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BRIEF COMMUNICATION

Development of Ethanol Tolerance not Altered by 6-OHDA Lesions of Dorsal Bundle

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STEKETEE, J. D., A. C. SWANN AND P. B. SILVERMAN. *Development of ethanol tolerance not altered by 6-OHDA lesions of dorsal bundle.* PHARMACOL BIOCHEM BEHAV **33**(3) 729–731, 1989.—It has been demonstrated via intraventricular (IVT) 6-OHDA infusions that central nervous system norepinephrine is necessary for the development of tolerance to ethanol (ETOH) in the mouse. Because 6-OHDA IVT infusions are not specific, we tested the effects of destruction of a specific NE pathway on development of ETOH tolerance. Rats received 6-OHDA lesions of the dorsal noradrenergic bundle (DB) and the development of ethanol tolerance was measured using the sleeptime test. The time between loss and recovery of the righting reflex was determined after an acute challenge dose of ETOH. Rats were then placed on ETOH diet for 13 days followed by a repeat of the sleeptime test. Sham and 6-OHDA-DB-lesioned rats exhibited the same sleeptime prior to ETOH diet, consumed similar amounts of ETOH diet over the course of 13 days, and exhibited similar significantly ($p<0.0005$) shorter sleeptimes after ETOH diet. Our data suggest that destruction of the DB does not alter development of ETOH tolerance as measured by the sleeptime test.

Norepinephrine Dorsal bundle Ethanol tolerance 6-Hydroxydopamine

RITZMANN and Tabakoff (4) first reported that intraventricular (IVT) infusions of 6-hydroxydopamine (6-OHDA) blocked the development of tolerance to the intoxicating effects of ethanol (ETOH) in mice. Tabakoff *et al.* (8) subsequently showed that IVT 6-OHDA administrations blocked the development of barbiturate tolerance. Infusions of 6-OHDA into either the dorsal noradrenergic bundle (DB) or ventral noradrenergic bundle (VB) have also been demonstrated to prevent development of barbiturate tolerance in the rat (7). The ability of 6-OHDA treatment to block development of tolerance to ETOH and barbiturates has been attributed to norepinephrine (NE) depletion (4,8). The individual contributions of DB and VB to the blockade of ETOH tolerance have not been investigated, however. As part of an ongoing series of experiments into the behavioral role(s) of DB in the rat, we have tested the effect of DB lesions in development of ETOH tolerance.

METHOD

Animals

Twenty-one male Sprague-Dawley rats weighing 150–200 g at the time of surgery were used in this experiment. Rats were housed on a 12-hour light/dark schedule and received food and water ad lib until beginning the ETOH diet regimen.

Surgery

Rats anesthetized with sodium pentobarbital (45 mg/kg, IP) were placed in a David Kopf stereotaxic frame. Eleven animals received bilateral infusions of 6-OHDA (2 μg base/μl of 0.1% ascorbate in saline, 0.5 μl/min, 2 μl/side) into the dorsal bundle (with incisor bar at −3.3 mm, coordinates were: posterior 5.8 mm from Bregma, lateral ±0.6 mm from midline and ventral 6.5 mm from the brain surface). The cannula remained in the brain for one minute after infusion to prevent 6-OHDA from flowing back up the cannula tract. The remaining 10 rats underwent the same procedure, except that the vehicle was infused in place of 6-OHDA.

Procedure

Three weeks after surgery rats were placed on a diet consisting

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of chocolate Carnation Slender with a vitamin supplement (3 g/l, ICN Corporation) and sucrose (96.8 g/l) for one day (5). The next day (day 22 postsurgical) the rats were given a challenge dose of ETOH (3 g/kg, IP) and sleeptime (the time between the loss and recovery of the righting reflex) was recorded as previously described (1). When rats regained their righting reflex, 70 μl of tail blood was taken for measurement of blood alcohol concentrations (BAC). The animals were then placed on the Carnation Slender diet with half the sucrose being replaced with 23.7 g/l 95% ETOH (2.25% w/v) for 3 days, followed by complete replacement of sucrose with 47.4 g/l 95% ETOH (4.5% w/v) for 10 days. After 13 days of ETOH diets, rats were switched to diet with ETOH being replaced by sucrose for 1 day prior to a repeat of the sleeptime test. At the end of the second sleeptime test, 70 μl of tail blood was taken for BAC and the rats were immediately sacrificed by guillotine. Brains were rapidly removed, cortex and hippocampus dissected out and frozen on dry ice. Animals who did not lose their righting reflex during the second sleeptime test, had tail blood taken at the same postinjection time at which they regained their righting reflex after the first sleeptime test.

Biochemistry

Tissue samples were homogenized in 2 ml of 0.32 M sucrose with 0.025 M Tris HCl (pH 7.5). A 0.5 ml aliquot of this homogenate was mixed with 0.5 ml of 0.1 N perchloric acid containing 7.9 mM sodium meta-bisulfite, 1.5 mM disodium EDTA, and 0.34 nM dihydroxybenzylamine (internal standard), and centrifuged at 40,000 × g for 30 minutes. The supernatant was removed and stored at −80°C for analysis by HPLC. The pellet was resuspended in 1.0 ml Tris HCl (pH 7.5) and stored at −80°C for assay of protein (2).

Norepinephrine was measured by high performance liquid chromatography (HPLC) with electrochemical detection using a modified version of the method described by Taylor *et al.* (9). The mobile phase consisted of 75% 10 mM disodium phosphate (pH 2.0) with 20 mM sodium dodecyl sulfate and 25% acetonitrile. The column was a 6.2 × 100 mm 3 μ ODS II column (Custom LC, Houston, TX). The detection system consisted of a Bioanalytical Systems electrochemical transducer and a LC-3A amperometric detector. The detector contained a glassy carbon electrode operated at an applied potential of 0.65 V relative to a Ag/AgCl reference electrode. Dihydroxybenzylamine was used as an internal standard.

Blood alcohol concentrations (BAC) were determined by the gas chromatographic head-space technique (10) using isopropyl alcohol as the internal standard.

Statistics

Diet consumption of both groups was analyzed by one-way Analysis of Variance (ANOVA). Sleeptimes and BAC's were analyzed by repeated measures ANOVA.

Conditions for Subject Removal From Study

Three control and 2 lesioned animals were removed from the study for the following reasons: 1) one lesioned rat had only a 40% depletion of cortical NE, 2) one control animal died after the first ETOH injection, 3) BAC measurements revealed that one control animal, who did not lose the righting reflex during the post-ETOH diet sleeptime test, had not received a complete dose of ETOH, and 4) the sleeptimes for one control (postdiet) and one lesioned (prediet) rat were more than 3 standard deviations away from their respective group means and were considered aberrant.

RESULTS

Norepinephrine Levels

HPLC analysis revealed that 8/9 rats had undetectable levels (less than 300 pg NE/mg protein) of cortical and hippocampal NE. The remaining animal had 78% and 99% depletion of cortical and hippocampal NE levels respectively (Table 1).

TABLE 1

NE LEVELS FOR CONTROLS AND 6-OHDA-DB LESIONED ANIMALS FOR CORTEX AND HIPPOCAMPUS IN ng/mg PROTEIN

	Cortex (ng/mg)	Hippocampus (ng/mg)
Shams	5.29 ± 0.35	7.29 ± 0.95
6-OHDA	0.12 ± 0.12*	0.03 ± 0.03*

Values represent means ± S.E.M. of 7 sham and 9 lesioned rats.
*6-OHDA rats had significantly less cortical and hippocampal NE than controls ($p<0.0005$).

Diet Consumption

Control and lesioned rats consumed the same amount of diet and stabilized at grater than 9.0 g ETOH/kg body weight/day by their eighth day on the diet.

Sleeptimes

Table 2 contains the results of pre and postdiet sleeptimes (interval of time between loss and recovery of righting reflex). Repeated measures ANOVA revealed no significant lesion effects ($F=1.61$, $p>0.05$), a significant diet effect ($F=21.85$, $p<0.0005$) and no interaction ($F=0.01$, $p>0.05$). Thus, control and lesioned rats had similar sleeptimes before exposure to ETOH diet and both groups similarly slept a significantly shorter period of time after two weeks on the ETOH diet.

Blood Alcohol Levels

BAC's at the time of recovery of righting reflex were not significantly different between groups after both sleeptime tests (Table 3).

DISCUSSION

Norepinephrine in the central nervous system (CNS) had been reported to be necessary for the development of tolerance to the hypnotic effects of ETOH in the mouse and of barbiturates in the mouse and rat. Destruction of the ascending NE pathways with 6-OHDA showed that both DB and VB play a role in blocking development of tolerance to barbiturates in the rat (7). We tested the effects of 6-OHDA-DB lesions on development of ETOH tolerance, as measured by sleeptime, in the rat. Our data suggest

TABLE 2

SLEEPTIMES AFTER A 3 g/kg (IP) DOSE OF ETOH BEFORE AND AFTER 13 DAYS OF ETOH DIET

	Pre-ETOH diet (min)	Post-ETOH diet (min)
Shams	73.14 ± 9.54	38.71 ± 14.14*
6-OHDA	89.82 ± 10.99	56.73 ± 8.36*

Values represent means ± S.E.M. of 7 sham and 9 lesioned rats. Sham and 6-OHDA rats had sleeptimes similar to each other before and after ETOH diet. Both groups slept significantly less after 13 days of ETOH diet and thus exhibited tolerance.
*$p<0.0005$.

TABLE 3

BAC'S FOR CONTROL AND 6-OHDA RATS AFTER RECOVERY OF RIGHTING REFLEX

	Pre-ETOH diet (mg%)	Post-ETOH diet (mg%)
Shams	234 ± 17	210 ± 14
6-OHDA	242 ± 9	213 ± 9

Values represent means ± S.E.M. of 7 sham and 9 lesioned rats. Both groups had similar BAC's at end of both pre- and post-ETOH diet sleep tests.

that the DB is not necessary for development of ETOH tolerance. Tabakoff *et al.* (6) have suggested that any significant loss of a single NE system in the CNS will effect development of tolerance to sedative hypnotics (i.e., ethanol and barbiturates). Our results do not support this hypothesis.

Development of tolerance to barbiturate and ETOH may have different mechanisms that involve NE. This suggestion is supported by a report that 6-OHDA-IVT infusions into the mouse-blocked development of ETOH tolerance but only partially blocked development of cross tolerance to pentobarbital (6).

It has been reported that increased stress via foot shock (3) or chronic dexamethasone treatment (11) facilitate development of tolerance. These data suggest a role for the hypothalamic-pituitary-adrenal cortical (HPAC) system in the development of ETOH tolerance. Since the ventral noradrenergic bundle provides the major NE innervation to the hypothalamus it is possible that this NE system may play a role in ETOH tolerance, either directly, or via its effects on the HPAC axis.

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