Ethanol intake is increased by injection of galanin in the paraventricular nucleus and reduced by a galanin antagonist

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Abstract

Ethanol intake stimulates expression of galanin in several hypothalamic sites, including the paraventricular nucleus. Because injection of galanin in the paraventricular nucleus induces eating, we hypothesized that galanin might also affect ethanol intake. Rats were given ad libitum access to 4% ethanol for 4 weeks and assigned to one of two groups according to levels of ethanol consumption: high levels (>1.5 g/kg/day) or low levels (<1.0 g/kg/day). In Experiment 1, galanin (1.0 nmol) or Ringer’s solution was injected unilaterally into the paraventricular nucleus, with food and water absent during the first 4 h. Galanin significantly increased ethanol intake only in rats that drank high levels of ethanol. In Experiment 2, injection of galanin (0.5 and 1.0 nmol) in the paraventricular nucleus dose-dependently increased ethanol intake with food and water available. The higher dose was also effective in eliciting ethanol intake when tested with food and water absent. In Experiment 3, a test of receptor specificity was provided by injecting rats with the galanin antagonist M-40 (0.5 nmol) or Ringer’s solution. Injection of M-40 in the paraventricular nucleus significantly decreased ethanol consumption. In Experiment 4, an anatomic control, with galanin injected 2 mm dorsal to the paraventricular nucleus in the same animals, caused no change in ethanol intake. In conclusion, injection of galanin in the paraventricular nucleus, at a dose known to induce feeding, acted by means of a galanin receptor to potentiate intake of 4% ethanol, even with food and water available as alternate sources of calories and fluid, respectively. Because ethanol can increase expression of galanin mRNA in the paraventricular nucleus, this could set the stage for a positive feedback loop between galanin and ethanol intake. © 2004 Elsevier Inc. All rights reserved.

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1. Introduction

Ethanol is a food containing calories as well as a drug of abuse. Findings from studies show that hypothalamic peptides are powerful controllers of the urge to eat and keep eating. In addition to stimulating food intake, these peptides are strongly affected by the food being ingested (Berthoud, 2000; Leibowitz & Hoebel, 2004). It is possible, therefore, that ethanol intake involves brain circuits that activate ingestive behaviors.

About 28 neuropeptides in the hypothalamus are known to modulate eating (Leibowitz & Hoebel, 2004). Two peptides known to induce eating, neuropeptide Y and galanin (Crawley et al., 1990; Kyrkouli et al., 1986; Leibowitz & Hoebel, 2004), have been suggested to play a role in ethanol intake (Badia-Elder et al., 2001; Caberlotto et al., 2001; Kelley et al., 2001; Leibowitz et al., 2003). The synthesis of neuropeptide Y is reduced by ethanol consumption (Caberlotto et al., 2001), and the injection of neuropeptide Y either suppresses or stimulates ethanol intake, depending on the site of administration (Badia-Elder et al., 2001; Katner et al., 2002a, 2002b; Kelley et al., 2001; Schroeder et al., 2003; Slawecki et al., 2000). Less work has been done with galanin. Systemic injection of ethanol or voluntary ethanol intake has been shown to increase galanin expression in certain hypothalamic nuclei, including the paraventricular nucleus (Leibowitz et al., 2003). This finding supports the suggestion that galanin injections might modulate ethanol intake.

To test a causal relation between the paraventricular nucleus–galanin system and ethanol intake, we examined the effect of galanin injection into the paraventricular nucleus, where this peptide is known to stimulate feeding and its expression is increased by ethanol. In the experiments, galanin was injected into rats with a baseline of moderate ethanol intake, to allow detection of both a stimulatory and an inhibitory effect of galanin.
2. Materials and methods

2.1. Animals and surgery

Naive, male, Sprague–Dawley rats (N = 35), bred at Princeton University from stock originating from Taconic Farms (Germantown, NY) and each weighing between 300 and 350 g at onset of experiments, were housed individually on a reversed 12-h light/12-h dark cycle with ad libitum access to LabDiet rodent chow (St. Louis, MO) and water. For surgery, animals were anesthetized with ketamine (80 mg/kg, i.p.) supplemented by xylazine (10 mg/kg, i.p.). Stainless steel guide shafts (21 gauge) were unilaterally implanted, half on the left side and half on the right side, above the paraventricular nucleus by using the following coordinates: B −1.8 mm, L 0.3 mm, and V 3.2 mm, with reference to bregma, midsagittal sinus, and the level skull. Stylets (26 gauge, 10-mm long) were inserted into the guide cannulas to keep them patent. Animals were allowed to recover for at least 1 week before the onset of ethanol access. Microinjectors were made of fused silica tubing (37 µm i.d. × 160 µm o.d.) that extended 5.0 mm beyond the guide shaft to reach a ventral coordinate of 8.2 mm below the skull surface. For the anatomic site control in Experiment 4, microinjectors were 2.0 mm shorter. Care and use of, as well as all procedures involving, animals were approved by the Princeton University Institutional Animal Care and Use Committee.

2.2. Ethanol access

Ethanol was presented ad libitum by means of a graduated cylinder fitted with a 4-in curved drinking tube with a steel ball to prevent leakage (Lab Products Inc., Seaford, DE). Ethanol solutions were diluted from 200 proof ethanol (AAPEX Alcohol and Chemical Co., Shelbyville, KY) to appropriate concentrations with tap water. Rats were introduced to ethanol at 1% (volume/volume), with an increase in concentration to 2% and, finally, 4% over a 1-week period. They were subsequently maintained on 4% ethanol for 4 weeks. Both chow and water were available ad libitum during this time. All rats were assigned to one of two groups according to levels of ethanol consumption on the basis of their ethanol intake during the last week of access: high levels (>1.5 g/kg/day) or low levels (<1.0 g/kg/day). The concentration of ethanol used in these experiments (4%) was selected on the basis of previous research findings from our laboratory, showing this concentration to be consumed in the highest quantity, per gram of body weight, by rats with ad libitum access to ethanol.

2.3. Microinjection procedures and ethanol measurements

For all experiments, galanin and the galanin antagonist M-40 (both from American Peptide Co., Inc., Sunnyvale, CA; dissolved in Ringer’s solution), or Ringer’s solution as a control, was injected into the paraventricular nucleus by means of a syringe pump set to deliver 0.5 µl in 45 s. The injectors were left in place for an additional 45 s to reduce reflux. Injection of freshly dissolved galanin (Experiments 1, 2, and 4) or M-40 (Experiment 3) was counterbalanced with Ringer’s solution, with half the rats receiving galanin or M-40 on the first day and Ringer’s solution on the next day, and the other half receiving these materials in a reverse pattern. When counterbalanced injections of drug versus Ringer’s solution were repeated by using the same animals, this second set of tests was started 5 days later. All injections were performed 4 to 5 h into the dark cycle when the rats were active. For each test, an observer blind to the experimental conditions measured ethanol intake hourly for the first 4 h and, subsequently, at 8 and 24 h after the injection. Food and water intake was not measured.

2.4. Experiment 1: galanin injection in rats consuming either high levels or low levels of ethanol

Galanin or Ringer’s solution (1.0 nmol) was injected unilaterally into rats that consumed either high levels (n = 6) or low levels (n = 4) of ethanol. Rats did not have access to food or water during the first 4 h after the microinjection but were given food and water for the remainder of the 24-h test period.

2.5. Experiment 2: galanin injection in rats consuming high levels of ethanol, with and without food and water available

To replicate and expand the finding of Experiment 1, a larger number of rats (n = 13) were injected with galanin or Ringer’s solution, as described above (see Section 2.3.). Because rats that consumed low levels of ethanol (<1.0 g/kg/day) failed to show a galanin response in Experiment 1, this experiment tested only rats that consumed high levels (>1.5 g/kg/day) of ethanol, which constituted 13 of a total of 25 rats. Three tests were performed. In the first test, we examined the effect of galanin (1.0 nmol) counterbalanced with Ringer’s solution, with food and water absent during the first 4 h after the injection. In the second test, 5 days later, we examined the same dose of galanin, but with food and water available. In the third test, we used the same design to examine a lower dose of galanin (0.5 nmol) counterbalanced with Ringer’s solution, with food and water available.

2.6. Experiment 3: injection of a specific galanin antagonist (M-40) in rats consuming high levels of ethanol

The rats in Experiment 2 (n = 13) were given an additional set of tests, with the galanin receptor antagonist M-40 (0.5 nmol), counterbalanced with Ringer’s solution, injected unilaterally into the paraventricular nucleus, with food and water available.

2.7. Experiment 4: injection of galanin in a control site

To test for possible leakage into the ventricle or reflux up the cannula to other sites, the rats from Experiment 2
were tested with galanin (1.0 nmol) or Ringer’s solution injected 2 mm dorsal to the original injection site in the paraventricular nucleus. Once again, food and water were available.

2.8. Blood alcohol levels, histologic studies, and statistical analyses

After completion of the microinjection experiments, the rats were maintained for 1 week on ad libitum access to 4% ethanol, chow, and water. Blood samples were collected from the tail vein 4 to 5 h into the dark cycle and subsequently cooled and centrifuged to obtain serum for analysis of blood alcohol levels (Analox, model GM7, Lunenburg, MA).

Histologic evaluation of rat brains was performed to verify microinjector location. Rats received an overdose of sodium pentobarbital and were perfused with 0.9% saline, followed by 10% formalin. Brains were removed, and frozen sections (40-μm thick) were taken from the anterior lobe caudally until probe tracks were identified. Results from comparison of galanin or M-40 with Ringer’s solution were analyzed by a two-tailed Student t test for paired samples or two-way analysis of variance (ANOVA) for repeated measures, with post hoc Student t tests when justified. Dose-response data were analyzed by one-way repeated-measures ANOVA, and correlations were computed by using Pearson correlation coefficient.

3. Results

3.1. Experiment 1: galanin increases ethanol intake in rats consuming high levels of ethanol

In rats with a history of drinking at least 1.5 g/kg/day, galanin (1.0 nmol) injected into the paraventricular nucleus significantly increased ethanol intake compared with findings for Ringer’s solution control \( F(1,16) = 4.93, P < .05; \) Fig. 1]. This effect was detected in the first hour after injection, with no change detected at 4, 8, or 24 h. In rats that consumed low levels (<1.0 g/kg/day) of ethanol, galanin infusion produced no change in ethanol intake (Fig. 1).

3.2. Experiment 2: galanin in rats consuming high levels of ethanol increased ethanol intake both with and without food and water available

Galanin (1.0 nmol) injected into the paraventricular nucleus of rats that consumed high levels of ethanol, without food and water available, again increased ethanol intake \( t(12) = 2.94, P < .02; \) Fig. 2. As in Experiment 1, this effect was seen in the first hour, with no significant differences in total intake evident at 4, 8, or 24 h after infusion.

This effect was larger when food and water were available. There was a dose-related response to galanin \( F(11,2) = 12.71, P < .01; \) Fig. 3]. Compared with findings for Ringer’s solution, galanin (1 nmol) significantly increased ethanol intake during the first hour \( t(12) = 4.11, P < .002 \). However, in contrast with the results with food and water absent, this increase in ethanol intake persisted up to the 8th hour after injection \( t(12) = 2.90, P < .02 \).

The lower dose of galanin (0.5 nmol) also increased ethanol intake \( t(12) = 4.19, P < .002 \). Rats infused with galanin consumed an average of 0.38 ± 0.03 g of ethanol in the first hour, compared with 0.26 ± 0.03 g with Ringer’s solution. The effect of 0.5 nmol of galanin lasted 1 h, compared with the longer lasting effect of 1 nmol of galanin. A dose dependency of this galanin effect in the first hour with food and water available was indicated by a strong, positive correlation between the doses of galanin (0.0, 0.5, and 1.0 nmol) and intake of ethanol \( r = 0.989, P < .01 \); Fig. 4).

3.3. Experiment 3: M-40 significantly decreased ethanol consumption

The specific galanin antagonist M-40 given unilaterally was sufficient to decrease ethanol intake significantly compared with findings for Ringer’s solution \( t(12) = 3.74, P < .005; \) Fig. 5]. This inhibitory effect was strongest during the first hour but was still significant after 2 h. It was not apparent in the measures taken at 4, 8, and 24 h.

3.4. Experiment 4: galanin had no effect on ethanol intake when injected dorsal to the paraventricular nucleus

The higher dose of galanin (1 nmol) injected 2 mm above the original paraventricular nucleus site did not modify...
ethanol intake (0.24 ± 0.03 g/kg, first hour) compared with findings for Ringer’s solution (0.29 ± 0.04 g/kg, first hour).

### 3.5. Ethanol intake, blood alcohol levels, and histologic studies

Rats that were categorized as drinking high levels (>1.5 g/kg/day) of ethanol consumed between 2.0 and 3.6 g/kg/day, with a mean of 2.6 ± 0.13 g/kg/day on the days when they were not handled for infusions. Their mean blood alcohol level was 25 ± 1.17 mg/dl.

Fig. 6 shows that the injector tips of all rats were located in the anterior portion of the paraventricular nucleus.

### 4. Discussion

Findings from the current experiments support the suggestion that galanin, a feeding-stimulatory peptide, participates in the induction of ethanol consumption. This is supported by the finding that injection of galanin significantly stimulated ethanol intake in a dose-dependent fashion. Although Experiment 1 was designed without food and water available to avoid competing stimuli, results of Experiment 2 show that galanin-induced ethanol intake was even greater with food and water available. The finding that galanin-induced ethanol intake was enhanced with food and water available is interesting because galanin is known to induce feeding (Crawley et al., 1990; Kyrkouli et al., 1986; Leibowitz & Hoebel, 2004). This finding, that galanin-injected rats drink ethanol despite the availability of chow and water, supports the possibility that galanin in ethanol-drinking rats increases the intake of ethanol, as opposed to ingestion of calories or fluids in general. In support of this possibility is the additional finding that the galanin receptor antagonist M-40 significantly inhibited the consumption of ethanol. Because these results were obtained by using unilateral injections, with galanin doubling ethanol intake and M-40 halving it, it is possible that bilateral treatments might have even larger effects.

The above-described results support the suggestion that the paraventricular nucleus is one site in which galanin may act to stimulate ethanol intake. Both galanin and M-40 were effective when injected into this site. Moreover, injections made 2 mm dorsal to the paraventricular nucleus had no apparent effect on ethanol intake. This off-target, control test was especially important, in light of evidence that galanin also stimulates ethanol intake when injected at higher doses into the third ventricle (Lewis et al., in press). From the current study results, it seems that the paraventricular nucleus is one site in which ventricular galanin is likely to diffuse and act. Results of the ventricular galanin injection study also showed that rats preferring 7% ethanol in a two-bottle-choice paradigm (ethanol vs. water) increase their consumption of ethanol in response to galanin. Thus, the stimulatory effect of this peptide on ethanol intake can occur...
Fig. 4. There is a dose dependency of the effect of galanin (GAL) on ethanol intake in the first hour with food and water available, as indicated by a strong, positive correlation between the doses of GAL (0.0, 0.5, and 1.0 nmol) and the intake of ethanol \((r = 0.98)\). Ethanol intake with 0 nmol of galanin is the average on the Ringer control at 0.5 and 1.0 nmol. Ringer = Ringer’s solution.

Fig. 5. The galanin antagonist M-40 significantly decreased ethanol intake. This effect persisted for 2 h after unilateral paraventricular nucleus injection \((n = 13; ^* P < .05)\). Ringer = Ringer’s solution.

Galanin-induced ethanol intake is not likely to be due to some nonspecific behavioral effect of the peptide. This is supported by evidence showing that injection of galanin in the ventral tegmental area or ventricles depresses, rather than excites, locomotor behavior (Ericson & Ahlenius, 1999; Wrenn & Crawley, 2001). As for a nonspecific stimulatory effect, this also seems unlikely in light of the current study results demonstrating that galanin increased ethanol consumption only in rats that showed avidity for ethanol, ruling out random hyperactivity with undirected licking. Further, galanin-injected animals drank more ethanol than consumed by the control rats, whether or not food and water were available.

Other brain peptides have been shown to contribute to ethanol intake. Opioid expression, receptors, and release are altered after long-term ethanol administration (Gianoulakis, 1989; Herz, 1997; Khatami et al., 1987; Lucchi et al., 1984; Winkler et al., 1998), and morphine at a low dose increases ethanol intake (Hubbell et al., 1986; Linseman & Harding, 1990; Reid & Hunter, 1984). Moreover, a mu-opioid agonist injected directly in the nucleus accumbens increases ethanol intake (Zhang & Kelley, 2002), and opioid antagonists (naloxone or naltrexone) decrease ethanol intake, both in animals and in human beings (Boyle et al., 1998; Marfaing-Jallat et al., 1983; Romach et al., 2002). Naloxone can also be used to precipitate withdrawal from ethanol, as shown by a decrease in extracellular dopamine and increase in acetylcholine concentrations in the nucleus accumbens, which is characteristic of opiate withdrawal (Rada et al., 1996, in press), confirming that the opioid system plays an important role in regulation of ethanol drinking (Gonzales & Weiss, 1998; Heyser et al., 1999). It is noteworthy that galanin and the opioids interact in several behavioral settings. For instance, galanin potentiates morphine analgesia (Przewlocka et al., 1995), and in studies of ingestive behavior, naloxone blocks galanin-induced feeding (Barton et al., 1996; Dube et al., 1994) and decreases paraventricular nucleus galanin mRNA in ethanol-drinking rats (Leibowitz et al., 2003). Galanin injection in the paraventricular nucleus releases dopamine and simultaneously inhibits acetylcholine release in the nucleus accumbens, and this occurs only in rats that exhibit galanin-induced feeding (Rada et al., 1998). This seems to indicate that ethanol consumption induced by paraventricular nucleus injections of galanin may be mediated through dopamine in the nucleus accumbens.

Findings from other studies indicate that not all feeding-stimulatory peptides induce ethanol drinking, nor are they all stimulated by the consumption of ethanol (Thiele et al., 2003). Intraventricular injection of neuropeptide Y has no effect on ethanol intake in Wistar or alcohol-preferring AA (Alko Alcohol) rats (Badia-Elder et al., 2001; Katner et al., 2002b; Slawekci et al., 2000), and it reduces ethanol intake in alcohol-preferring (P) rats (Badia-Elder et al., 2001). However, when neuropeptide Y is given locally into the...
paraventricular nucleus of Long–Evans rats, it can significantly increase ethanol intake (Kelley et al., 2001). The mechanisms through which neuropeptide Y and galanin operate are likely to be different, in light of evidence obtained in studies of feeding behavior. For example, neuropeptide Y and galanin have differential effects on the ingestion of carbohydrate and fat (Leibowitz, 1985; Tempel et al., 1988), on circulating hormones, and on neurotransmitter release in the nucleus accumbens (Baranowska et al., 1999; Kyrkouli et al., 1990; Leiva & Crozatto, 1994). Dopamine is released in the nucleus accumbens by paraventricular nucleus injection of galanin, but not of neuropeptide Y (Rada et al., 1998). It is particularly relevant that ethanol suppresses neuropeptide Y gene expression in the arcuate nucleus of the hypothalamus while increasing galanin expression in the paraventricular nucleus (Leibowitz et al., 2003; Pandey et al., 2003).

There may be positive feedback between galanin in the paraventricular nucleus and the intake of ethanol. Not only does galanin elicit ethanol intake, but ethanol intake increases galanin expression in several hypothalamic sites, including the paraventricular nucleus (Leibowitz et al., 2003). Thus, galanin injected into the paraventricular nucleus, at a dose known to increase food intake and release dopamine in the nucleus accumbens, also potentiates ethanol intake in rats that have learned to drink. Under the influence of galanin, intake of 4% ethanol nearly doubled in the first hour, with or without food and water available as competing stimuli. Because ethanol intake can increase both the expression of galanin mRNA and the levels of galanin peptide in the paraventricular nucleus, this could set the stage for positive feedback between galanin and ethanol intake, requiring other systems to bring drinking to a halt. This positive feedback may be a contributing factor leading from moderate ethanol intake to excessive intake and alcoholism in susceptible individuals.

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References


Fig. 6. Black dots show the injection site in the paraventricular nucleus for all animals used in the experiments. Adapted from The Rat Brain (compact third edition), G. Paxinos and C. Watson, figs. 25 and 26, Copyright 1997, with permission from Elsevier.


