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Increased Alcohol Intake in Low Alcohol Drinking Rats After Chronic Infusion of the β-Carboline Harman Into the Hippocampus

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ADELL, A. AND R. D. MYERS. *Increased alcohol intake in low alcohol drinking rats after chronic infusion of the β-carboline harman into the hippocampus.* PHARMACOL BIOCHEM BEHAV 49(4) 949–953, 1994. – Harman (1-methyl-β-carboline) has been shown to induce volitional drinking of ethyl alcohol in the rat. The purpose of this study was to examine the long-term effect of sustained delivery of harman into the dorsal hippocampus on the subsequent preference for alcohol in the genetically bred low alcohol drinking (LAD) rat. The individual pattern of preference for alcohol was first determined following a standard 3–30% alcohol self-selection test for 10 days. Thereafter, a cerebral cannula for constant infusion was implanted stereotaxically into the dorsal hippocampus. The cannula was attached to an osmotic minipump implanted subcutaneously, which was filled with either an artificial cerebrospinal fluid (CSF) vehicle or harman. Harman was delivered at a rate of 1.0 or 3.0 μg/h (i.e., 5.5 or 16.5 nmol/h, respectively) for a period of 14 days. Four days after surgery, the rats underwent a second 3–30% alcohol preference test for 10 days. Both doses of harman induced a threefold increase in the voluntary consumption of alcohol, expressed as g/kg per day. This effect of the β-carboline seems to be specific for ethanol because its intake by the LAD rats was increased significantly only when concentrations from 11% to 30% were presented. Harman also enhanced the daily intake of food in a dose-dependent manner, but did not affect body weights or the volumes of water and total fluid consumed. These results, thus, demonstrate that the long-term exposure of hippocampal neurons to harman induces a preference for high concentrations of alcohol even in a line of rats lacking such a genetic predisposition.

Alcohol drinking Harman Osmotic minipump Ethanol preference Hippocampus β-Carboline
Aldehyde adduct 1-Methyl-β-carboline Condensation product Alcoholism Limbic system

PREVIOUS studies have demonstrated that systemic as well as intracerebroventricular (ICV) administration of several β-carbolines induce drinking of ethyl alcohol in the rat. Acute injections as well as recurrent ICV infusions of tetrahydro-β-carboline (THBC) increase the voluntary intake of alcohol in the Sprague-Dawley rat (14,15). A similar drinking response also occurs in the Wistar strain after THBC is infused continuously by the ICV route by means of osmotic minipumps (1,19,26). Further, it is apparent that the hippocampus also plays a pivotal role in the development of alcohol consumption in the rat because periodic microinjections of THBC into the dorsal hippocampus evoke spontaneous alcohol drinking in the rat (6). Differences in the potency of the β-carbolines on alcohol consumption may also exist. For example, harman (1-methyl-β-carboline) was found to be more effective than other compounds of the same family in inducing a dose-dependent increase in the intake of alcohol (1,19). However, in another study, the efficacy of both harman and THBC was virtually identical (26).

Harman occurs in vivo in the brain of the rat and is probably formed by the condensation reaction of tryptamine with acetaldehyde (25), the proximal metabolite of alcohol. Evidence exists that harman is not only excreted into the urine of rats pretreated with tryptamine and alcohol (24), but also is present in the urine of healthy volunteers and alcoholic patients (23) and in the brain and urine of rats given alcohol intragastrically (20). Harman is also one of the most potent inhibitors of benzodiazepine receptor binding, thus suggesting

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that this compound and possibly other related β-carbolines could act as the endogenous ligands of the benzodiazepine receptor (10,21). However, harman and other compounds of the β-carboline family exert anxiogenic rather than anxiolytic effects (5,17,18). These findings prompted some investigators to consider β-carbolines as agonists at benzodiazepine receptors, where benzodiazepines themselves would act as antagonists (10,21).

At present, the action of harman on the brain of a selectively bred line of an alcohol-avoiding animal is not yet clear. The purpose of the present study, therefore, was to examine whether a β-carboline is able to induce volitional alcohol drinking in the low alcohol drinking (LAD) line of rat. In this study, we determined a) whether the reported increase in alcohol intake evoked by chronic ICV infusion of harman (19) could be replicated by means of its sustained infusion into the hippocampus over time; and b) whether alcohol drinking could be induced in LAD rats genetically selected for drinking very little if any alcohol (9).

METHOD

Male LAD rats (Indiana University Alcohol Research Center, Indianapolis, IN) weighing 300–450 g were housed individually in wire mesh cages. They were kept on a 12 L : 12 D cycle, with lights on at 0700 h, and at an ambient temperature of 22 to 23°C. Water and Purina Rat Chow were always available throughout the experiments. Measures of body weight and the intakes of food, water, and alcohol were recorded daily at 0900 to 1000 h. The rats were assigned randomly to either the control or one of the harman groups before the start of the experiments.

Alcohol Preference Tests

The voluntary intake of alcohol was determined by a standard three-bottle, two-choice self selection technique (12). One bottle contained a solution of alcohol that was increased in percent concentration (v/v) on each of 10 consecutive days as follows: 3, 4, 5, 7, 9, 11, 13, 15, 20, and 30%. Each solution was prepared in tap water with 95% alcohol. A second bottle contained water and a third one was empty and served as a dummy. The position of the bottles was randomly interchanged on each day to prevent the development of a position habit (8).

Chronic Intrahippocampal Infusion

Harman (Sigma, Saint Louis, MO) was dissolved in three drops of glacial acetic acid and made up to volume with artificial cerebrospinal fluid (CSF) containing 0.1 mg/ml ascorbic acid. The solution was then adjusted to pH 5.2–5.4 with NaOH. The same solution without harman was used as the vehicle for the sham-operated rats. The composition of artificial CSF (11) was 128 mM NaCl, 2.55 mM KCl, 1.26 mM $CaCl_2$ and 0.93 mM $MgCl_2$. Each solution was passed through a pyrogen free filter with a 0.2 μm pore size membrane (Gelman Acrodisc).

Each of the rats was anesthetized with 55 mg/kg sodium pentobarbital given intraperitoneally and then placed in a Kopf stereotaxic apparatus. Under aseptic conditions, an intracerebral infusion cannula (Alzet Brain Infusion Kit, Alza Corp., Palo Alto, CA) was implanted unilaterally in the dorsal hippocampus, according to the following stereotaxic coordinates (16): 4.0 mm posterior to bregma; 3.0 mm ventral to the dura mater; and 2.5 mm lateral to the midline. The cannula

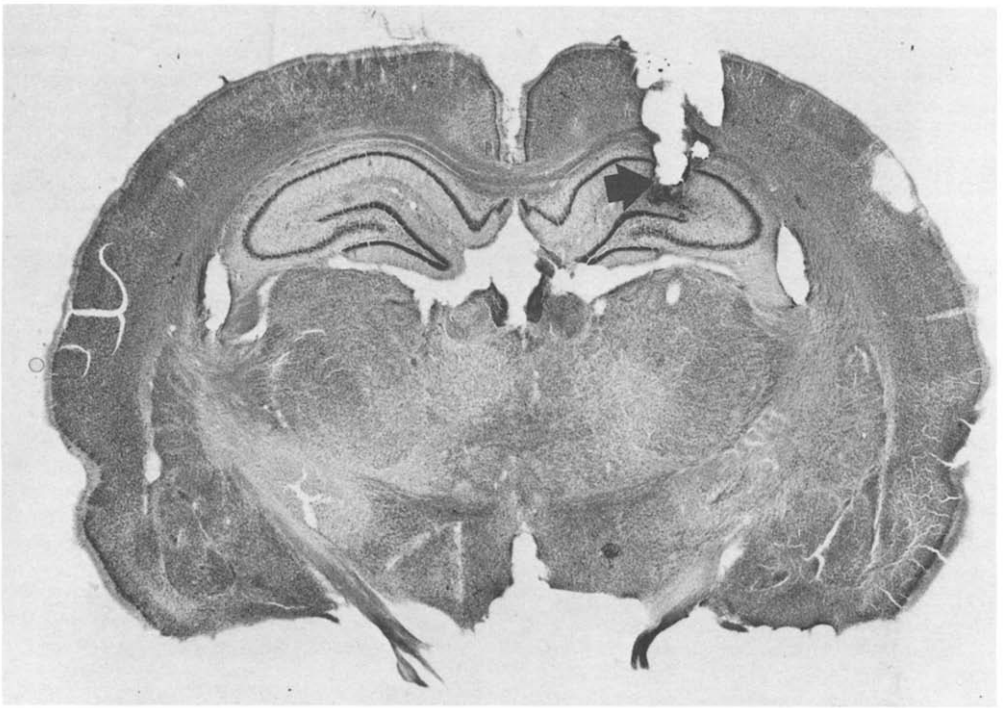

FIG. 1. Representative histological section cut in the coronal plane at 50 μm and stained with cresyl violet. The site of cannula placement for the infusion of harman is denoted by the arrow. Magnification × 8.

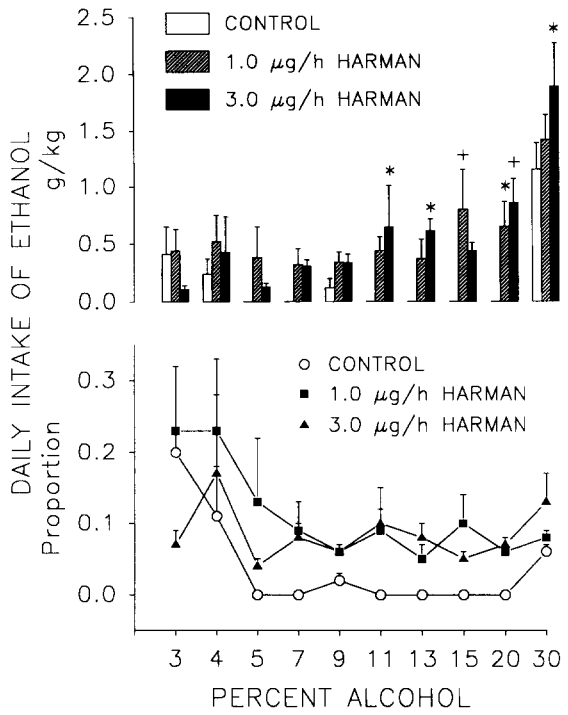

FIG. 2. Daily intakes of ethanol expressed as g/kg (top) and proportion to total fluid (bottom) during the 3-30% alcohol preference test following surgery. Each data point represents the mean ± SEM for control (n = 5), 1.0 μg/h harman (n = 7) and 3.0 μg/h harman (n = 6) infused animals. *$p < 0.05$ and $^{+}p < 0.01$ as compared to the control group by Tukey's HSD test.

was attached by means of a PVC catheter to an osmotic minipump (Alzet model 2002), which was implanted within a subcutaneous (SC) pocket opened at the scapular level. Each osmotic minipump was filled with either the control vehicle solution (vide supra) or a solution of 2.0 μg/μl or 6.0 μg/μl harman. Then the pump was incubated in sterilized 0.9% NaCl at a temperature of 22°C for 18 h prior to its implantation. Because the nominal delivery rate of the minipumps was 0.5 μl/h, harman was, therefore, infused into the dorsal hippocampus at a rate of 1.0 μg/h or 3.0 μg/h (i.e., 5.5 or 16.5 nmol/h, respectively) for a period of 14 days.

Experimental Design

After the rats were given the 10 day alcohol preference test (vide supra), each animal was maintained on only water for the next 5 days. Then the osmotic pumps, filled with either the harman solution or control vehicle, were implanted SC. After 4 days had elapsed, a second alcohol preference test was undertaken.

Histological and Statistical Analysis

At the end of the experiment, representative rats were given an overdose of sodium pentobarbital and then perfused transcardially with 0.9% saline followed by 10% buffered formalin. Each brain was removed immediately, stored in formalin for 3 days, and then cut on a cryostat in the coronal plane at 50 μm. Each of the sections was then stained with cresyl violet, according to standard procedures, for localization of the cannula track and site of infusion.

All data were calculated as the mean ± the standard error of the mean (SEM). The effects of harman on the different variables studied were assessed by analysis of variance (ANOVA) followed by Tukey's HSD tests using GBSTAT software (Dynamic Microsystems, Silver Spring, MD). A p-value of < 0.05 was considered statistically significant.

RESULTS

A representative site of a cannula placement is depicted in Fig. 1. The implantation of the infusion cannulae per se produced no apparent effects on any of the variables examined in this study. There were no significant changes observed in the vehicle control group between the two 10-day 3-30% alcohol preference tests undertaken both before and after surgery.

Overall, the chronic infusion of harman into the dorsal region of the hippocampus produced a significant increase in the drinking of alcohol expressed as both the mean daily g/kg, $F(2, 179) = 7.40, p < 0.01$, and mean daily proportion of alcohol to total fluid consumed, $F(2, 179) = 5.17, p < 0.01$. The rise in alcohol intake, however, was independent of the dose of harman infused because both doses of the β-

TABLE 1

OVERALL MEAN ± SEM OF DAILY MEASURES OF BODY WEIGHT AND THE INTAKES OF FOOD, WATER, ETHANOL AND TOTAL FLUID DURING THE 3-30% ALCOHOL PREFERENCE TEST

		Harman for 14 Days	
	Control	1.0 μg/h	3.0 μg/h
Ethanol (g/kg/day)	0.19 ± 0.06	0.57 ± 0.07*	0.58 ± 0.09*
Ethanol (proportion)	0.04 ± 0.02	0.11 ± 0.02*	0.08 ± 0.01
Body weight (g)	373 ± 8	379 ± 6	369 ± 5
Food (g)	21.9 ± 0.4	24.4 ± 0.5*	29.6 ± 0.8*†
Water (ml)	26.5 ± 1.3	24.2 ± 1.0	24.1 ± 0.8
Total fluid (ml)	27.6 ± 1.3	27.3 ± 1.0	26.4 ± 0.8

*Different from control ($p < 0.01$)
†Different from 1.0 μg/h harman ($p < 0.01$)

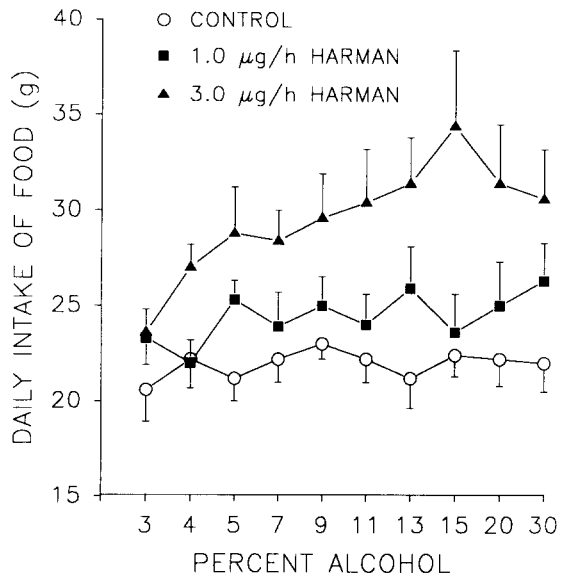

FIG. 3. Daily intake of food during the 3-30% alcohol preference test after surgery. Each data point represents the mean ± SEM for control (n = 5), 1.0 μg/h harman (n = 7) and 3.0 μg/h harman (n = 5) infused animals. The overall means of the three groups are significantly different from each other (Table 1).

carboline induced a similar augmentation in alcohol drinking (Table 1).

An analysis of the intakes of alcohol examined according to individual concentrations is presented in Fig. 2. The harman-induced increase in the mean daily g/kg intake was significant consistently at concentrations equal or higher than that of 11% (Fig. 2, top). Although harman also induced an increase in the mean daily proportion of alcohol to total fluid consumed, no statistically significant differences in proportion were found among the three groups at any of the 10 concentrations of alcohol tested (Fig. 2, bottom).

A further analysis of variance showed also that the amount of food eaten daily by the LAD rats, as portrayed in Fig. 3, increased significantly in a dose-dependent manner as a result of the infusion of harman, $F(2, 169) = 37.47$, $p < 0.0001$. However, as shown in Table 1, harman produced no significant effect on the body weight, $F(2, 179) = 0.73$, NS, volume of water ingested, $F(2, 179) = 1.52$, NS, or the total daily amount of fluid consumed, $F(2, 179) = 0.39$, NS.

DISCUSSION

Previously, it was reported that acute injections of tetrahydro-β-carboline in the hippocampus enhanced the intake of alcohol in the Sprague–Dawley rat (6). Because that response was found to be independent of whether THBC was given either unilaterally or bilaterally, in the present study the cannulae were implanted unilaterally for the chronic 14-day infusion of harman into the hippocampus. This strategy minimized both the trauma to the brain as well as the necessity of implantation of a second osmotic pump.

The basal level of alcohol drinking of the LAD rats in the present study of 0.19 g/kg per day agrees well with the values reported previously of 0.28 to 0.64 g/kg per day (7,9). Thus, the observation is confirmed further that the LAD line of rats possesses little or no inherent preference for alcohol. In the present investigation, the sham-operated rats failed to consume any significant amount of alcohol at the concentrations of 5, 7, 11, 13, 15, or 20%.

In spite of the genetically based avoidance of alcohol, the continuous infusion of harman into the dorsal hippocampus caused a threefold increase in the voluntary consumption of the fluid. This shift in preference was even more conspicuous when alcohol was offered in a gustatorily aversive range of concentrations, i.e., higher than 11%. It is also of interest that the sharp increase in alcohol drinking of the LAD rats induced by harman occurred after very small amounts of only 1.0–3.0 μg per hour of the β-carboline were infused into the hippocampus. Ordinarily, a higher concentration of a drug is required to evoke an effect when it is infused ICV. A twofold increase above the basal intake of alcohol occurred after the ICV infusion of 5.0 μg of harman per hour for 14 days (19).

At present, the mechanism of harman-induced augmentation of food intake is not yet known. Although the most prominent neuropharmacological property of harman is its high affinity for benzodiazepine receptors (2,10,21), its primary action is anxiogenic rather than anxiolytic (17,18). In fact, the benzodiazepine compounds generally antagonize convulsive episodes (22) and the anxiogenic action elicited by harman (17,18). Because the benzodiazepines themselves can enhance the consumption of food (3,4), the harman-induced hyperphagia is not likely due to an action on benzodiazepine receptors. In that an intravenous injection of harman lowers body temperature in a dose-dependent manner (10), an alternative neural mechanism involving a metabolic process could be responsible for the hyperphagic response. Should the sustained infusion of harman induce hypothermia, a compensatory increase in the intake of food could reflect the homeostatic attempt of the rat to cope with this imposed caloric demand. For the same reason, it is conceivable that the enhanced intake of alcohol could be due, in part, to an increase in caloric requirement for which alcohol provides a readily available source.

On the other hand, the effect of harman is apparently specific for the drinking of ethanol for two reasons. First, the shift in preference for alcohol of the harman-infused animals occurs as the concentrations of alcohol become more aversive in the range of 11% to 30%. Second, Rommelspacher and colleagues (19) have demonstrated that harman does not alter the volitional selection and subsequent intake of other substances with known dependence potential such as etonitazene.

In conclusion, the present observations underscore further the crucial role played by the hippocampus in the cerebral circuitry involved in the voluntary intake of alcohol (6,13). Further, the long-term exposure of hippocampal neurons to harman leads to the preference for high concentrations of alcohol, the pattern of which is not unlike that of the genetically high alcohol drinking (HAD) or alcohol preferring (P) lines of rat (7,8). Because harman and other β-carbolines are also able to induce an anxiety-like state in the rat (5,17,18), the mechanism precipitating the increase in the consumption of alcohol could reflect a compensatory response to the anxiogenic state of the animal (13).

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