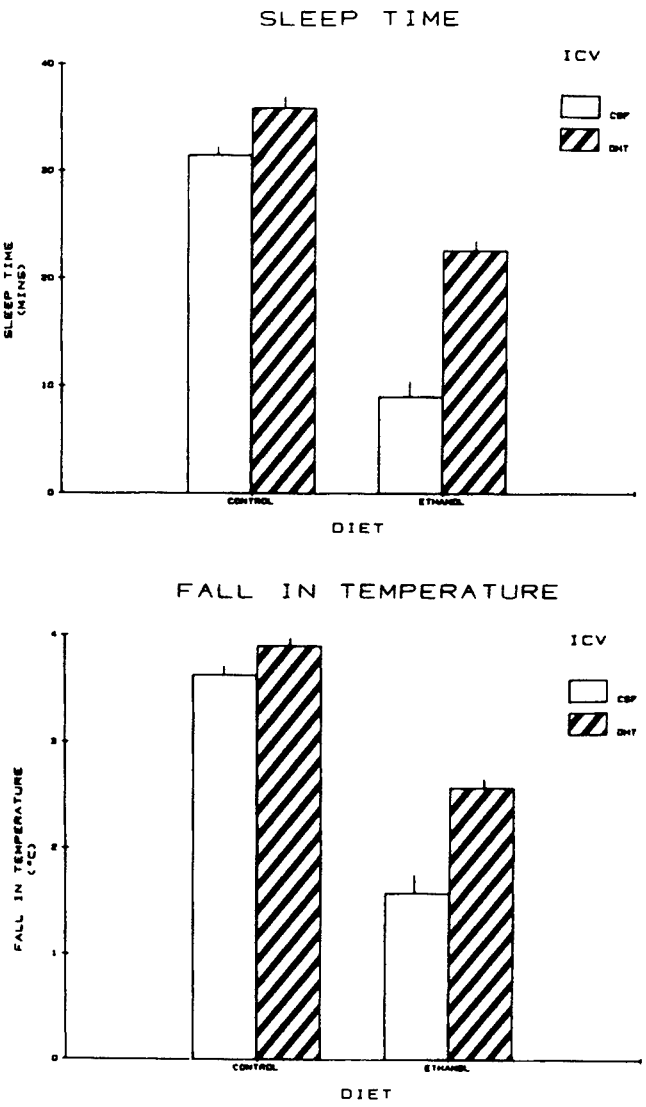

FIG 5 Mice given ICV injections of CSF or 12 5 μg of 5,7-DHT were placed on a liquid diet containing ethanol or a control liquid diet for five days At 24 hours after withdrawal, all mice were injected with 3 5 g/kg ethanol and sleep time and fall in body temperature were measured The data are means±S.E M s. N=18–30 per group All groups are different from all others ($p<0.01$ Newman-Keuls tests)

parametric analysis, a t-test showed that the CSF group had developed significantly more tolerance than the 5,7-DHT-treated mice (Sleep-time: $t(54)=5.043$, $p<0.01$; temperature· $t(54)=3.263$, $p<0.01$)

Response to ICV Ethanol

As found after IP injections of ethanol, the response to ethanol administered ICV was greater in the mice treated with 5,7-DHT than those treated with CSF (Table 3). This was observed across all dietary treatments, $F(1,51)=325.520$, $p<0.01$

The CSF-treated mice given the ethanol diet showed tolerance compared to CSF-treated mice given the control diet The 5,7-DHT mice, in contrast, did not demonstrate tolerance after exposure to the ethanol diet

DISCUSSION

This study shows that the serotonergic system is important for determining the response to both acute and chronic treatment with ethanol in the mouse

An alteration in the disposition of ethanol was a critical feature of the 5,7-DHT treatment Since the disposition of ethanol may be affected by changes in nutritional status and body water content [7, 18, 19], this finding may be a reflection of changes in the internal state of the animals which are suggested by the influence of serotonin depletion on body weight and fluid intake While there were dose-dependent changes in body weight following ICV injections which were consistent with the established effects of serotonin depletion on food intake [1], within the time period in which the responses to ethanol were tested, the CSF and 12 5 μg 5,7-DHT-treated groups had similar body weights. However, all the 5,7-DHT-treated mice were polydipsic compared to the

CSF mice Although there are conflicting reports [2,27], polydipsia has previously been shown to occur following different types of treatments which reduce brain serotonin levels [3,34]

Previous studies of the influence of serotonin depletion on the initial response to the hypnotic effects of ethanol or barbiturates have reported either an enhanced response [4, 10, 11, 25, 35], like that seen in this study, or no effect [12,21] The higher levels of ethanol in the brains of the 5,7-DHT mice undoubtedly contribute to the greater response to ethanol in these mice compared to the mice given CSF However, the 5,7-DHT group recovers righting reflex at higher ethanol levels than the CSF group This may simply reflect the fact that the 5,7-DHT group has a greater impetus to develop acute tolerance [25,31], or indicate that the 5,7-DHT-treated mice are actually *less* sensitive to the hypnotic effects of ethanol than the CSF group The latter possibility is certainly not true with regard to the hypothermic effects of ethanol, since the responses observed following ICV administration of ethanol were far greater in the 5,7-DHT group

During exposure to the ethanol-containing liquid diet, ethanol consumption was similar for the CSF- and 5,7-DHT-treated mice However, the mortality rates indicated that the 5,7-DHT group was more affected than the CSF group The high mortality rate for the 5,7-DHT group during exposure to the diet may be explained by both the presence of higher levels of ethanol in the bodies of the animals and an increased sensitivity to the hypothermic effect of ethanol

This study was designed to parallel previous studies from this laboratory examining the effects of NE depletion in the mouse on tolerance to ethanol so that comparisons could be made using the same model However, the high mortality rate of the 5,7-DHT-treated mice on the ethanol diet dictated that the duration of ethanol exposure be reduced from seven days to five days Maximal tolerance has previously been shown to occur within this time [24] Accordingly, the CSF group in this study demonstrated a significant degree of tolerance on both the sleep time and temperature measure The 5,7-DHT-treated mice were less tolerant than the CSF group when considered either in terms of magnitude of response compared to the CSF group exposed to ethanol, or in terms of differences in response of mice given the control vs ethanol diet in the two treatment groups The difference in the degree of tolerance developed cannot be explained on the basis of differential exposure to ethanol, since exposure to higher levels of ethanol should lead to the development of more, rather than less, tolerance [7] The higher mortality rate during withdrawal in the 5,7-DHT group, however, could be explained by greater exposure to ethanol Since withdrawal severity is increased while tolerance is decreased, these results support the idea that dependence and tolerance are not determined by identical mechanisms [29,30]

In the mice used for these experiments the 12 5 μg dose of 5,7-DHT resulted in approximately 50 percent depletion of central serotonin levels A similar degree of depletion of NE was effective in blocking the development of tolerance in mice [30] In the studies of serotonin depletion in rats, a much greater depletion of serotonin was reported to be required to be effective [11] While it has been hypothesized that the hippocampus is a critical site in mediating the development of tolerance in the rat [15], the anatomical sites important in the development of tolerance in the mouse have not been defined It would, therefore, be difficult to discuss the relative importance of the serotonergic systems in tolerance development in rats and mice based on comparisons of levels of serotonin in the whole brain

In conclusion, these studies show that depletion of serotonin with 5,7-DHT in the mouse causes significant changes in the animals' response to ethanol An increase in the initial response to ethanol appears due to both the presence of higher ethanol levels in mice injected with 5,7-DHT as well as an increase in sensitivity to ethanol The ability to develop tolerance is also significantly impaired

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