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Ethanol-Induced Changes in Tyrosine Hydroxylase Activity in Brains of Mice Selectively Bred for Differences in Sensitivity to Ethanol[1]

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BAIZER, L., J. M. MASSERANO AND N. WEINER. *Ethanol-induced changes in tyrosine hydroxylase activity in brains of mice selectively bred for differences in sensitivity to ethanol.* PHARMAC. BIOCHEM. BEHAV. 15(6) 945–949, 1981.—The effects of ethanol on tyrosine hydroxylase (TH) activity in five brain areas were analyzed in two lines of mice selectively bred for their differences in sensitivity to ethanol. Following a 4.1 g/kg dose of ethanol, intraperitoneally, short sleep (SS) mice lose their righting reflex for a duration of 20 minutes and long sleep (LS) mice fail to regain their righting reflex until 120 minutes. A significant increase in TH activity occurred in the striatum, locus coeruleus and frontal cortex in both lines of mice approximately 25 minutes following ethanol administration. A decrease in TH activity occurred in the substantia nigra of SS mice at 5 minutes following ethanol administration. However, there was no significant difference in TH activity in any of these four brain regions between LS and SS mice at any time following ethanol administration. In contrast, hypothalamic TH activity was significantly increased at 25 minutes in the SS mice and at 125 minutes in the LS mice following the administration of ethanol, times which coincided with the regaining of the righting reflex. These data suggest that activation of TH in the hypothalamus of LS and SS mice in response to ethanol is associated with arousal from ethanol induced narcosis.

Genetics Ethanol Tyrosine hydroxylase Hypothalamus

McCLEARN and Kakihana [19] have developed by genetic selection two lines of mice that differ in the duration of the loss of the righting reflex following the administration of ethanol. The mice have been designated long sleep (LS) and short sleep (SS). Heston *et al.* [11] and Erwin *et al.* [9] concluded that the two lines of mice differ in their central nervous system (CNS) sensitivity to ethanol since there is no difference in the rates of ethanol metabolism or in the distribution of ethanol in the two lines. Furthermore, the durations of loss of righting reflex produced by nonalcoholic sedative-hypnotic compounds [9] and the general anesthetic, halothane [1], are similar in the two lines of mice.

A number of studies suggests that the differences in loss of righting reflex produced by ethanol between the two lines of mice may be related to differences in responses in catecholamine systems in the brain [4, 5, 7, 8]. Dibner *et al.* [7] reported that β-adrenergic receptor density was lower in cortices of LS mice as compared to the SS mice. Gamma-butyrolactone, which is known to inhibit impulse transmission in dopaminergic neurons, produced a loss of the righting reflex of greater duration after ethanol injection in the LS than in the SS mice [8]. Church *et al.* [4] observed that salsolinol, the dopamine-acetaldehyde condensation product, injected intracisternally into LS and SS mice in the absence of ethanol, produced a loss of righting reflex for a significantly longer time in the LS mice than in the SS mice. Collins *et al.* [5] observed that the turnover rate of norepinephrine was unaffected following ethanol administration, whereas the turnover rate for dopamine was significantly reduced in both lines of mice. However, the LS mice exhibited a larger decrease (65%) in turnover rate than the SS mice (35%). They concluded that the dopamine neural systems in the brain may be involved in the enhanced sensitivity of the LS mice to ethanol.

In our laboratory (J. M. Masserano and N. Weiner, submitted for publication) we found that the injection of norepinephrine or dopamine intraventricularly into the LS mice produced a decrease in the duration of the ethanol induced loss of righting reflex. In contrast, the injection of the catecholaminergic antagonist phentolamine intraventricularly resulted in a significant prolongation of the ethanol induced loss of righting reflex. The SS mice responded differently to the intraventricular injection of the catecholamines. Norepinephrine, dopamine, epinephrine or isoproterenol injected intraventricularly into the SS mice produced a significant increase in the duration of the ethanol induced loss of righting reflex. These results suggest that a relative deficit in either or both the norepinephrine or dopamine systems in the

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brain of LS mice, compared with the SS mice, might be responsible for the increased sensitivity of the LS mice to ethanol.

In an attempt to examine further possible differences in the catecholaminergic systems that appear to exist between the two lines of mice, we chose to investigate the effects of ethanol on tyrosine hydroxylase (TH) activity in several brain areas of the LS and SS mice in the presence and absence of ethanol. The enzyme TH was chosen since it is the rate-limiting enzyme in the pathway concerned with the synthesis of catecholamines [23], and it is activated acutely following a number of treatments including: stimulation of the isolated hypogastric nerve-vas deferens preparation [20,24], in the adrenal gland after stress [18], in the hippocampus following stimulation of the locus coeruleus [21] and in the striatum following administration of antipsychotic agents [15,26].

The present study reports a correlation between the regaining of the righting reflex in the LS and SS mice following a hypnotic dose of ethanol and the activation of TH in the hypothalamus.

METHOD

Animals

Male short sleep (SS) and long sleep (LS) mice weighing between 20 and 30 grams were obtained from the Institute of Behavioral Genetics, University of Colorado, Boulder, CO. The mice were housed 15 per cage and allowed free access to food and water. The mice were of the 25th generation.

Ethanol Treatment

Animals were injected with 4.1 g/kg ethanol (20% v/v ethanol:H_2O), intraperitoneally, and sacrificed at 5, 15, 25, 65, and 125 minutes following ethanol administration. These times were chosen, based on the average duration of the loss of righting reflex for the SS (20 minutes) and LS (120 minutes) mice [11]. Loss of righting reflex is defined as the inability of the mice to right themselves after being placed on their backs. The test was conducted three times in rapid succession every 5 minutes until the righting reflex returned. All mice had loss of the righting reflex 5 minutes following ethanol administration, when the first test of the righting reflex was performed.

Control animals received an injection of saline and were sacrificed 5 minutes following this injection. At the indicated times the animals were decapitated, the brains removed and maintained at 4° on ice. The frontal cortex, striatum, hypothalamus, substantia nigra and locus coeruleus were dissected out according to the methods of Glowinski and Iversen [10] and Zigmond *et al.* [25] and immediately frozen on dry ice.

Tissue Preparation and Assay of Tyrosine Hydroxylase

The frozen brain regions were weighed and homogenized in 3 volumes (frontal cortex), 4 volumes (substantia nigra, locus coeruleus, hypothalamus) and 9 volumes (striatum) of 0.05 M Tris-acetate buffer, pH 6, containing 0.2% Triton X-100. The homogenates were centrifuged at 40,000×G for 30 min at 4°.

TH activity was determined in the supernatant fraction by the coupled decarboxylase assay [22] as modified by Kapatos and Zigmond [13]. The standard assay mixture (15 μl) contained: 200 mM Tris HCl buffer (pH 6), 0.417 mM 6-methyl-5,6,7,8-tetrahydropterin (6-$MePtH_4$), 0.833 mM reduced nicotinamide adenine dinucleotide (NADH), 1300 units of catalase, 0.167 mM 1-^{14}C-L-tyrosine (specific activity approximately 56 mCi/mmole) and 3 μl sheep liver pteridine reductase (prepared according to the method of Kaufman [14]). The 6-$MePtH_4$ was dissolved in 0.01 N HCl and the NADH was dissolved in 10 mM Tris HCl (pH 7.5). Ten μl of the brain supernatant, containing 40–150 μg protein, was added to each assay tube followed by 15 μl of the assay mixture. All constituents of the assay tubes were maintained at 4° during these manipulations.

Following these additions, the reaction was initiated by placing the tubes into a 30° bath. The samples were incubated for 20 minutes and then transferred to an ice bath. The reaction was linear with protein concentration (40–150 μg) and time for at least 30 min for each brain region. At the end of the 20 min incubation period, 10 μl of a mixture containing: 300 mM Tris HCl (pH 6.8), 1.5 mM pyridoxal phosphate, 3.9 mM 3-iodotyrosine and 2 μl hog kidney L-aromatic amino acid decarboxylase (prepared according to Waymire *et al.* [22]) was added. The pyridoxal phosphate and 3-iodotyrosine were dissolved in 10 mM Tris HCl (pH 7.5). The tubes were capped with a rubber septum from which a plastic well containing 100 μl of the CO_2 trapping reagent, NCS® tissue solubilizer (Amersham, Arlington Heights, IL), was suspended, and the second step of the reaction (DOPA decarboxylation) was initiated by placing the stoppered tubes into a 30° bath for 30 min to allow for the quantitative conversion of ^{14}C-DOPA to $^{14}CO_2$ and dopamine. The reaction was stopped by injecting 0.1 ml 10% trichloroacetic acid into the assay tubes, and the tubes were incubated for an additional hour at 30° to trap quantitatively the liberated CO_2. The plastic well was then removed and placed into a scintillation vial to which was added 10 ml scintillation fluid containing: 0.5 g 1,4-bis (2-(4 methyl-5-phenyloxazolyl)) benzene (di-methyl POPOP), 4.9 g 2,5-diphenyloxazole (PPO) and 5 ml ethanol per liter of toluene. Radioactivity was counted by liquid scintillation spectrometry. Counting efficiency was 85%. Protein was determined by the method of Lowry *et al.* [16]. Results are expressed as nmols product formed per mg protein per hour.

RESULTS

No significant differences in TH activity between saline treated LS and SS mice in any of the five brain areas were observed. Figure 1 illustrates the effects of ethanol on TH activity in the substantia nigra. A significant decrease in TH activity in the substantia nigra was observed at the onset of ethanol narcosis in the SS mice. However, no significant differences in TH activity in substantia nigra were apparent at any time after ethanol administration between LS and SS mice.

Figures 2 and 3 illustrate the effect of ethanol on TH activity in the striatum and locus coeruleus. TH activity was significantly increased above control values in the striatum and locus coeruleus in both lines of mice at 15 minutes and 25 minutes following ethanol administration. Likewise, TH activity in the frontal cortex in both lines of mice (Fig. 4) was significantly increased over control at 25 minutes following ethanol. Sixty minutes after ethanol administration, the increase in TH activity in the three brain areas had returned to control values. There was no significant difference in TH activity between the LS and SS mice among the three brain areas at any time after ethanol administration.

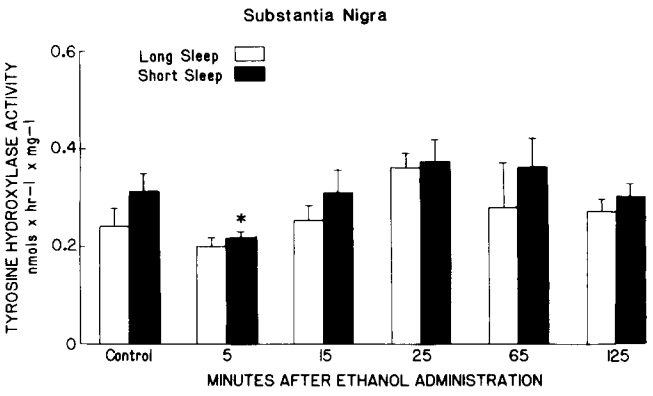

FIG. 1. Tyrosine hydroxylase activity in the substantia nigra after the administration of ethanol. Ethanol was administered by intraperitoneal injection in a dose of 4.1 g/kg. The results are means from ten experiments±SEM. All values were determined at 0.1 mM (1-^{14}C)L-tyrosine and 0.25 mM 6-MePtH$_4$. *$p<0.05$ vs corresponding control value.

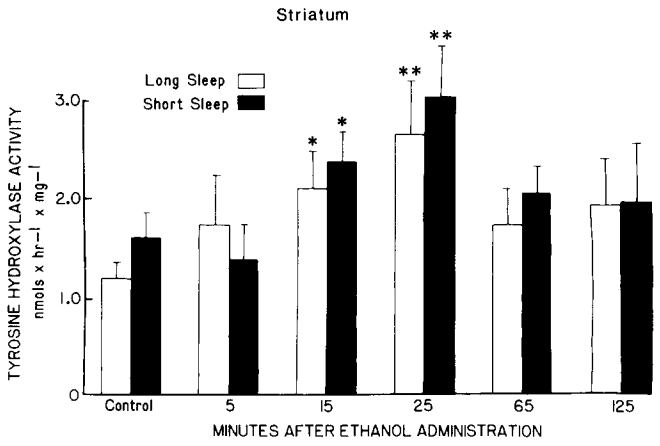

FIG. 2. Tyrosine hydroxylase activity in the striatum after the administration of ethanol. For details, see legend to Fig. 1. **$p<0.01$ vs corresponding control values.

Figure 5 illustrates the effects of ethanol on TH activity in the hypothalamus. In contrast to the other regions of the brains of LS and SS mice, TH in the hypothalamus was differentially activated in the two lines of mice following ethanol injection. A significant increase in TH activity occurred at 25 minutes following ethanol administration in the SS mice and at 125 minutes in the LS mice. These increases in TH activity coincided with the average times for the recovery of the righting reflex in each line. No other changes in TH activity were apparent in this brain area in either line of mice following ethanol.

DISCUSSION

No significant differences were found in basal TH activity of LS and SS mice in the five brain areas examined: striatum,

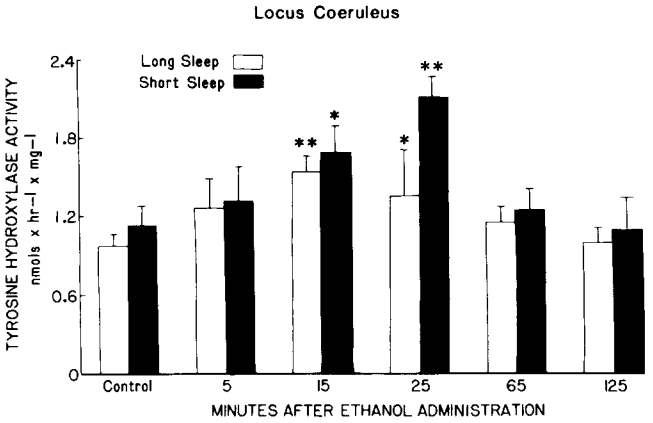

FIG. 3. Tyrosine hydroxylase activity in the locus coeruleus after the administration of ethanol. For details, see legends to Figs. 1 and 2.

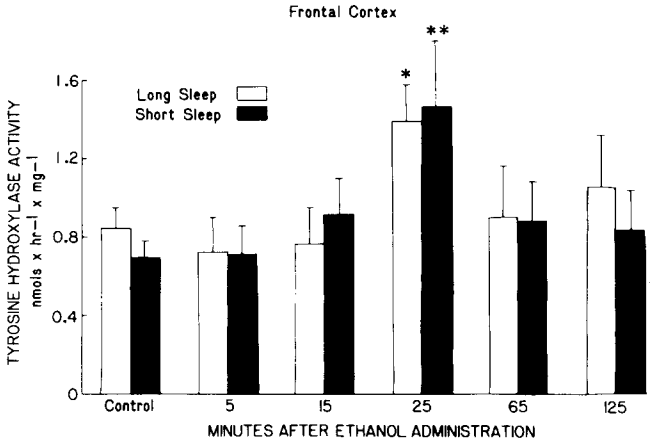

FIG. 4. Tyrosine hydroxylase activity in the frontal cortex after the administration of ethanol. For details, see legends to Figs. 1 and 2.

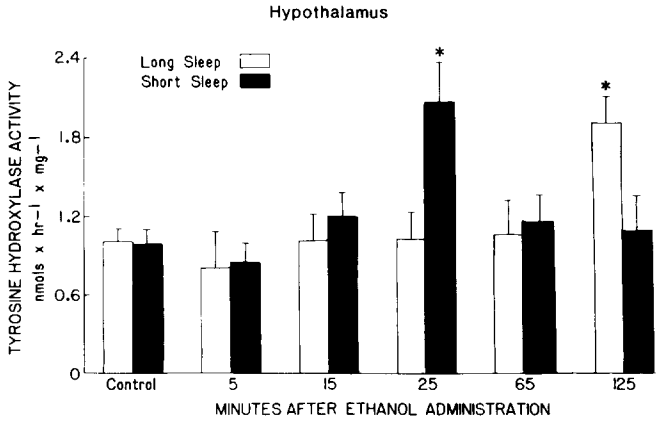

FIG. 5. Tyrosine hydroxylase activity in the hypothalamus after the administration of ethanol. For details, see legend to Fig. 1.

locus coeruleus, frontal cortex, substantia nigra and hypothalamus. This is consistent with the data of Collins *et al.* [5] who reported that whole brain TH activity is not different in the two lines of mice.

The major purpose of the present study was to relate changes in brain TH activity in the LS and SS mice after ethanol to the markedly different durations in the loss of righting reflex exhibited by these lines of mice following ethanol administration. Among the five brain areas assayed for TH activity following ethanol, a relationship between TH activation and the loss of righting reflex of LS and SS mice was noted only in the hypothalamus (Fig. 5). Twenty-five minutes after the administration of ethanol, TH activity increased significantly in the hypothalamus of the SS mice, at the time these animals were recovering from ethanol. An analogous change in TH activity was not seen in the LS mice 25 minutes after ethanol administration. Likewise, at 125 minutes following ethanol, when the LS mice are regaining their righting reflex, the TH activity in the hypothalamus is significantly increased in the LS, but not in the SS, mice. These data suggest that activation of TH in the hypothalamus of the LS and SS mice in response to ethanol is associated with the arousal of the mice from ethanol induced narcosis. The effects of ethanol on TH activity in the hypothalamus of the two lines appears to be peculiar to alcohol since no changes in hypothalamic TH activity were observed in these two lines of mice during induction of, or recovery from, sleep induced by pentobarbital (R. Wimer, J. M. Masserano and N. Weiner, unpublished observation).

A significant increase in TH activity also occurred in the striatum, locus coeruleus and frontal cortex in both lines of mice approximately 25 minutes following the administration of ethanol (Figs. 2, 3, 4). These increases in TH activity were sustained for less than 65 minutes after ethanol and were comparable in both strains of mice. These data suggest that the activation of TH by ethanol in the striatum, locus coeruleus and frontal cortex is not due to a genetically determined difference in ethanol sensitivity in these two outbred lines of mice.

Since ethanol increased TH activity in 4 of the 5 brain areas studied, an increase in brain catecholamine turnover following ethanol administration might be anticipated. Several groups of investigators have reported that a single dose of ethanol given to rats produces an increase in norepinephrine turnover in the brain [3, 6, 12]. Carlsson and Lindqvist [3] also reported that, in the striatum, ethanol administration is associated with an increase in dopamine turnover. The LS and SS mice, however, exhibit no change in norepinephrine turnover and a decrease in dopamine turnover after the administration of a hypnotic dose of ethanol [5]. The exact relationships between the depressant effect of ethanol and its actions on brain catecholamine turnover and brain TH activation remain to be fully clarified.

Ethanol may activate brain TH either directly or indirectly, possibly as a consequence of enhanced catecholaminergic neuronal activity in both SS and LS mice. Bustos *et al.* [2] reported that incubation of rat striatal slices with ethanol (0.2% to 0.8%, w/v) did not affect TH activity. Masserano *et al.* [17] studied the effects of ethanol administration on tyrosine hydroxylase activity in the adrenal gland. No significant change in the activity of adrenal TH was apparent 15 minutes following the oral administration of different doses of ethanol. In contrast, in the present study, we have observed a rapid activation of brain TH in several brain regions following the administration of ethanol.

The present results provide further evidence to support the notion that catecholaminergic neuronal systems may be implicated in the different sensitivities of LS and SS mice to ethanol. The activation of TH in the hypothalamus is temporally correlated with arousal of both lines of mice from ethanol induced narcosis. This effect on hypothalamic TH is not seen in LS and SS mice depressed with pentobarbital. The exact relationship of hypothalamic TH to arousal from ethanol induced sleep remains to be determined.

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