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Inhibition of Nitric Oxide Synthesis Attenuates Alcohol Consumption in Two Strains of Alcohol-Preferring Rats

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REZVANI, A. H., D. R. GRADY, A. E. PEEK AND O. PUCILOWSKI. *Inhibition of nitric oxide synthesis attenuates alcohol consumption in two strains of alcohol-preferring rats.* PHARMACOL BIOCHEM BEHAV **50**(2) 265-270, 1995.—The effect of the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) on voluntary alcohol consumption was examined in two different strains of alcohol-preferring rats, in a continuous-access, two-bottle-choice paradigm. Compared with the vehicle, intraperitoneal injections of L-NAME significantly and dose-dependently (10, 30, and 60 mg/kg) suppressed alcohol intake and preference in both alcohol-preferring (P) and Fawn-Hooded (FH) rats. The effect of the highest dose of L-NAME was nonspecific; it caused general decreases in consumption of alcohol, water, and food. Repeated injection of L-NAME (30 mg/kg) for 4 consecutive days significantly attenuated alcohol intake, but tolerance developed after 3 days of treatment. A single administration of a high dose of L-NAME (60 mg/kg) did not influence the blood alcohol concentrations, which suggests a possible central effect. Furthermore, a moderate dose of 30 mg/kg L-NAME, which selectively inhibited alcohol intake, did not exert a significant effect on telemetrically measured heart rate, core body temperature, and gross motor activity of alcohol naive Fawn-Hooded rats. These results suggest an involvement of nitric oxide in alcohol drinking behavior. Although the true mechanism(s) of action is not yet clear, it can be speculated that L-NAME may exert its action indirectly by modulating neurotransmitters proposed to be involved in alcohol drinking and/or by influencing other neuronal factors, such as neuronal Ca^{2+} channels, which have been shown to be involved in alcohol drinking behavior.

Nitric oxide Nitric oxide synthase Alcohol Alcohol consumption Alcohol-preferring rats Fawn-Hooded rats Alcohol drinking

THE NEUROTRANSMITTER function for nitric oxide (NO) in the CNS, proposed by Bredt et al. in 1992, opened a new frontier in many areas of brain research. It has been demonstrated that NO, a simple gas with free radical chemical properties, is synthesized in mammalian central tissue (4). Furthermore, it has been shown that although it is unconventional, NO satisfies the major criteria for a neurotransmitter in the CNS (18,39,40). There is a growing body of evidence indicating the involvement of NO in various neurophysiologic functions (21). L-arginine : NO pathways have been linked to the stimulation by the excitatory amino acids of specific receptors in the CNS (39,40). In addition, NO has been implicated in several other functions in the brain, including modulation of the wakefulness and circadian rhythms (26), learning and memory (7), feeding (23), drinking (6), anxiety (31), and regulation of release and uptake of neurotransmitters such as norepinephrine and dopamine (5,17). A modulatory role for NO in the development of cellular (14) and behavioral (16) tolerance to chronic opiate treatment has also been proposed. NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) also prevented the acquisition of tolerance to the ataxic effect of alcohol in rats (15).

The potential utility of novel pharmacologic treatments to curb alcohol drinking and preference has been the focus of several studies from this and other laboratories (29,34,36,38). We have demonstrated that several chemically different Ca^{2+} channel inhibitors or blockers effectively decrease alcohol intake and preference in selectively bred alcohol-preferring rats

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(27,30,33,34,38) and alcohol-drinking monkeys (32). Because neuronal NO synthesis by NO synthase is Ca^{2+} dependent, it is possible that these agents exert their attenuating effects on alcohol intake by reducing intracellular Ca^{2+}, which leads to a reduction in NO synthesis. We hypothesized that modulation in NO concentrations in the brain may lead to the attenuation of alcohol intake. Because NO itself is highly labile, its behavioral and physiologic effects are inferred from the results of studies performed on the enzyme, NO synthase, which transforms L-arginine into NO and citrulline. To test this hypothesis, we examined the effects of different doses of L-NAME, an NO synthase inhibitor, on alcohol intake/preference in two lines of rats that exhibit high alcohol intake, the P rats (42) and the Fawn-Hooded rats (37).

METHOD

Animals

Alcohol-drinking Fawn-Hooded (FH) rats, from a colony established at the University of North Carolina, and alcohol-preferring (P) rats, from a colony established at Indiana University, were used for these experiments. Both of these strains exhibit a high preference for alcohol and drink substantial amounts of alcohol in a free-choice situation (20,37). In addition, separate groups of alcohol naive FH rats were used to investigate the effect of L-NAME on heart rate, core body temperature, gross motor activity, and blood alcohol concentrations.

Adult, male Fawn-Hooded rats weighing 0.358 ± 0.054 kg and P rats weighing 0.578 ± 0.068 kg were housed individually in wire mesh single cages (26 × 24 × 20 cm) under a constant temperature of 21 ± 1°C and a 12 : 12 dark-light reversed cycle (1000–2200 h dark). Rats were fed Agway/Prolab Rat/Mouse/Hamster 3000 Formula (Agway, Syracuse, NY) and water ad lib.

Preparation of Drugs

Solutions of L-NAME were prepared in sterilized isotonic saline and passed into a pyrogen-free glass bottle and stoppered. Three doses of L-NAME (10, 30, and 60 mg/kg body wt.) were used. The volume of vehicle or drug injected was 1 ml/kg body wt. Solutions of 10 and 16% (vol./vol.) alcohol were prepared daily from 95% reagent grade alcohol and distilled water.

Experimental Protocol

Alcohol intake. Following a standard method used in our laboratory, animals were screened and tested for alcohol preference (37,42). Each rat was given free access to tapwater in a graduated 100-ml Richter tube for 1 day. Thereafter, the animals were given free access to a solution of 10% (vol./vol.) alcohol as the sole source of fluid for 3 consecutive days. After this period, the animals were given free access to both tapwater and a solution of 10% alcohol for at least 3 consecutive weeks. During the course of the experiments, food was available ad lib.

After establishing a stable baseline for alcohol intake, each of the P (n = 7) and Fawn-Hooded rats (n = 11) was injected IP at 0930 h with either saline or one of the three doses of L-NAME (10, 30, or 60 mg/kg) in a pseudorandomized order. The interval between injections was at least 5 days. In another series of experiments, to determine the effect of subchronic administration of L-NAME, P rats were injected with either 30 mg/kg L-NAME or saline once a day for 4 consecutive days. Food, water, alcohol intake, and preference were recorded every day between 0900 and 0930 h throughout. Alcohol preference was calculated as the total alcohol intake (ml)/total fluid (alcohol + water) intake (ml) × 100 (38,42).

Blood alcohol concentrations. To determine the effect of L-NAME on blood alcohol concentrations, the following experiments were carried out with two groups of alcohol-naive Fawn-Hooded rats. Rats were injected IP with either 60 mg/kg L-NAME or saline, and 15–20 min later with a dose of 2.0 g/kg alcohol (16% vol./vol.). Twenty-microliter blood samples were collected from the tip of the tail of each rat at 1, 3, and 5 h after alcohol injection. Blood samples were transferred immediately to a microcentrifuge tube containing 180 ml *tert*-butanol (0.3 mg/ml) as an internal standard and stored at −20°C until gas chromatography analysis (38).

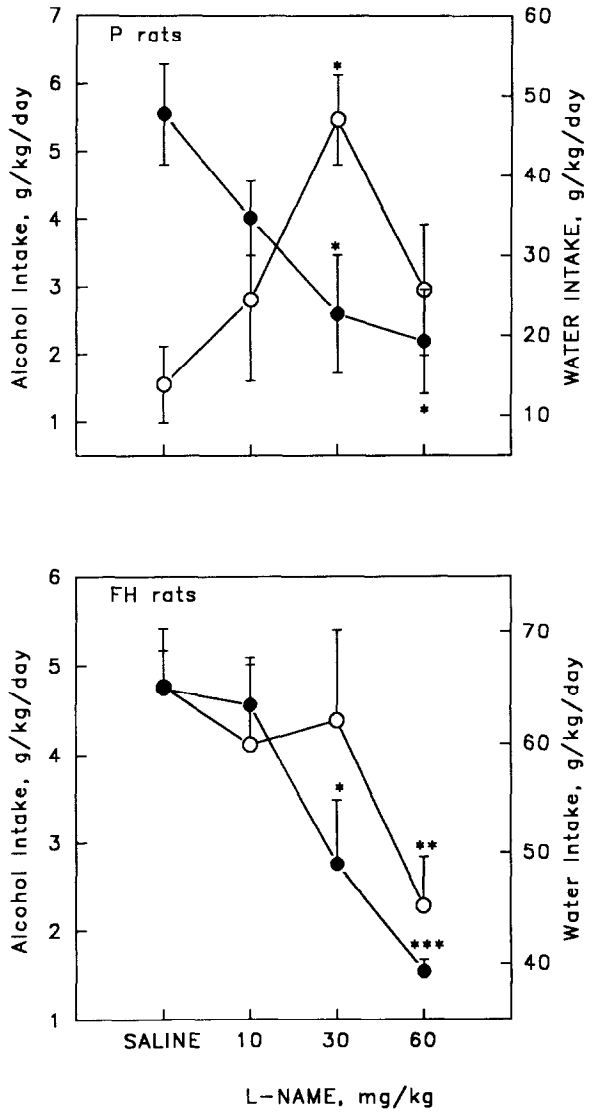

FIG. 1. Effects of different doses of L-NAME on the consumption of alcohol (●) and water (○) in P (upper panel) and FH (lower panel) rats. Data are means ± SEM from seven P and 11 FH rats per dose of L-NAME and 10 P and 11 FH rats for saline. *$p < 0.05$, **$p < 0.02$, and ***$p < 0.01$ compared with corresponding control saline group (Newman-Keuls test).

Autonomic responses. To determine the effect of L-NAME on autonomic responses such as heart rate, core body temperature, and gross motor activity, a radiotransmitter (model TA-11ETA-F40-L20; Data Sciences, Inc., St. Paul, MN) weighing about 7.0 g was surgically implanted into alcohol-naive Fawn-Hooded rats (n = 5). The transmitter was inserted into the abdominal cavity and sutured to the peritoneum with 4-0 silk thread. Core body temperature, heart rate, and gross motor activity were collected automatically every 15 min and stored in a computer using DATA Quest IV Software (Data Sciences, Inc.) for analysis. A detailed description of the methodology has been published elsewhere (35). After recovering from the surgery and establishing a stable baseline for body temperature and heart rate, rats were injected intraperitoneally with either a dose of 30 mg/kg L-NAME or the control vehicle, and autonomic responses were recorded in their home cages over the next 4 h.

Statistical analysis of data. The results are expressed as means ± SEM, and the statistical differences between L-NAME-treated and saline-treated groups were determined by using analysis of variance followed by Newman-Keuls test.

RESULTS

Acute Administration

Both P and Fawn-Hooded rats drink significant amounts of alcohol in a free-choice situation. In the present experiment, when given free access to alcohol and water, P rats and Fawn-Hooded rats consumed an average of 6.0 ± 1.0 and 5.4 ± 1.3 g/kg per day alcohol, respectively. Compared with the control vehicle, a single systemic administration of L-NAME significantly and dose-dependently attenuated alcohol intake (Fig. 1) and alcohol preference (Fig. 2) in both P and Fawn-Hooded rats.

A single administration of L-NAME, but not saline, into P rats resulted in an increase in water intake (Fig. 1, upper panel) with no change in total fluid intake (Table 1). However, in Fawn-Hooded rats the two lower doses of L-NAME did not exert significant effects on water intake, but the high dose of 60 mg/kg significantly ($p < 0.02$) reduced water intake (Fig. 1, lower panel).

Compared with saline, acute administration of L-NAME at 10 and 30 mg/kg did not affect the food intake, but the dose of 60 mg/kg of the drug significantly ($p < 0.05$) reduced food consumption in P rats.

TABLE 1

EFFECTS OF DIFFERENT DOSES OF L-NAME ON FOOD AND TOTAL FLUID INTAKE IN ALCOHOL-PREFERRING (P) AND FAWN-HOODED (FH) RATS

	Food Intake (g/kg per day)		Total Fluid Intake (ml/kg per day)	
	P	FH	P	FH
Saline	45 ± 4	63 ± 3	82 ± 7	128 ± 6
L-NAME				
10 mg/kg	38 ± 2	53 ± 5	66 ± 5	120 ± 9
30 mg/kg	32 ± 5	42 ± 6**	80 ± 11	100 ± 10**
60 mg/kg	27 ± 4*	41 ± 3**	58 ± 10	81 ± 7†

Data are the means ± SEM.

*$p < 0.05$, **$p < 0.01$, †$p < 0.001$ comparing L-NAME with the corresponding saline values (Newman-Keuls Test).

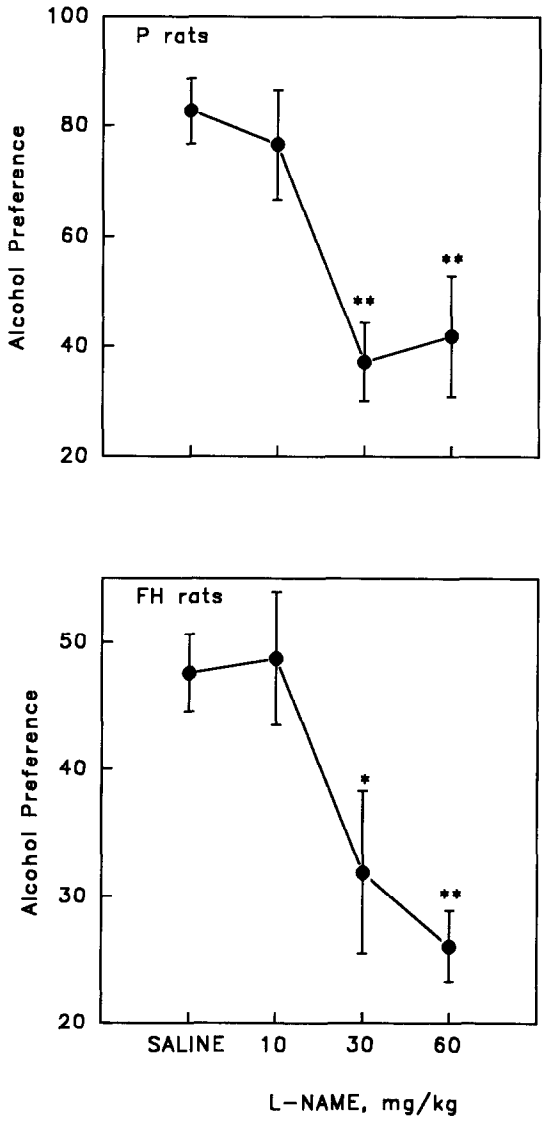

FIG. 2. Effects of different doses of L-NAME on alcohol preference in P (upper panel) and FH (lower panel) rats. Data are means ± SEM from 7 P and 11 FH rats per dose of L-NAME and 10 P and 11 FH rats for saline. *$p < 0.05$ and **$p < 0.01$ compared with corresponding control saline group (Newman-Keuls test).

In Fawn-Hooded rats, food intake was suppressed following the administration of 30 and 60 mg/kg L-NAME ($p < 0.01$). No correlation was found between the suppression of food and alcohol consumption in both strains of rats treated with 60 mg/kg L-NAME.

Blood Alcohol Concentrations

Compared with control vehicle, administration of 60 mg/kg L-NAME 15–20 min before alcohol injection did not significantly affect blood alcohol concentrations in Fawn-Hooded rats (Fig. 3).

Subchronic Administration

Repeated administration of 30 mg/kg L-NAME for 4 consecutive days, but not control saline, significantly ($p < 0.001$)

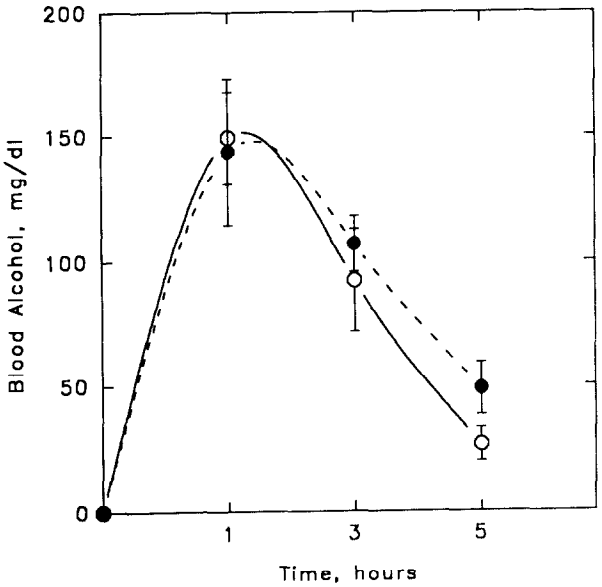

FIG. 3. Effects of 60 mg/kg L-NAME (●) and an equal volume of saline (○) on blood alcohol levels in FH rats injected (IP) with 2.0 g/kg alcohol. Data are means ± SEM; $n = 5$ in each group.

reduced alcohol intake and concomitantly increased water intake for only 2 consecutive days. However, after the third injection, tolerance developed to the attenuating effect of L-NAME on alcohol intake, and both alcohol and water intake started to return to the pretreatment baseline (Fig. 4). Subchronic administration of L-NAME did not exert a significant effect on food intake (data not shown).

Autonomic Responses

Compared with saline, a single injection of 30 mg/kg L-NAME into alcohol-naive Fawn-Hooded rats did not exert a significant effect on core body temperature, heart rate, and gross motor activity. However, there was a slight and transient rise in these parameters immediately after the administration of both the vehicle and L-NAME, possibly as a result of the handling and injection per se. One hour after the injection, the heart rate was 342 ± 29 and 334 ± 36 beat/min for saline and L-NAME, respectively. Corresponding values for the core body temperature were 37.33 ± 0.2 and 37.0 ± 0.5°C, respectively.

DISCUSSION

The present study demonstrates for the first time that when it is injected, L-NAME systemically reduces alcohol intake and preference in a dose-dependent fashion in alcohol-preferring rats. The fact that even a high dose of L-NAME did not exert a significant effect on blood alcohol concentrations suggests that L-NAME may exert its effect centrally. However, the present data cannot exclude the peripheral action of L-NAME on alcohol intake. There are several possible mechanisms that may explain the inhibitory action of L-NAME on alcohol-seeking behavior in rats. It is possible that by reducing NO production, L-NAME exerts its inhibitory action on alcohol intake. Because NO synthesis in the brain is Ca^{2+} dependent, it is possible that Ca^{2+} channel blockers also exert their inhibitory effects on alcohol intake (11,12,30,32,33) by impairing NO production in the brain. An additional similarity between the effects of NO synthase blockade and inhibition of Ca^{2+} influx via the L-type channels is the impaired development of tolerance to the depressant actions of alcohol in rodents treated with L-NAME (15) or various calcium channel blockers (10,28,43). Both L-NAME (16) and calcium channel blockers (7) were found to decrease tolerance to the analgesic effect of morphine and to prevent opiate withdrawal syndromes (1,2,16). Thus, it is conceivable that NO synthesis might be the next step in the cascade of Ca^{2+}-dependent intracellular events involved in processes of tolerance to and dependence on sedative or hypnotic drugs. However, there is not yet direct biochemical evidence for the interaction of calcium channel blockers with the activity of NO synthase.

Another possible mechanism relates to L-NAME's interaction with certain neurotransmitters or neuromodulators proposed to be involved in alcohol drinking. Along with other neurotransmitters and neuromodulators such as serotonin (20,37) and opioid systems in the brain, the dopaminergic system has also been implicated in the reinforcing properties of alcohol. Ethanol has been shown to release dopamine in the mesolimbic system of rats (9,13). Alcohol-preferring rats exhibit about 25% deficiency in the concentrations of dopamine and its metabolites in the nucleus accumbens (24). Furthermore, the systemic administration of bromocryptine (a preferential D_2 agonist) and GBR 12909 (a dopamine uptake inhibitor) attenuate alcohol intake in alcohol-preferring rats (19,25). Thus, it has been speculated that the alcohol-induced facilitation of dopamine release may be one of the neurochemical mechanisms responsible for its positive reinforcing properties (9,13). NO has been reported to be present in brain regions containing dopaminergic terminals, and it has been shown to inhibit dopamine transport in vivo and to alter dopaminergic neurotransmission (17). Thus, one can speculate that

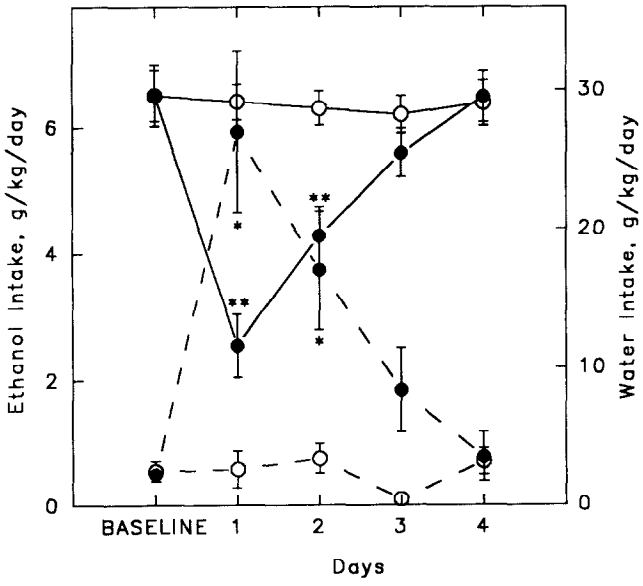

FIG. 4. Effects of IP repeated administrations of saline (○) and 30 mg/kg L-NAME (●) on alcohol (—) and water (- -) intake in P rats. Rats were injected IP either with 30 mg/kg L-NAME or saline once a day for 4 consecutive days. Data are means ± SEM; $n = 10$ for each group. *$p < 0.01$ and **$p < 0.001$ comparing L-NAME with corresponding saline values.

L-NAME may exert its inhibitory action on alcohol intake by interfering with the dopamingeric system. Recently, it has been demonstrated that NO synthase inhibitors *N*-nitro-L-arginine and *N*-monomethyl-L-arginine blocked the NMDA-mediated release of neurotransmitters norepinephrine and L-glutamate (22), indicating an important role for NO in the process of neurotransmitter release. However, despite reports that NO may modulate dopamine release in the brain, it was recently shown that NO is not involved in the dopamine-dependent rewarding effect of electrical brain stimulation or the reward facilitation produced by cocaine's enhancement of dopamine activity (3).

One other possible mechanism is related to the involvement of NO pathways in other consumatory behaviors such as drinking and feeding. The involvement of the L-arginine-nitric oxide pathway in the regulation of drinking behavior has been evaluated by the cerebral injection of L-arginine and L-NAME in rats (5). In this study, it was shown that L-NAME given ICV antagonized the antidipsogenic effect of L-arginine in water-deprived rats. However, central administration of L-NAME by itself did not increase thirst (5). Thus, it is unlikely that L-NAME exerts its inhibitory action on alcohol intake by modulating thirst.

There is some evidence that NO may play a physiologic role in appetite regulation. It has been shown that L-arginine, a substrate for NO synthesis, increases food intake in mice, whereas L-NO Arg, an inhibitor of NO synthesis, inhibits food intake in food-deprived mice (23). Furthermore, it has been shown that acute or repeated administration of L-NO Arg, which also inhibits brain NO synthase, reduced food intake and body weight in both obese and lean rats (41). One might speculate that L-NAME, similar to other NO synthase inhibitors, possesses anorectic properties that might be an important factor in the reduction of alcohol intake. Indeed, a high dose of L-NAME (60 mg/kg) reduced food intake in both strains of alcohol-preferring rats. However, in the present experiment, no correlation was found between alcohol intake and food consumption following L-NAME treatment, indicating a more specific effect of L-NAME on alcohol intake. Because the administration of L-NAME did not exert a significant effect on autonomic responses, it can be concluded that the reduction in alcohol intake may not be associated with these parameters.

When one studies the effect of a particular agent on alcohol intake, it is important to determine whether the agent exerts any effect on alcohol metabolism. If an agent reduces the rate of alcohol breakdown, it will consequently elevate blood alcohol concentrations, which may lead to the attenuation of alcohol intake. An agent of this kind is not suitable for the treatment of alcoholism because, although it may reduce alcohol intake, the increased blood alcohol concentration may have severe toxic consequences. Thus, it is important to know whether the compound exerts any effect on alcohol metabolism. The fact that L-NAME, even at the highest dose used in this experiment, did not influence blood alcohol concentrations suggests that L-NAME probably exerts its attenuating effect on alcohol intake centrally. However, our data on blood alcohol levels only demonstrate the effect of L-NAME and saline on alcohol elimination. The effect of this compound on alcohol absorption and distribution is not known.

In conclusion, the present findings demonstrate that acute or subchronic administration of the NO synthase inhibitor, L-NAME, caused a rapid and significant reduction in alcohol intake and preference in alcohol-preferring rats without affecting blood alcohol concentrations and autonomic responses. Thus, it would suggest that NO systems may be operative in the control of alcohol preference. Although it is possible that L-NAME exerts its action by altering the activity of neurotransmitters proposed to be involved in alcohol intake, the exact nature of such an interaction remains to be systematically investigated.

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