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The Effect of Intragastric Ethanol on Meal Size in the Rat

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SEELEY, R. J., L. M. SHARON AND S. C. WOODS. *The effect of intragastric ethanol on meal size in the rat.* PHARMACOL BIOCHEM BEHAV **56**(3) 379–382, 1997.—Although ethanol is a calorically dense substance, little is known about the mechanisms by which those calories are detected, nor how they effect subsequent intake of other calories. In the current study, four doses of ethanol (0, 1, 2, and 3.5 g/kg) were administered to rats prior to 30-min access to a sucrose solution. The effect of ethanol (EtOH) to impact sucrose intake was compared to that of another carbohydrate, glucose, matched for calories, and to NaCl solutions matched to the osmotic properties of the glucose. A final set of conditions provided calories in the form of EtOH as well as osmoles in the form of NaCl to match the combined caloric and osmotic properties of the glucose solutions. Aqueous solutions of EtOH and of glucose suppressed food intake in a dose-dependent fashion with glucose tending to be more effective than EtOH. NaCl in water did not suppress intake. Surprisingly, when NaCl was added to the EtOH solution the effect of EtOH to suppress food intake was completely ameliorated. Subsequent analysis of plasma EtOH levels showed that adding NaCl also reduced the rate at which EtOH appeared in the plasma. The results suggest that changes of short-term food intake caused by EtOH calories are produced by different inhibitory signals than those produced by other carbohydrates. While other carbohydrates generate preabsorptive signals within the stomach, ethanol appears to have to leave the stomach to inhibit further food intake.

Food intake Ethanol Meal size Gastric emptying Stomach Satiety

ETHANOL (EtOH), particularly wine, has long been considered as an appetite enhancer in humans (11). This belief occurs despite the fact that EtOH is calorically dense, and proportionally has more energy than equal amounts of other carbohydrates. In fact, in heavy drinkers, EtOH can provide a substantial proportion of the daily caloric intake (7) and impacts subsequent food ingestion in humans and animals under many conditions (3,5,6,13,18). Such observations strongly imply that calories from EtOH generate signals that impact ingestion of food. Despite the strength of these conclusions, little is known about the nature or location of caloric signals generated by EtOH, nor how they impact subsequent ingestion.

The current study used a standard pre-load paradigm to compare the food intake suppressive effects of EtOH to those of another carbohydrate, glucose. In the pre-load paradigm, rats are trained to consume a test meal by providing them 30-min access to a highly palatable food at the same time each day. Rats adapted to such a regimen begin consuming the food as soon as it becomes available and they consume comparably sized meals each day. Once animals have been trained in this way, a caloric solution can be delivered directly into the stomach via an indwelling gastric catheter prior to the 30-min food access and its effect on meal size measured.

This paradigm has the advantage of minimizing any impact of long-term regulatory systems that might alter compensatory changes in food intake after EtOH calories. It is likely that calories from EtOH are taken into account once they are either burned for energy or stored in the form of adipose tissue, just as occurs for other metabolic substrates. The question addressed by the current study is whether calories from EtOH, like those of other carbohydrates, can be detected preabsorptively and influence subsequent food intake. In this way, any role that various inhibitory feedback signals from gastric and post-gastric sites play in the suppressive effects of EtOH on food intake can be assessed.

EXPERIMENT 1: THE EFFECT OF EtOH ON SUCROSE INTAKE

Methods

Subjects. Subjects were eight naive male, Long-Evans rats, weighing between 350 and 450 g. All animals were housed in individual, hanging, wire-mesh cages in a temperature-controlled vivarium, with a 12:12 h light:dark photoperiod. Pelleted rat chow and tap water were available ad libitum.

Surgical Procedures. Gastric catheters were constructed

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TABLE 1
DOSES OF THE MATCHED GLUCOSE AND NaCl SOLUTIONS USED IN EXPERIMENT 1

EtOH dose (g/kg)	Glucose (g/kg)	NaCl (g/kg)
1.00	1.78	0.29
2.00	3.55	0.58
3.50	6.21	1.01

from 15 centimeters of silastic tubing. A small amount of silastic bonding was applied approximately 2 cm from the gastric end of the catheter. Next, a dacron and silastic mesh disc about 1 cm in diameter was threaded down the tubing. The disc and bonding together served as an anchor for the catheter in the stomach. All rats were food deprived for 24 h prior to surgery, and anesthetized with 3.7 mg/kg equithesin. A 1-cm incision was made at the skull and another 2-cm incision along the midline just below the rib cage. The muscle wall was cut and the stomach exteriorized. A short incision was made in the greater curvature of the stomach, the disc end of the catheter was inserted into the stomach, and the incision sealed with two small stitches. The catheter was then threaded through the stomach wall and subcutaneously routed to the incision at the top of the skull. A 1.5-cm length of 23-gauge metal tubing was force fit into the silastic tubing of the catheter. Four screws were placed and the metal tubing was anchored to the screws with dental acrylic.

Experimental Procedure. Rats were given 30 min access to 0.1 molar sucrose solution each day for a period of 12 days. By the 12th day, all animals were responding immediately to the sucrose when it was presented and intake was consistent across days. Starting on Day 13, animals were administered a 10-ml injection of one of 16 solutions via the gastrostomy tube, 30 min prior to sucrose exposure. Each animal received every condition with solution order counterbalanced across subjects according to a latin-square design such that in any two days all 16 conditions were represented.

To ascertain the effect of intragastric EtOH on sucrose intake, three ethanol (EtOH) doses were used (Table 1). These EtOH solutions were prepared by using either 1, 2, or 3.5 g of EtOH per kg of the animal's body weight mixed in distilled water to a total volume of 10 ml. To assess the impact of intragastric glucose on sucrose ingestion, three glucose concentrations were administered. The glucose solutions were prepared by calculating the number of calories in each of the EtOH conditions, determining the amount of glucose needed to provide the equivalent number of calories, and mixing the appropriate amount of glucose with distilled water to make a 10-ml total solution. Since the osmotic properties of glucose in the stomach may be at least partially responsible for its suppression of sucrose intake and since EtOH does not change the osmotic properties of water, it was necessary to assess independently the impact of the osmotic stimulus on intake by administering a solution with the same osmotic properties as the glucose, but without the calories. Thus, three NaCl solutions were also administered. For NaCl solution preparation, moles of glucose administered in each of the three glucose concentrations were calculated. Since the number of moles of NaCl in solution doubles as it dissociates in solution, half this number of moles of NaCl were weighed, and sufficient distilled water added to make 10 ml. Hence, total osmoles were equal for the glucose and NaCl solutions. To determine the impact

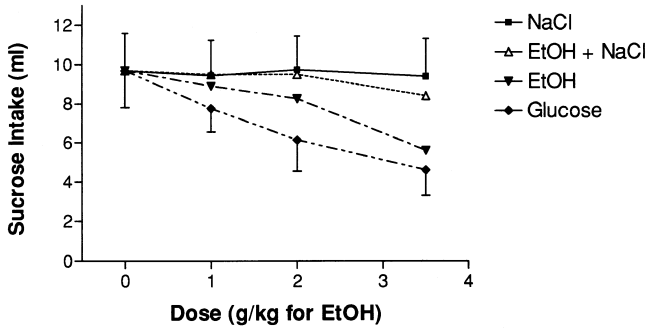

FIG. 1. Thirty-min intake of 0.1 M sucrose solution after gastric preloads of either EtOH, glucose, NaCl or EtOH plus NaCl. The glucose condition was matched to the caloric value of the various doses of EtOH and both NaCl conditions were matched to the osmoles of the corresponding glucose condition. Dose 0 is the response following 10 ml of only water. Data are expressed as means ± SEM with some error bars omitted for clarity.

of a solution reproducing, as closely as possible, the combined osmotic and caloric properties of the glucose conditions, three EtOH plus NaCl conditions were also administered. These solutions were made by combining the low, medium and high doses of EtOH and NaCl respectively each in a total volume of 10 ml. Hence, there were three EtOH plus water conditions, three glucose plus water conditions matched calorically to the EtOH, three saline conditions matched osmotically to the glucose, and three EtOH plus saline conditions matched osmotically and calorically to the glucose. Interspersed among these sessions were four conditions consisting of 10 ml of water as a control.

Results

As seen in Fig. 1, rats administered aqueous solutions of either glucose or EtOH displayed a dose-dependent suppression of sucrose intake, with equicaloric glucose being more effective than EtOH at suppressing sucrose intake at each dose. NaCl, by itself, had no impact on sucrose intake at any concentration. Interestingly, however, the EtOH + NaCl condition also caused no suppression at all. Data were analyzed by two-way repeated measures ANOVA using condition (EtOH vs glucose vs NaCl vs EtOH plus NaCl) and dose. There was a significant main effect for condition [$F(3, 21) = 5.89$, $p < 0.01$] but not for dose [$F(3, 21) = 1.60$, $p = 0.22$]. The lack of a main effect for dose is probably due to the inclusion of two conditions (NaCl and EtOH +NaCl) that had no effect on intake. A one-way repeated measures ANOVA on only the highest dose of each condition yielded a significant effect [$F(3, 21) = 6.05$, $p < 0.01$] and planned comparisons using paired t-tests revealed that while the effects of aqueous solutions of EtOH and glucose do not differ from each other, both were significantly different from NaCl and EtOH plus NaCl, which in turn did not differ from one another. The overall effectiveness of EtOH to suppress food relative to glucose was compared using a two-way ANOVA excluding the zero dose for both conditions. This analysis showed an almost significant effect of glucose vs EtOH [$F(1, 7) = 4.45$, $p = 0.07$]. Additionally, there was no evidence that in this paradigm, EtOH produced a conditioned taste aversion. Sucrose intake after the water only preload throughout the

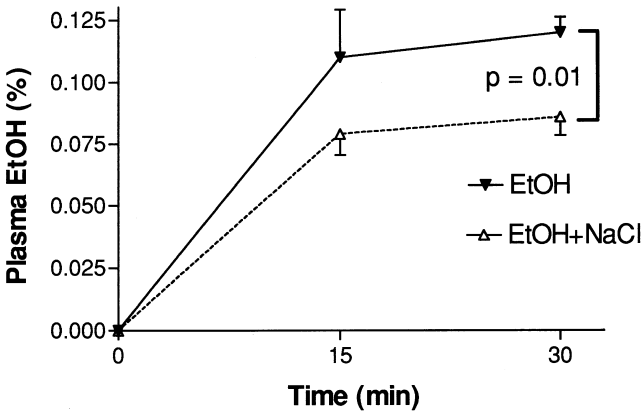

FIG. 2. Plasma ethanol concentrations after either a 3.5 g/kg dose of aqueous EtOH or after a 3.5 g/kg dose of EtOH plus NaCl. Data are expressed as means ± SEM.

duration of the experiment was the same as baseline intakes before any EtOH was administered.

EXPERIMENT 2

Subjects. Six rats, weighing between 460 and 580, g were housed and maintained in the same environment and received gastrostomy tubes as described in Experiment 1.

Experimental Procedure. On two consecutive days, rats were administered either EtOH in a concentration of 3.5 g EtOH per kilogram body weight (the highest dose used in Experiment 1), or EtOH + NaCl using the same concentration of EtOH , plus the appropriate amount of NaCl as used in Experiment 1. Condition order was counterbalanced across subjects. Following administration, blood samples were taken from the tail vein 15 and 30 min after administration of the solutions. Blood was immediately centrifuged and the plasma refrigerated for later assay of plasma EtOH. Plasma EtOH was determined on all samples using Sigma Diagnostics Quantitative, Enzymatic Determination of Alcohol Test.

Results

As depicted in Fig. 2, plasma EtOH concentrations were 32% less in EtOH plus NaCl at 15 min and 28% less at 30 min relative to the EtOH alone condition. Data were analyzed with a two-way ANOVA using time (15 vs 30 min) and condition EtOH vs EtOH+ NaCl) as main effects. There was a significant effect for condition [$F(1, 16) = 7.99$, $p = 0.01$].

DISCUSSION

The current results indicate that aqueous solutions of EtOH suppress food intake in a dose-dependent fashion when introduced directly into the stomach. Similarly, equicaloric aqueous solutions of glucose also suppress intake, with a trend towards glucose being more effective than EtOH. The ability of glucose to suppress food intake, however, cannot be interpreted solely on the basis of calories since, unlike EtOH, glucose has both caloric and osmotic properties. An impact of osmotic factors to reduce food intake is well documented (17). Therefore, to assess the impact of osmotic stimuli on sucrose intake independently, NaCl was added to both the water and the EtOH solutions. No dose of NaCl in water changed subsequent sucrose intake relative to the water control. Adding NaCl to the EtOH solution not only does not produce additional suppression of intake, it completely ameliorated the ability of EtOH to suppress food intake. These results were counterintuitive and suggest that rather than more closely simulating the effect of glucose, adding NaCl to EtOH reverses the suppressive effect of EtOH on food intake in the 30 min test. The results of Experiment 2 suggest that the rate at which EtOH leaves the stomach (either through the pylorus or through the stomach wall) and appears in the blood is slowed by the addition of NaCl in the highest doses used in Experiment 1. A natural conclusion is that this slowed rate of absorption may be the explanation for EtOH's reduced suppressive effect in the presence of NaCl. If so, changing the distribution of EtOH in the body has a profound impact on the ability of EtOH to alter subsequent food intake. The more EtOH that remains sequestered in the stomach, the less that food intake is suppressed.

This conclusion is quite different than the one that would be drawn for other carbohydrates such as glucose. Manipulations that bias the relative distribution and absorption of glucose during a meal do not alter the amount consumed in subsequent test meals (8,14). Glucose can be completely sequestered in the stomach by the use of a pyloric cuff which, when inflated, prevents glucose from emptying through the pylorus. The ability of intragastric glucose to alter subsequent intake, however, is not changed by this manipulation (15). That is to say, glucose calories that are completely held in the stomach suppress food intake equivalently to glucose calories that are distributed throughout the gastrointestinal tract and beyond. These results imply that glucose calories can be detected in the stomach itself, and that signals arising from the stomach effect subsequent food intake.

EtOH calories apparently follow different rules than calories derived from glucose. Their ability to suppress food intake depends critically on EtOH or its effects being sensed in locations other than in the stomach. Several possibilities exist as to the location of signals related to EtOH calories. EtOH produces elevations in the gastrointestinal hormone cholecystokinin (CCK) (10). Several lines of evidence now point to CCK being a satiety hormone that suppresses further food intake (for a review see (16)). It is therefore possible that the suppressive effect of EtOH is a result of its action in the duodenum to cause the release of CCK. Another possibility is that EtOH calories are detected in the liver. Again, several lines of evidence point to a role for the liver in the control of food intake (4) and it is only in the liver that EtOH is processed into products that can be used as metabolic fuels. Finally, it is possible that EtOH suppresses food intake by direct effects on areas of the central nervous system. This could either be in the form of producing greater general intoxication which results in impaired ability to produce ingestive behaviors or by ethanol acting directly in CNS regions important to the regulation of food intake. The possibility that EtOH 's food-suppressive effect is related to its pharmacological effects in the CNS rather than to its caloric properties is yet to be explored.

The current data provide no evidence that EtOH facilitates subsequent food intake. This is despite that the current study closely simulates the situations where EtOH is purported to have this effect anecdotally. EtOH was delivered in close temporal proximity to an anticipated and highly palatable meal. Across a wide dose range that would be similar to moderate to heavy EtOH usage in humans, rats never consumed more of the test meal after EtOH administration relative to the control conditions. What is true, however, is that the combined

calories from the EtOH and the sucrose test meal were higher in the EtOH condition and especially in the EtOH plus NaCl condition. In fact, when EtOH is served in the context of a meal, it is likely that food serves to sequester additional EtOH in the stomach just as the NaCl does in the current experiment. Eating food with EtOH would therefore be anticipated to reduce the ability of consumed EtOH to suppress subsequent food intake. Consequently, while EtOH by itself does not produce an enhancement of food intake in these conditions, the total calories consumed in a meal that includes EtOH may be substantially higher than a meal that does not include EtOH.

While there is little evidence in humans or animals for the contention that EtOH is an appetite enhancer, human studies have disagreed about whether or not EtOH displaces other calories in the diet. Several studies have reported that EtOH calories cause a reduction in other carbohydrate intake, especially in relatively heavy drinkers (3,6,18), while others find that EtOH calories are simply added on to the total caloric intake in more moderate drinkers (1,2,7,9). The current results offer two potential explanations for these contradicting results. First, the dose-dependency of the ability of EtOH to suppress subsequent food intake suggests that at moderate EtOH levels suppression is minimal. Consequently, it may be that EtOH only suppresses food intake beyond some critical threshold that is not attained in moderate drinkers in the human studies. Second, the ability of EtOH to suppress food intake in the current study was clearly dependent on the context in which it was delivered. EtOH delivered with NaCl has no impact whatsoever on food intake. It is likely that moderate drinkers are consuming a greater proportion of their EtOH with meals than are heavy drinkers. Accompanying food presumably acts much like the NaCl in the present experiment and sequesters more of the EtOH in the stomach, thereby substantially reducing its effect on food intake. Further research in both animals and humans is needed to delineate the caloric signals generated by EtOH and how and when such signals are integrated with other signals concerning food intake and body weight regulation.

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REFERENCES

1. Colditz, G. A.; Giovannucci, E.; Rimm, E. B.; Stampfer, M. I.; Rosner, B.; Speizer, F. E.; Gordis, E. Willet, W. Alcohol intake in relation to diet and obesity in women and men. Am. J. Clin. Nutr. 54:49–55; 1991.
2. de Castro, J. M.; Orozco, S.; The effects of moderate alcohol intake on the spontaneous eating patterns of humans: Evidence of unregulated supplementation. Am. J. Clin. Nutr. 52:246–253; 1990.
3. Foltin, R. W.; Keeley, T. H.; Fischman, M. W. Ethanol as an energy source in humans: Comparison with dextrose-containing beverages. Appetite 20:95–110; 1993.
4. Friedman, M. I. Body fat and the metabolic control of food intake. Int. J. Obesity 14:53–66; 1990.
5. Giner, M.; Meguid, M. M. Effect of intragastric and intravenous ethanol on food intake in rats. Physiol. Behav. 54:399–401; 1993.
6. Hillers, V. N.; Massey, L. K. Interrelationships of moderate and high alcohol consumption with diet and health status. Am. J. Clin. Nutr. 41:356–362; 1985.
7. Jones, B. R.; Barrett–Conner, E.; Criqui, M. H.; Holdbrook, M. J. A community study of calorie and nutrient intake in drinkers and nondrinkers of alcohol. Am. J. Clin. Nutr. 35:135–141; 1982.
8. Kaplan, J. M.; Siemers, W.; Grill, H. J. Ingestion, gastric fill, and gastric emptying before and after withdrawal of gastric contents. Am. J. Physiology 267:R1257–1265; 1994.
9. Le Marchand, L, Kolonel, L. N.; Hankin, J. H.; Yoshizawa, C. N. Relationship of alcohol consumption to diet: A population-based study in Hawaii. Am. J. Clin. Nutr. 49:567–572; 1989.
10. Liddle, R. A.; Goldfine, I. D.; Williams, J. A. Bioassay of plasma cholecystokinin in rats: Effects of food, trypsin inhibitor and alcohol. Gastroenterology 87:542–549; 1984.
11. McDonald, J. T.; Margen, S. Wine versus ethanol in human nutrition: Nitrogen and calorie balance. Am. J. Clin. Nutr. 29:1093–1103; 1976.
12. Orozco, S.; de Castro, J. M. Effects of alcohol abstinence on spontaneous feeding patterns in moderate alcohol consuming humans. Pharmacol. Biochem. Behav. 40:867–873; 1991.
13. Richardson, A.; Rumsey, R. D.; Read, N. W. The effect of ethanol on the normal food intake and eating behavior of the rat. Physiol. Behav. 48:845–848; 1990.
14. Seeley, R. J.; Kaplan, J. M.; Grill, H. J. Effects of interrupting an intraoral meal on meal size and meal duration in rats. Appetite 20:13–20; 1993.
15. Seeley, R. J.; Kaplan, J. M.; Grill, H. J. The effect of occluding the pyloris on intraoral intake: a test of the gastric hypothesis of meal termination. Physiol. Behav. 58:245–249; 1995.
16. Smith, G. P.; Gibbs, J. Satiating effect of cholecystokinin. Ann. NY Acad. Sci. 713:236–241; 1994.
17. Stricker, E. M.; Verbalis, J. G. Caloric and noncaloric controls of food intake. Brain Res. Bull. 27:299–303; 1991.
18. Windham, C. T.; Wyse, B. W.; Hansen, R. G. Alcohol consumption and nutrient density of diets in the Nationwide Food Consumption Survey. J. Am. Diet. Assoc. 82:365–372; 1983.