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Norepinephrine and Serotonin Receptors in the Paraventricular Nucleus Interactively Modulate Ethanol Consumption

Clyde W. Hodge, Craig J. Slawecki, and Amy S. Aiken

Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, North Carolina

Abstract

The homeostatic function of the hypothalamus has long been recognized. In particular, the role of the paraventricular nucleus (PVN) in regulating ingestive behavior has been of interest. Infusions of serotonin and norepinephrine into the PVN are correlated with nutrient selective decreases and increases in consumatory behavior, respectively. Given the wide range of homeostatic functions of the hypothalamus, it is plausible that similar hypothalamic mechanisms may also be involved in the regulation of ethanol intake. This study examined the effects of PVN infusions of serotonin (5-HT) and norepinephrine (NE) on ethanol intake in a 1-hr limited-access two-bottle paradigm. When intake of 6% (v/v) ethanol versus water stabilized, male Wistar rats were implanted with stainless steel guide cannulae aimed at the PVN. After recovery, NE (20, 50, and 100 nmol), 5-HT (5 and 25 nmol), and their combination (NE 50 nmol + 5-HT 5 nmol) were microinjected into the PVN in a volume of 0.5 μ L/side over 1 min. Baseline ethanol intake was approximately 1.0 g/kg and ethanol preference (milliliters ethanol per total milliliters) was approximately 60%. Both 20 and 50 nmol of NE significantly increased absolute ethanol intake by 50% and relative ethanol intake by approximately 30%. Corresponding decreases were observed in water intake. Neither dose of 5-HT when administered alone altered ethanol or water consumption, but the 5-nmol dose of 5-HT attenuated the increase observed after NE (50 nmol) administration. These data demonstrate that NE and 5-HT receptors in the PVN interact in the modulation of ethanol ingestion. This finding suggests that homeostatic regulatory functions of the hypothalamus are involved in ethanol intake, and a perturbation of this system may influence excessive drinking.

Keywords

Alcohol Drinking; Ethanol; Self-administration; Serotonin; Norepinephrine; Paraventricular nucleus; Hypothalamus; Rats

THE NEUROBIOLOGICAL mechanisms that control alcohol seeking and the magnitude of alcohol intake are not well characterized. Mesolimbic systems that regulate drug self-administration¹ have recently been shown to influence responding maintained by ethanol reinforcement.²⁻⁸ However, the dopaminergic system alone does not regulate ethanol self-

administration.⁷ 6-OHDA lesions of the nucleus accumbens (NAcc) did not alter ethanol reinforced responding,⁶ and site specific pharmacological manipulations of the meso-accumbens dopamine (DA) system do not influence the onset of ethanol reinforced responding.^{3,4} These data raise questions concerning the extent of this system's involvement in alcohol seeking behavior.

Recent data suggest that mesolimbic systems similarly regulate responding maintained by ethanol and nondrug reinforcers such as sucrose. When ethanol and sucrose reinforced behavioral baselines are equated, administration of the D2-like agonist quinpirole in the NAcc shifts the pattern of both ethanol and sucrose reinforced responding from high response rates with early termination to slow continuous rates with delayed termination.² Quinpirole infusions in the ventral tegmental area (VTA) decrease ethanol and sucrose reinforced responding with identical effects on response patterns.³ Raclopride infusions into the NAcc decrease both ethanol⁹ and sucrose⁴ reinforced responding by producing early termination. The finding that ethanol and nondrug (i.e., food related) reinforcement processes are regulated in a similar manner by the mesolimbic system suggests that brain systems known to regulate appetitive and consumatory behavior may influence ethanol intake.⁷

Research on appetitive and consumatory behavior has focused for many years on the hypothalamus.^{10, 11} Recent studies have demonstrated that the paraventricular nucleus (PVN) may play a significant role in feeding and drinking behavior. Infusions of norepinephrine (NE) into the PVN selectively increased carbohydrate intake, decreased protein intake, and did not change fat consumption.¹²⁻¹⁴ Similar increases in carbohydrate intake were found after peripheral injection of the α_2 agonist clonidine.^{15,16} Peripheral and PVN administration of serotonin (5-HT) or fluoxetine resulted in a nutrient selective reduction in carbohydrate intake.^{17,18} Suppressive effects on feeding behavior by central injection of 5-HT are not observed in other brain areas including the amygdala, NAcc, septum, and the nucleus reuniens, which is dorsal to the PVN.¹⁷ Injections of 5-HT into the PVN inhibit α_2 noradrenergic induced eating.¹⁹ Thus, serotonergic and noradrenergic receptors in the PVN have been shown to regulate feeding in a reciprocal manner.

Although studies of the hypothalamus have been key to the understanding of the neurobiology of consumatory behavior in general, there are virtually no data indicating the specific role of the PVN in ethanol drinking behavior. However, noradrenergic and serotonergic neurochemical systems are involved in the behavioral effects of ethanol in a manner consistent with their role in feeding behavior. Ethanol induced intoxication, as measured by exploration, loss of righting reflex, and an observer-rated intoxication score, was antagonized by the α_2 antagonists atipamezole and idazoxan.²⁰ Ethanol withdrawal symptoms are associated with a transient 24-hr increase in plasma noradrenaline,²¹ suggesting that ethanol seeking behavior, which occurs during acute withdrawal, may be related to increased synaptic availability of noradrenaline. Furthermore, administration of 5-HT agonists^{22,23} and uptake inhibitors²⁴⁻²⁷ decrease ethanol intake. Accordingly, selectively bred ethanol preferring (P) rats show chronic reduction of 5-HT function²⁸ and reduced 5-HT binding in some brain areas.^{22,29,30}

Although the general involvement of NE and 5-HT receptor systems in ethanol-related behavior is consistent with data from studies on appetitive behavior, the specific involvement of these systems within the PVN of the hypothalamus in ethanol intake has not been demonstrated. This experiment tested the effects of microinjections of exogenous NE and 5-HT, both alone and in combination, in the PVN on ethanol and water intake using a two-bottle limited access model.

MATERIALS AND METHODS

Animals

Male Wistar rats ($n = 15$) were housed individually in standard hanging stainless steel cages with food available ad libitum. The colony room was maintained on a 12-hr light:12-hr dark cycle with lights on at 7:00 AM. Fluid access was limited to 1hr per day during the lights-on portion of the light:dark cycle. Initial body weights (mean $\pm$ SE) were $316 \text{ g} \pm 12$. All experimental sessions were conducted during the light portion of the cycle. Before each daily session, rats were weighed and inspected for general health. All rats were experimentally and drug naive.

Apparatus

Daily drinking sessions were conducted in the home cage ($35 \text{ L} \times 18 \text{ H} \times 18 \text{ W cm}$). Two plastic 50-ml graduated (1-ml graduations) drinking tubes were mounted on the front of each cage before daily sessions. For intra-PVN injections, stainless steel injectors (33-gauge) were connected with PE 20 tubing to 1.0- μl syringes (Hamilton, Reno, NV) mounted on a microdrive pump (Harvard Apparatus, model 22).

Procedure

Rats were allowed 1 week to adapt to individual housing conditions and handling. During this period ad libitum food and water were available. For 3 days after the initial adaptation period, 10% ethanol (v/v) was the only fluid made available (24 hr/day). Then, fluid access was restricted to 1hr each day (12:00 PM to 1:00 PM) during which time water and an ethanol solution were concurrently available. Two-bottle drinking sessions were conducted 5 days/week (Monday-Friday). On weekends, water alone was available for 1hr on the home cage. During the first 15 days of two-bottle drinking, the ethanol concentration was increased from 2.0% (2 days) to 4.0% (2 days), to a final concentration of 6% as previously described.³¹ When intake of 6% ethanol stabilized, rats were surgically implanted with stainless steel guide cannulae aimed bilaterally at the PVN. Microinjection test sessions began when ethanol intake levels restabilized. Drugs were administered on Tuesday and Friday. Thursdays were used as no-injection control days. Before the start of each test session, the rats were weighed and placed in individual holding chambers while the fluids were attached to the home cage. Initial fluid levels were recorded to the nearest milliliter at the beginning and end of 1-hr fluid access periods. The position (left or right) of each solution was alternated daily to control for side preferences. Rats were sacrificed at the end of the study and injection sites were determined histologically.

Stereotaxic Surgery

Rats were anesthetized with pentobarbital (40 mg/kg, intraperitoneally) and chloral hydrate (127.5 mg/kg, intraperitoneally) and placed in a stereotaxic device (David Kopf Instruments, model 1404) with the incisor bar set –3.3 mm below the interaural line. Stainless steel guide cannulae (13-mm, 26-gauge) were lowered into both sides of the brain and secured to the skull with stainless steel cranial screws and dental cement. The guide cannulae terminated 1.0 mm above the PVN. Removable 13-mm obturators (33-gauge) were inserted in the cannulae to prevent obstruction by foreign substances and to limit infection. Plastic caps were affixed around the cannula area to prevent disruption of the obturators. The stereotaxic coordinates used for the PVN were –1.8 mm from bregma, +1.0 mm lateral to the midline, and –6.5 mm ventral to the cortical surface at 5 degrees lateral to the vertical plane.³²

Microinjection Procedure

Unanesthetized rats were placed in a plastic tub (27 × 17 × 12 cm) to minimize movement. Obturators were removed and the cannula area was swabbed with sterile physiological (0.9%) saline. Bilateral infusions of artificial cerebrospinal fluid (ACSF) and each drug were performed through 14-mm stainless steel hypodermic injectors (33-gauge). The injectors extended 1 mm past the end of the guide cannulae. The infusion rate of the pump was 1.0 μ l/minute, and a total fluid volume of 0.5 μ l was delivered into each side of the brain. NE (20–100 nmol), 5-HT (5–25 nmol), and 5-HT (5 nmol) + NE (50 nmol) were infused bilaterally in the PVN. Injectors remained in place for 30 additional seconds to allow for diffusion. New sterile obturators were inserted after removal of the injectors. Sham injections were conducted in a similar manner except the injectors were the same length as the guide cannulae (i.e., 13 mm) to prevent brain penetration, and, although the pump was operated, the syringes were not driven. Test sessions began 10 min after the injections. All animals first received ACSF followed by a randomized order of NE, 5-HT, and their combination for a total of seven injections.

Drugs and Solutions

Norepinephrine hydrochloride and serotonin creatine complex (RBI Chemicals, Natick, MA) solutions were prepared immediately before injection. The nanomole amount of NE and 5-HT infused were the amounts of free neurotransmitter determined from the formula weight of the compounds. Drugs for the microinjections were prepared in ACSF vehicle and vortexed on a mechanical shaker. Tap water and ethanol (6% v/v) in tap water were the two solutions used in the two-bottle test sessions.

Histology

Rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 60 ml of sodium phosphate buffer solution (pH 7.5) followed by 60 ml of 10% formalin. Brains were removed immediately and stored in 10% formalin for at least 5 days. The brains were cut into 60- μ m coronal sections, allowed to stand in 10% formalin vapor for approximately 3 days, and subsequently stained with cresyl violet. Cannula placement and injection location was verified with the aid of a light microscope. Only data obtained from rats with bilateral cannulae placement in the PVN³² were used in the data analysis.

Data Analysis

The collected data consisted of total consumption (milliliters) of water and ethanol during the 1 hr session. Total session ethanol intake (grams per kilogram) was calculated according to the individual body weight of each rat immediately before the session. Relative ethanol intake ratios were calculated as ethanol milliliters per total milliliters. Statistical analysis of fluid volumes consumed, relative ethanol intake, and ethanol intake was accomplished by means of within animal one-way repeated measures analysis of variance (ANOVA). When necessary, a Student NewmanKeuls post hoc multiple comparisons test was used ($p < 0.05$). Preliminary analysis (ANOVA) of no-injection control data showed no variation in baseline ethanol and water intake across the 7-week injection period. Thus, the no-injection control associated with the ACSF injection was used as the control for graphic display and statistical analysis.

RESULTS

Histology

Histological analysis of brain sections showed that injections occurred bilaterally in the PVN in 10 of the 15 animals tested. Figure 1 shows the range of injection sites considered to be bilateral hits in the PVN.³²

Fluid Intake

Total fluid consumed during no-injection control sessions was $20.1 \text{ ml} \pm 4.3$ and was not altered by any experimental manipulation indicating that all changes in ethanol intake were associated with compensatory changes in water intake.

Ethanol Intake

The average 24-hr ethanol intake during the 3 days of forced ethanol was $6.36 \pm 0.29 \text{ g/kg/day}$. This corresponded to an average intake of $23.24 \pm 0.84 \text{ ml of ethanol/day}$. Control ethanol intake (mean $\pm$ SE) during limited access was $1.02 \pm 0.15 \text{ g/kg}$ resulting in preference ratios of 0.61 ± 0.08 , indicating that the procedure resulted in pharmacologically significant levels of ethanol intake during the 1-hr drinking sessions. ACSF vehicle injections had no effect on ethanol intake. Repeated measures ANOVA indicated a main effect for NE infusions into the PVN [$F(4,36) = 5.8, p < 0.001$] that resulted in increases in ethanol intake (Fig. 2A). Post hoc comparisons showed that 20- and 50-nmol doses of NE increased intake in comparison with no-ACSF control, $p < 0.05$. Infusion of 5-HT (5 and 25 nmol) produced no significant change in ethanol intake in comparison with ACSF control. When coadministered with NE (50 nmol), 5-HT (5 nmol) completely blocked the NE induced increase in ethanol intake (Fig. 2A, right).

Water Intake

ACSF vehicle injections had no effect on water intake. NE infusions into the PVN resulted in a significant decrease in water intake [$F(4,36) = 7.8, p < 0.001$]. Post hoc comparisons showed that both the 20- and 50-nmol doses of NE significantly decreased water intake relative to no-injection and ACSF controls, $p < 0.05$. Water intake was unaffected by 5-HT

infusions into the PVN. Combined administration of NE (50 nmol) and 5-HT (5 nmol) resulted in complete blockade of the NE induced decrease in water intake (Fig. 2B).

Relative Ethanol Intake (Preference)

ACSF vehicle injections had no effect on ethanol preference (Fig. 2C). NE infusions into the PVN resulted in a significant increase in ethanol preference [$F(4,36) = 3.9, p < 0.01$]. Post hoc comparisons showed that both the 20- and 50-nmol doses of NE significantly increased relative ethanol intake compared with no-injection and ACSF controls, $p < 0.05$. Relative ethanol intake was unaffected by 5-HT infusions into the PVN. Combined administration of NE (50 nmol) and 5-HT (5 nmol) resulted in complete blockade of the NE-induced changes in relative ethanol intake (Fig. 2C, right).

DISCUSSION

The principal finding from the present study is that the PVN of the hypothalamus is involved in the regulation of ethanol intake. NE infusions into the PVN increased ethanol intake and resulted in a concomitant decrease in water intake. 5-HT infusions alone did not influence ethanol or water intake. A dose of 5-HT (5 nmol) that was ineffective when administered alone, completely blocked the effects of NE (50 nmol) on ethanol and water intake. These data complement other findings that showed involvement of NE^{33,34} and 5-HT²²⁻³⁰ systems in the regulation of alcohol intake and suggest that some of the effects seen in systemic administration studies may involve receptor activity in the PVN.

The finding that NE infusions in the PVN increase ethanol intake is in agreement with numerous studies on ingestive behavior. Intra-PVN infusions of NE selectively and robustly increased carbohydrate intake, decreased protein intake, and did not change fat intake.¹²⁻¹⁴ The nutrient selective role of NE in the facilitation of feeding appears to be regulated by α_2 receptors because the antagonist idazoxan selectively increased carbohydrate intake after intra-PVN administration.³⁵ This corresponds with the dense population of α_2 receptors in the PVN³⁶ and related hypotheses regarding the role of the PVN in energy homeostasis.³⁷

Because NE transmission in the PVN selectively regulates carbohydrate intake, the present data warrant consideration of whether changes in ethanol intake may reflect energy regulation. Although the present study did not directly control for this possibility, several lines of data suggest that the enhanced ethanol intake may have been dissociated from the feeding cycle. For example, PVN control of carbohydrate ingestion through NE transmission is associated with corticosterone (CORT).³⁸ Plasma CORT levels peak in rats at the onset of the dark cycle³⁹ and NE infusions in the PVN are ineffective in eliciting feeding during the light cycle, when CORT levels are low.⁴⁰ The present study was conducted during the middle of the light cycle (11:00 AM), when CORT levels are low,³⁹ suggesting that the effects observed in this study were not dependent on CORT and possibly unrelated to feeding. In addition, hypothalamic nuclei influence general behavioral processes that are unrelated to energy regulation. For instance, the PVN has been shown to be involved in sensory and spatial learning,⁴¹ aversive learning tasks,⁴² and morphine self-administration.⁴³ Thus, the involvement of the hypothalamus in general behavioral functions

supports the notion that the observed effects on ethanol consumption in the present study may not be solely related to ethanol's caloric value.

One aspect of the present data that should be considered is that low doses of NE (20 and 50 nmol) influenced ethanol and water intake, but a higher dose of NE (100 nmol) was without effect. A consistent finding is that α_1 and α_2 receptors show differential distribution ($\alpha_2 > \alpha_1$) in the PVN.⁴⁴ These receptor subtypes also exhibit differential affinities ($\alpha_2 > \alpha_1$) for endogenous NE and have different functional properties (inhibitory versus excitatory).⁴⁵ Thus, one explanation for the differential effects of NE is that the low doses selectively activated the more prevalent highaffinity α_2 receptors in the PVN, whereas the lack of effect at the higher dose may reflect the loss of subtype specificity and opposing function of α_1 receptors. Additional studies with selective antagonists are required to address this question.

Infusions of 5-HT alone into the PVN had no effect on ethanol or water intake in the present study. This finding is in contrast with data showing that 5-HT agonists^{22,23} and uptake inhibitors²⁴⁻²⁶ decrease ethanol intake. Injections of the selective 5-HT_{1A} agonist 8-OH-DPAT (0-2.5 μ g) into the dorsal raphe increased ethanol intake possibly by decreasing 5-HT transmission through activation of somatodendritic autoreceptors.⁴⁶ These data also contrast with evidence showing that intra-PVN administration of 5-HT, at concentrations comparable to those used in the present study, selectively reduced carbohydrate intake.¹⁷⁻¹⁸ Thus, the present data do not support a role for 5-HT transmission alone in the PVN in ethanol drinking. However, when considering the behavioral effects of exogenous ligands, it is important to take into account the possible role of uptake mechanisms. For instance, the highest density of 5-HT transporters in rat brain ($B_{\max} = 1240$ fmoVmg of protein) is found in hypothalamic regions,⁴⁷ but NE transporter densities are somewhat lower ($B_{\max} = 847$ fmol/mg protein) in the PVN.⁴⁸ This finding raises the possibility that synaptic clearance may have influenced the present results and suggests that higher concentrations, or coadministration of a 5-HT uptake inhibitor, may have revealed involvement of PVN 5-HT receptors in ethanol intake.

Although 5-HT infusions in the PVN had no effect on ethanol or water intake, 5-HT (5 nmol) blocked the effects of NE (50 nmol) on ethanol and water intake and ethanol preference. This finding suggests that 5-HT receptors in the PVN may modulate NE transmission or the behavioral effects of NE receptor activation during ethanol self-administration. Evidence for an interactive role of NE and 5-HT in the PVN in ingestive behavior is supported by the finding that intra-PVN infusion of 5-HT inhibits NE induced eating.¹⁹ These and related findings have led to the hypothesis that the α_2 noradrenergic receptor system is a potential site and mechanism through which 5-HT may influence satiety mechanisms involved in feeding and drinking.¹⁹ The present data support the hypothesis that an interactive regulatory mechanism involving 5-HT and NE receptors in the PVN influence ethanol self-administration in a manner that is consistent with the neuroregulation of feeding and drinking.

Other evidence supports the notion that ethanol selfadministration and food intake are subject to similar neurobehavioral regulation.³⁻⁴ For example, when rates of responding maintained by 75% sucrose and 10% ethanol are equated, intra-NAcc infusions of the D2

receptor antagonist raclopride decrease to a similar extent lever press responding maintained by sucrose and ethanol reinforcement.⁴ Infusions of the D2 agonist quinpirole in the VTA decrease both ethanol and sucrose reinforced responding by producing early termination.³ Furthermore, increases in dopamine release in the NAcc have been reported in rats whose behavior is maintained by food⁴⁹ and ethanol⁵⁰ reinforcement. Hypothalamic nuclei receive afferent connections from the brain stem and mesolimbic DA regions, such as the VTA, and there are numerous efferent connections, via the median forebrain bundle, to the prefrontal cortex, hippocampus, and amygdala.⁵¹ It is plausible, therefore, that PVN outputs to the mesolimbic system may represent a critical link in the influence of the hypothalamus on ethanol reinforcement.

In conclusion, the present data show that the PVN of the hypothalamus is involved in the neuroregulation of ethanol drinking. Pharmacological, biochemical, and behavioral evidence from the "feeding" literature indicates that NE and 5-HT receptors in medial hypothalamic regions, particularly the paraventricular nucleus (PVN), function in an antagonistic manner in regulating increases and decreases in eating, respectively.¹¹ This study provides the first evidence that ethanol and food intake may be regulated in a similar manner by hypothalamic mechanisms. However, the limited access procedure uses fluid restriction conditions, which raises questions concerning the motivation to drink ethanol (i.e., fluid needs versus pharmacological effect). Whether the PVN is also involved in alcohol seeking behavior (i.e., reinforcement processes) in the absence of fluid restriction remains to be determined.

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Fig. 1. Representation of coronal section of rat brain corresponding to 1.8 mm caudal to Bregma.³² The shaded areas show the range of injection sites (i.e., injector location) in the PVN. Dye was infused in unanesthetized animals before histological procedures to estimate pattern of drug diffusion. Histological analysis of dye distribution suggested that the microinjections of NE, 5-HT, and their combination spanned the dorsomedial cap, medial parvocellular, lateral magnocellular, and ventral regions of the PVN.

Fig. 2.

Effects of PVN infusions of NE, 5-HT, and their combination on ethanol intake (A), water intake (B), and relative ethanol intake (C). All bars, including no-injection (N) and vehicle M controls, represent the average intake of $n = 10$ animals during 1-hr test sessions with ethanol (6% v/v) and water available. Horizontal reference lines are drawn at the mean of the N control value. Error bars represent SE. * indicates significantly different from both N and V control, $p < 0.05$, Student Newman-Keuls procedure. Comparison of NE (50 nmol) to combined administration of NE and 5-HT was conducted with paired t test.