Pharmacology Biochemistry & Behavior, Vol. 35, pp. 465–468. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

RAPID COMMUNICATION

Central Action of an Inhibitor of Brain Dopa-Decarboxylase, NSD-1015, on Cyanamide-Induced Alcohol Drinking in Rats

F. J. MIÑANO,[1] B. A. McMILLEN AND R. D. MYERS[2]

Departments of Pharmacology and Psychiatric Medicine
East Carolina University School of Medicine, Greenville, NC 27858

Received 24 April 1989

MIÑANO, F. J., B. A. McMILLEN AND R. D. MYERS. *Central action of an inhibitor of brain dopa-decarboxylase, NSD-1015, on cyanamide-induced alcohol drinking in rats.* PHARMACOL BIOCHEM BEHAV **35**(2) 465–468, 1990.—A cannula for repeated intracerebroventricular (ICV) infusion was implanted stereotaxically in 16 male Sprague-Dawley rats. Subsequently, an alcohol preference test was given to each animal to establish its preferred concentration in the presence of water. After the alcohol solution was removed, 15 mg/kg cyanamide was injected subcutaneously for 4 days to maximize volitional intake of the single preferred solution of alcohol, which ranged from 7–15% in these animals. The L-aromatic amino acid decarboxylase inhibitor, NSD-1015 (3-hydroxybenzylhydrazine dihydrochloride) was then given ICV twice daily in a volume of 5.0 μl in the following doses: 0.005, 0.01, 0.1 and 1.0 μg. NSD-1015 in all doses attenuated the g/kg alcohol intake of the rats; however, this decline was significant only at the lowest dose, which was pharmacologically specific, since neither food nor water intakes were altered by the treatment. Following the ICV infusions of NSD-1015, alcohol drinking returned essentially to postcyanamide levels. Further, during the interval of administration of NSD-1015, the cyanamide-induced decline in food consumption was reversed. These observations are in agreement with previous findings obtained under similar experimental conditions with the L-aromatic amino acid decarboxylase inhibitor, benserazide (Ro4-4602). They suggest that central decarboxylation or other effects of this drug on limbic system structures involved in the intake of alcohol could comprise a part of the mechanism underlying the induction of drinking. Further support is also provided for the involvement of brain dopamine and/or serotonin in the specific pattern of alcohol consumption in the rat.

Alcohol drinking NSD-1015 l-DOPA Cerebral ventricle 3-Hydroxybenzylhydrazine dihydrochloride Brain Ethanol Dopa-decarboxylase inhibition Dopamine Serotonin

IN a series of previous investigations, the potential role of L-aromatic amino acid decarboxylase inhibition in the brain was determined on either the basal consumption of a preferred solution of alcohol or intake of the fluid induced by cyanamide or tetrahydropapaveroline (THP). When a potent l-DOPA decarboxylase inhibitor, Ro4-4602 (benserazide), is administered to the rat by the intracerebroventricular (ICV) route, the animal's consumption of alcohol is attenuated in a dose-dependent manner (13,14). This finding implicates enzymatic inhibition of the neuronal synthesis of dopamine (DA), serotonin (5-HT) and perhaps norepinephrine (NE) as a potentially important mechanism in the development of the syndrome of chronic alcohol drinking.

The purpose of the present experiments was to explore further the effect of a pharmacologically induced perturbation in the metabolic synthesis of monoamine neurotransmitters (2,6) on the volitional intake of alcohol. Because of its similarity to Ro4-4602, 3-hydroxybenzylhydrazine dihydrochloride (NSD-1015) was selected since it acts to inhibit the decarboxylation of aromatic amino acids, which is a rate limiting step in the conversion of 5-HTP to 5-HT and l-DOPA to dopamine (1,2). In the present experiments, adult male rats were treated initially with cyanamide in order to augment the animals' self-selection of alcohol (4,5). Then, NSD-1015 was injected by the ICV route in one of four doses. Throughout the study, measures of food and water intakes as well

[1]Present address: Department of Pharmacology, Faculty of Medicine, University of Seville 41009, Spain.
[2]Requests for reprints should be addressed to R. D. Myers.

as body weight were obtained on a daily basis.

METHOD

Male Sprague-Dawley rats (N = 16), weighing from 500–600 g, were housed in individual cages in the vivarium of the Department of Comparative Medicine. The colony room was maintained at a temperature of 22–24°C and illuminated on a 12-hour light cycle. Water and a diet of NIH-07 Zeigler rat food were freely available throughout the experiments. Measures of food and fluid intakes as well as body weight were recorded daily between 0830–0930 hr.

Surgery

Standard aseptic procedures were used to surgically implant a 20-gauge stainless steel guide tube for ICV infusions (8,15). The cannula was positioned just above the lateral cerebral ventricle according to the following stereotaxic coordinates: AP 5.8; LAT 1.5; and HOR −2.0 below the dura mater (4). Postoperatively, a recovery period of 7 days elapsed prior to the beginning of the experiments.

Alcohol Preference Determination

The inherent pattern of preference for alcohol was determined initially for each animal by the standard self-selection method used in our laboratory (8). In this three-bottle, two-choice procedure, one bottle contained water, a second was empty, and the third bottle was filled with a v/v solution of one of 10 solutions of alcohol. The test concentration of alcohol was increased on each of 10 consecutive days as follows 3%, 4%, 5%, 7%, 9%, 11%, 13%, 15%, 20% and 30%. The position of each bottle was changed daily to prevent the development of a position habit (8,11).

After completion of the 10-day preference test, a single test concentration of alcohol was selected for each rat. This constant solution was individually determined as the concentration of alcohol, compared with water, which comprised 50% or more of the rat's total fluid intake during the initial preference test (12,18). In this group of animals, these single concentrations ranged from 7 to 15%.

Administration of Drugs

Cyanamide (Sigma) was prepared daily in a 0.9% saline vehicle and administered subcutaneously in a volume of 0.5 ml/kg. As based on previous experiments (4), an optimal dose of 15 mg/kg was injected twice daily at 1000 and 1600 hr on each of 4 consecutive days. During the administration of cyanamide, the tube containing alcohol was removed from each cage so that the toxicity of the drug when given in combination with alcohol was averted (4). Following treatment with cyanamide, the same concentration of alcohol was reintroduced to each rat and the same test procedure was followed.

A repeated measures design was utilized (13) in which each rat served as its own control. Thus, an individual deviation from the postcyanamide level of alcohol intake could be ascertained. On the day after the last injection of cyanamide, the same individually selected, single concentration of alcohol again was presented according to the following experimental protocol: for a 4-day period of infusion of an artificial CSF solution (15) alone; for 4 days of infusion of a given dose of NSD-1015; and for 4 days of infusion of CSF vehicle alone. The CSF solution was not substituted for the drug since once the pattern of alcohol consumption stabilizes, repeated ICV infusions of this vehicle fail to alter intake (4, 5, 19).

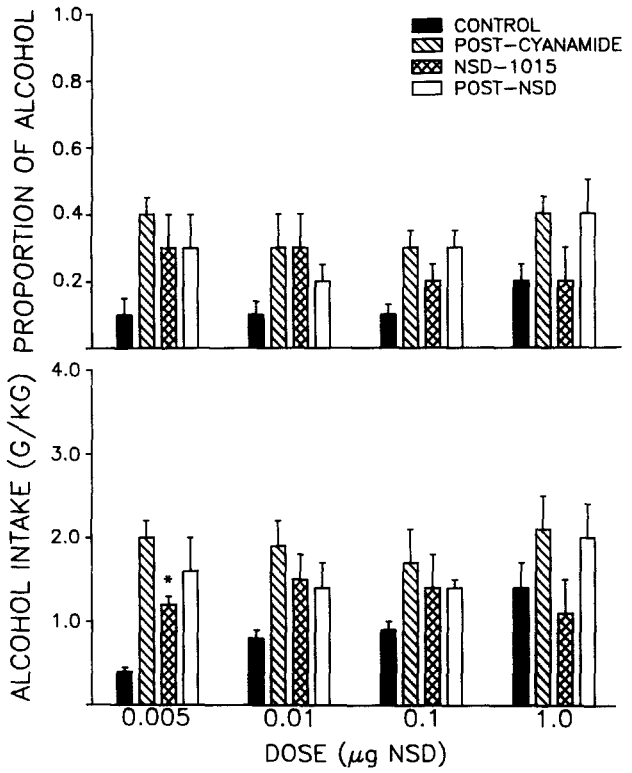

FIG. 1. Mean ± S.E.M. alcohol intake in four groups of rats (N = 4) in terms of g/kg and proportion of alcohol to total fluid 4 days before cyanamide treatment (PRE-CONTROL), 4 days postcyanamide treatment (30 mg/kg, SC), 4 days during ICV injections of NSD-1015 (0.005, 0.01, 0.1 and 1.0 μg), and 4 days postdrug (POST-CONTROL). *$p<0.05$ compared to cyanamide.

Each concentration of NSD-1015 (Regis) for ICV infusion was prepared freshly on each day in CSF. As based on earlier findings with Ro4-4602 (12–14), another L-aromatic amino acid decarboxylase inhibitor (1,2), NSD-1015 was given ICV in one of four doses of the salt: 0.005, 0.01, 0.1, and 1.0 μg. The drug was infused by gravity flow in a volume of 5.0 μl through a 23-ga injector needle lowered beyond the tip of the ICV guide tube into the ventricular lumen (4,13). Infusions were scheduled twice daily at 900 and 1700 hr for 4 consecutive days. For both pre- and post-NSD-1015 control infusions, the CSF vehicle was administered by the same procedures, same schedule, and in the same volume.

Statistical Analyses

The results were analyzed statistically on a computer using the Stat-Mate program. A one-way analysis of variance (ANOVA) followed by Newman-Keuls tests as well as two-tailed paired Student's *t*-tests were run. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

As shown in Fig. 1, when NSD-1015 was infused ICV, the g/kg consumption of alcohol was suppressed by all doses of the l-DOPA decarboxylase inhibitor, but significantly only at the lowest dose of 0.005 μg, $F(3,60) = 8.78$, $p<0.01$. The proportional values of alcohol intake, as depicted in Fig. 1, following all

TABLE 1

BODY WEIGHT, WATER AND FOOD INTAKE DURING ALCOHOL DRINKING UNDER SUCCESSIVE EXPERIMENTAL CONDITIONS

	Body Weight	Water Intake	Food Intake
Precyanamide Control	570.6 ± 27.8	41.5 ± 1.9	39.0 ± 2.8
Postcyanamide	568.9 ± 26.5	24.7 ± 0.7†	22.6 ± 0.1*
NSD-1015 (0.005 μg)	570.0 ± 30.0	22.5 ± 2.5†	25.0 ± 0.7†
Post-NSD-1015 Control	575.0 ± 31.8	26.5 ± 2.8†	21.5 ± 0.4†
Precyanamide Control	550.0 ± 9.7	41.5 ± 2.5	31.5 ± 2.5
Postcyanamide	537.5 ± 5.3	35.0 ± 2.8*	20.5 ± 0.4†
NSD-1015 (0.01 μg)	543.8 ± 2.7	32.8 ± 2.1†	25.3 ± 0.2†‡
Post-NSD-1015 Control	550.0 ± 9.8	39.5 ± 1.4	29.5 ± 1.4§
Precyanamide Control	559.3 ± 10.3	45.2 ± 3.3	29.8 ± 2.8
Postcyanamide	552.5 ± 12.0	33.3 ± 1.3†	20.7 ± 1.4†
NSD-1015 (0.1 μg)	548.3 ± 20.3	27.8 ± 2.4†	29.7 ± 2.1§
Post-NSD-1015 Control	550.0 ± 18.2	32.8 ± 1.2†	25.3 ± 1.3
Precyanamide Control	562.5 ± 26.8	42.1 ± 1.9	35.3 ± 0.5
Postcyanamide	558.8 ± 15.0	31.7 ± 0.7†	25.9 ± 0.7†
NSD-1015 (1.0 μg)	555.4 ± 15.6	34.2 ± 1.7†	26.2 ± 2.5†
Post-NSD-1015 Control	550.0 ± 16.1	21.9 ± 1.2†‡	20.4 ± 2.0†‡

*$p<0.05$ and †$p<0.01$ compared to control group.
‡$p<0.05$ and §$p<0.01$ compared to cyanamide group.

ICV doses of NSD-1015, except the 0.01 μg, also declined similarly to a level below that of the cyanamide-treated animals; however, these changes were not statistically significant ($p>0.05$). During the 4-day period following the ICV infusions of NSD-1015, both g/kg intake and proportion of alcohol to water were not significantly different from cyanamide values (Fig. 1).

Table 1 presents the mean ± S.E. measures of body weight, water and food intakes of the rats during successive alcohol preference tests. The values were calculated for the intervals prior to and following cyanamide treatment, and during and after the 4 doses of NSD-1015 were injected ICV. Water and food intakes declined significantly under both postcyanamide and NSD-1015 treatment conditions when compared to the respective control groups. No significant changes occurred in water intake in NSD-1015-treated rats in comparison to their respective cyanamide-treated groups. Although treatment with NSD-1015 did not reverse the effect of cyanamide on food intake in any of the groups of rats when compared to the controls, the doses of 0.01 and 0.1 μg of NSD-1015 given ICV augmented significantly the consumption of food above that of the postcyanamide level, $F(3,60)=11.3$, $p<0.001$ and $F(3,60)=4.71$, $p<0.01$, respectively.

In a separate experiment, the concentrations of l-DOPA in the corpus striatum were determined in four additional rats. Striatal tissue was extracted by standard batch alumina procedures, then estimated by HPLC with electrochemical detection as described previously (9). In contrast to a control value of l-DOPA of 0.055 μg/g, the level of striatal l-DOPA increased to 1.25 μg/g after 100 mg/kg NSD-1015 was administered peripherally. Following the acute ICV injection of 5.0 μg bilaterally to rats under sodium pentobarbital anesthesia, the tissue concentration of l-DOPA rose above the control value to 0.143 μg/g. This approximates 10% of the concentration of l-DOPA in the striatum following the complete inhibition of l-DOPA-decarboxylase.

DISCUSSION

The present results extend our previous findings in which Ro4-4602 infused ICV in the rat suppressed the volitional selection of alcohol induced in the animal by cyanamide (12,13). In that the lowest dose of 0.005 μg of NSD-1015 attenuated the absolute intake of alcohol significantly in the cyanamide-treated animal without altering food or water intakes, relative to the postcyanamide interval, the effect of the drug in terms of ingestive function was specific. Although the higher doses of the l-DOPA decarboxylase inhibitor also reduced the intake of alcohol by as much as one-half, these changes were not statistically different from postcyanamide levels.

In an earlier study, Bartholini and Pletscher (1,2) compared the efficacy of several compounds to inhibit l-DOPA decarboxylase. Ro4-4602 enhanced the l-DOPA-induced rise in cerebral catecholamine activity of the rat in doses which reached asymptote rapidly. In contrast, however, only the lowest dose of NSD-1015 caused a marked enhancement of the l-DOPA-induced increase in catecholamines (2); in fact, a high dose of the drug produced a steady decline in the catecholamine levels because of the central inhibition of DOPA decarboxylase due to the penetration by NSD-1015 of the blood-brain barrier.

In terms of the pharmacological effect of NSD-1015, it is possible that the mechanism of action of the drug is similar to that of Ro4-4602. Because NSD-1015 inhibits concurrently the decarboxylation of l-DOPA to DA as well as 5-HTP to 5-HT (1,2), the metabolic synthesis of functionally active amine-aldehyde adducts would consequently be reduced. Certain of these adducts, including specific tetrahydroisoquinolines and β-carbolines, can induce intense alcohol drinking when injected either ICV or directly into limbic system pathways in the brain (8, 16, 17). Thus, an infinitesimal modification of l-DOPA-decarboxylase in brain tissue could lead to the alteration of the consumption of alcohol which had been elevated earlier by cyanamide pretreatment (22).

Another explanation of the central effect of NSD-1015 is that the drug could block the synthesis of one of the isozymes responsible for the degradation of acetaldehyde. Although NSD-1015 does not contain a pyrogallol moiety, as does Ro4-4602, it is possible that NSD-1015 may secondarily inhibit aldehyde dehydrogenase and thereby increase the level of an alcohol-derived acetaldehyde (3). Thus, because of a toxic accumulation of an aldehyde from the presence of NSD-1015 centrally, further intake of alcohol simply would exacerbate the deleterious effect of any additional ingestion of alcohol. Theoretically, a pharmacological corollary thus could be envisaged with that of the aldehyde dehydrogenase (ALDH) inhibitor, cyanamide, which in the presence of alcohol, raises the level of endogenous acetaldehyde (21); this deters alcohol drinking because of its ensuing accumulation.

Finally, whether the central action of NSD-1015 involves a perturbation of either the normal release of a neurotransmitter or receptors on catechol- or indoleamine-containing neurons in TIQ or β-carboline reactive sites (8,20) is not yet known. The question of which enzymes or receptor sites are altered by such a low dose of this hydrazine-containing drug also requires clarification. Our preliminary findings indicate that the largest ICV dose of NSD-1015 used in this study does not produce a significant accumulation of l-DOPA in the striatum of the rat. Nevertheless, the residual concentrations of l-DOPA in neurons of those structures contiguous to the ependymal wall of the cerebral ventricles, which are in direct contact with the DOPA decarboxylase inhibitor following an ICV injection, are as yet unknown. Notwithstanding the potential inhibition of monoamine oxidase by NSD-1015, a concentration of 5×10^{-4} is required for a 50% inhibition of the conversion of kynurenine to 4-hydroxyquinoline (unpublished observation). Thus, additional research will be required to deter-

mine the neuroanatomical substrate underlying the effects of NSD-1015 on normal as well as the pharmacologically induced drinking of alcohol.

ACKNOWLEDGEMENTS

This research was supported in part by Grant AA-04200-09 to R.D.M. from NIAAA and Grant DA-04895 to B.A.M. from NIDA. F.J.M. was partially supported by a research grant from the Spanish Ministry of Education and Research. The authors wish to thank Kelly Hines, Amy Roscoe and Tammy Saffel for their excellent technical assistance.

REFERENCES

1. Bartholini, G.; Pletscher, A. Effect of various decarboxylase inhibitors on the cerebral metabolism of dihydroxyphenylalanine. J. Pharm. Pharmacol. 21:323–324; 1969.
2. Bartholini, G.; Pletscher, A. Decarboxylase inhibitors. Pharmacol. Ther. 1(3):407–421; 1975.
3. Critcher, E. C.; Hepler, J. R.; Myers, R. D. Induction of alcohol preference in the rat after aldehyde elevation in brain by ICV infusion of cyanamide. Alcohol.: Clin. Exp. Res. 7:246; 1983.
4. Critcher, E. C.; Myers, R. D. Cyanamide given ICV or systemically to the rat alters subsequent alcohol drinking. Alcohol 4(5):347–353; 1987.
5. Critcher, E. C.; Myers, R. D. Evidence for the hippocampus as a site of action of tetrahydropapaveroline (THP)- or cyanamide-induced alcohol drinking. Alcohol 1:167; 1984.
6. Collins, M. A.; Custod, J. T.; Rubenstein, J. A.; Tabakoff, B. Studies on the effects of pyrogallol and the structurally related Dopa decarboxylase inhibitor RO4-4602 on acetaldehyde metabolism. Ann. NY Acad. Sci. 273:227–233; 1976.
7. Deitrich, R. A.; Troxell, P. A.; Worth, W. S.; Erwin, V. G. Inhibition of aldehyde dehydrogenase in brain and liver by cyanamide. Biochem. Pharmacol. 35:2733–2737; 1976.
8. Huttunen, P.; Myers, R. D. Anatomical localization in hippocampus of tetrahydro-β-carboline-induced alcohol drinking in the rat. Alcohol 4(3):181–187; 1987.
9. McMillen, B. A. Acute and subchronic effects of MJ-13859, a potential antipsychotic drug, on rat brain dopaminergic function. J. Pharmacol. Exp. Ther. 233:369–375; 1985.
10. Melchior, C.; Collins, M. A. The route and significance of endogenous synthesis of alkaloids in animals. CRC Crit. Rev. Toxicol. 1982:313–356.
11. Melchior, C. L.; Myers, R. D. Genetic differences in ethanol drinking of the rat following injection of 6-OHDA, 5,6-DHT or 5,7-DHT into the cerebral ventricles. Pharmacol. Biochem. Behav. 5(1):63–72; 1976.
12. Miñano, F. J.; Myers, R. D. Central and peripheral Dopa decarboxylase inhibition: effects on alcohol drinking in the rat. Alcohol.: Clin. Exp. Res. 12(12):304; 1988.
13. Miñano, F. J.; Myers, R. D. Inhibition of brain dopa-decarboxylase by RO 4-4602 infused ICV blocks alcohol drinking induced in rats by cyanamide. Psychopharmacology (Berlin) 98:176–182; 1989.
14. Miñano, F. J.; McMillen, B. A.; Myers, R. D. Interaction of tetrahydropapaveroline with inhibition of dopa-decarboxylase by Ro 4-4602 in brain: Effects on alcohol drinking in the rat. Alcohol 6(2):133–137; 1989.
15. Myers, R. D. Methods for chemical stimulation of the brain. In: Myers, R. D., ed. Methods in psychobiology, vol. 1. London: Academic Press; 1971:247–280.
16. Myers, R. D. Alkaloid metabolites and addictive drinking of alcohol. In: Chang, N. C.; Chao, H.M., eds. NIAAA Res. Monogr. 17. 1985:268–284.
17. Myers, R. D. Multiple metabolite theory, alcohol drinking and the alcogene. In: Collins, M. A., ed. Aldehyde adducts in alcoholism. New York: Alan Liss; 1985:201–220.
18. Myers, R. D.; Melchior, C. L. Alcohol drinking: abnormal intake caused by tetrahydropapaveroline in brain. Science 196:554–556; 1977.
19. Myers, R. D.; Melchior, C. L. Differential actions on voluntary alcohol intake of tetrahydroisoquinolines or a β-carboline infused chronically in the ventricle of the rat. Pharmacol. Biochem. Behav. 7:381–392; 1977.
20. Myers, R. D.; Privette, T. L. A neuroanatomical substrate for alcohol drinking: Identification of tetrahydropapaveroline (THP)-reactive sites in the rat brain. Brain Res. Bull. 22(5):899–911; 1989.
21. Sinclair, J. D.; Gibble, P. A. Cyanamide injections during alcohol deprivation increase alcohol drinking. Alcohol 2:627–630; 1985.
22. Sinclair, J. D.; Lindros, K. O. Suppression of alcohol drinking with brain aldehyde dehydrogenase inhibition. Pharmacol. Biochem. Behav. 14:377–383; 1981.