ELSEVIER

Peptides 20 (1999) 737–741

PEPTIDES

Gastrin-releasing peptide suppresses independent but not intraoral intake

Paul A. Rushing*, Thomas A. Houpt

Department of Psychiatry, Cornell University Medical College, and Edward W. Bourne Behavioral Research Laboratory, New York Hospital-Cornell Medical Center, White Plains, NY 10605, USA

Received 7 January 1999; accepted 10 February 1999

Abstract

Independent and intraoral intake tests have been used to separate the effects of various substances on the appetitive and consummatory phases of ingestive behavior. This study compared the ability of gastrin-releasing peptide$_{1–27}$ (GRP) to suppress intraoral intake of nutrient solutions versus independent intake of the same solutions from a bottle. In a series of experiments, adult male Sprague–Dawley rats implanted with anterior sublingual chronic intraoral catheters were injected intraperitoneally with saline control or 28 μg/kg GRP before 20-min intraoral and 20-min one-bottle intake tests of a sucrose (0.1 M) and milk solution (1.2 kcal/ml). GRP potently reduced independent intake of both sucrose and milk from a bottle but had no significant effect on intraoral intake of either solution. From these results, we conclude that GRP affects appetitive-related aspects of the feeding process to reduce food intake. © 1999 Elsevier Science Inc. All rights reserved.

Keywords: Satiety; Ingestion; Bombesin; Cholecystokinin; Rat

1. Introduction

Manipulations that affect ingestion can act during either the appetitive or the consummatory phase [2]. The appetitive phase includes the search for food, meal initiation, preference choices, and somatic behaviors that compete with ingestion and terminate meals. The consummatory phase includes reflexive licking, chewing, and swallowing during ingestion and reveals the animal's direct orosensory evaluation of food or drink leading to acceptance or rejection.

These two phases of ingestion have been experimentally dissociated by examining ad libitum intake from a bottle and intake during infusions via an intraoral catheter. Independent intake from a bottle includes both the appetitive and consummatory phases, whereas intraoral infusions evoke the consummatory phase of ingestion independent of appetitive behaviors. These two paradigms have produced evidence suggesting that the neural circuitry underlying these phases can be independently activated. For example, unlike appetitive behaviors, unconditioned consummatory behaviors can be maintained solely by the neural circuitry of the hindbrain in chronic decerebrate rats [5].

Most manipulations that affect ad libitum or independent intake produce parallel effects on intraoral intake. Intraoral intake of a sucrose solution, for example, is increased by food deprivation [6]. This parallelism, however, is not evident in all manipulations. For example, neuropeptide Y does not increase intraoral intake of sucrose [11], although it is one of the most potent known orexigenics [1], and amphetamine decreases ad libitum but not intraoral intake [13].

Gastrin-releasing peptide$_{1–27}$ (GRP), a mammalian homolog of the amphibian-derived peptide bombesin [3], decreases feeding in humans and a variety of other species including rodents (for review, see Smith and Gibbs [12]). In the following series of experiments, we compare the ability of GRP to suppress intraoral versus independent intake of the same nutrient solutions. The results provide evidence that aids in the understanding of what aspect of the feeding process (i.e. appetitive or consummatory) GRP affects to regulate intake.

* Corresponding author. College of Medicine, Department of Psychiatry, University of Cincinnati, PO Box 670559, Cincinnati, OH 45267-0559. Tel.: +1-513-558-3431; fax: +1-513-558-8990.

E-mail address: Paul.Rushing@uc.edu (P.A. Rushing)

PII: S0196-9781(99)00056-X

2. Methods

2.1. Subjects and housing

Adult male Sprague–Dawley rats (300–400 g; Charles River, Wilmington, MA, USA) were individually housed under a 12-h light/dark cycle. Ad libitum food (rat chow) and water were provided except as noted below.

2.2. Surgery

Rats were implanted with anterior sublingual chronic intraoral catheters under methoxyflurane anesthesia as previously described [7]. In brief, polyethylene tubing (Intramedic PE 50, Clay Adams, Parsippany, NJ, USA) was heat-flared at one end and drawn through the floor of the mouth midway between the root of the lower incisors and the base of the tongue. The unflared end of the catheter was externalized between the scapulas on the dorsal surface of the rat's neck, and held in place with an outer sleeve of silicone tubing (1.02-mm inside diameter, 2.16-mm outside diameter; Technical Products, Decatur, GA, USA) attached to a surgical mesh disk (Marlex, Bard Implants, Billerica, MA, USA) sutured to the dorsal neck musculature.

2.3. Peptides

Gastrin-releasing peptide$_{1–27}$ (porcine GRP$_{1–27}$; Bachem, Torrance, CA, USA) was dissolved in bacteriostatic saline (0.9% benzyl alcohol, 0.9% NaCl; Abbott Laboratories, Chicago, IL, USA) to a concentration of 28 μg/ml (10 nmol/ml). The GRP solution was then aliquoted and frozen at −80°C for subsequent use in intake tests. In a similar manner, cholecystokinin (sulfated CCK octapeptide; a kind gift of the Bristol-Myers-Squibb Pharmaceutical Research Institute) was dissolved in bacteriostatic saline to a concentration of 8 μg/ml (7 nmol/ml), aliquoted, and frozen for later use.

2.4. Test solutions

Two test solutions were used in intake tests, i.e. sucrose (0.1 M) and a milk diet consisting of 396 g of sweetened condensed milk, 792 ml of water, and 3 ml of vitamins (Poly Vi Sol, Mead Johnson, Evansville, IN, USA). Green food coloring was added to the sucrose solution to aid in the detection of dripping or rejection of the sucrose solution. Before testing, rats received a daily intraoral infusion of sucrose or milk for at least 5 days, or were given daily access to sucrose or milk in a bottle for at least 5 days.

2.5. Intraoral feeding tests

Rats were tested in a glass aquarium separated into subchambers by Plexiglas dividers, so that four rats could be tested at the same time. During intraoral infusions, syringe pumps (Harvard Apparatus, Holliston, MA, USA) infused test solutions (0.1 M sucrose or milk) from 20-ml syringes at a rate of 1.1 ml/min for 20 min through polyethylene (PE 50) tubing attached to the externalized end of the implanted intraoral catheters. The latency for the solution to begin dripping from the rats' mouths during the infusion was measured; if no dripping was observed, a latency of 1200 s was assigned. Rats were weighed immediately before and after each infusion (along with all feces produced during the infusion) with the difference in weight after infusion serving as a measure of intraoral intake during the infusion. Intraoral catheters were flushed with distilled water after each intraoral infusion, and the rats returned to their home cages. All tests were conducted at midlight cycle; rats were not food or water deprived before the tests.

2.6. Independent feeding tests

Food and water were removed from the home cages, and the rats were weighed. Preweighed bottles (with sipper tubes) containing a test solution (0.1 M sucrose or milk) were placed on the home cages, and the rats were given ad libitum access to the solution. After 20-min access, the bottles were removed, weighed, and intakes (g) were recorded. Food and water were then returned to the rats. As with the intraoral paradigm, all tests were conducted at midlight cycle; rats were not food or water deprived before the tests.

2.7. Statistical analyses

For all experiments, paired *t* tests ($P < 0.05$, two-tailed) were used to assess potential differences between testing conditions.

2.8. Experiment I: GRP and intraoral sucrose intake

GRP potently reduces intake from bottles of sucrose or glucose solutions, milk, and artificial diets [12]. Our initial experiment tested the effects of GRP on intake of 0.1 M sucrose, an innately palatable solution, during an intraoral infusion.

2.8.1. Procedure

Rats ($n = 8$) received an intraperitoneal injection of 28 μg/kg GRP or vehicle (bacteriostatic saline, 1 ml/kg). Five min later, rats were placed in the test chamber and intraorally infused with 0.1 M sucrose for 20 min at a rate of 1.1 ml/min. Latency to drip sucrose and intraoral intake (weight gain during the infusion) were measured. All rats received both a GRP and a vehicle injection, administered in a cross-over design on 2 consecutive days.

Table 1
Summary of intraoral and independent intake tests

	Intake (g)	Latency to Drip (s)
Exp. I. GRP and intraoral sucrose ($n = 8$)		
Saline control	11.8 ± 1.4	477 ± 94
28 μg/kg GRP	11.8 ± 2.9	333 ± 63
Exp. II. CCK and intraoral sucrose ($n = 8$)		
Saline control	11.5 ± 1.3	521 ± 79
8 μg/kg CCK	3.3 ± 0.8*	34 ± 11*
Exp. III. GRP and independent sucrose ($n = 8$)		
Saline control	10.9 ± 1.5	
28 μg/kg GRP	4.3 ± 1.4*	
Exp. IV. GRP and intraoral milk ($n = 6$)		
Saline control	20.1 ± 0.6	1027 ± 90
28 μg/kg GRP	19.0 ± 1.2	849 ± 114
Exp. V. GRP and intraoral milk after preload ($n = 6$)		
Saline control	13.9 ± 1.6	582 ± 184
28 μg/kg GRP	11.9 ± 1.6	337 ± 131
Exp. VI. GRP and independent milk ($n = 6$)		
Saline control	18.0 ± 2.6	
28 μg/kg GRP	13.1 ± 2.1*	

Data are expressed as mean ± SEM values. GRP, gastrin-releasing peptide$_{1–27}$; CCK, cholecystokinin.
*Significantly different from saline control, $P < 0.05$, paired t test, two-tailed.

2.8.2. Results

GRP failed to suppress intraoral intake of a 20-min infusion of 0.1 M sucrose (see Table 1). Further, no effect of GRP was observed on the latency to drip.

2.9. Experiment II: CCK and intraoral sucrose intake

It is well established that CCK decreases nutrient intake in bottle tests as well as intraoral intake tests [12]. Therefore, we tested the effects of CCK in the group of rats from Experiment I as a positive control for peptide-induced suppression of intraoral intake.

2.9.1. Procedure

The rats from Experiment I ($n = 8$) received an intraperitoneal injection of 8 μg/kg CCK or vehicle (bacteriostatic saline, 1 ml/kg). Five min later, rats were placed in the test chamber and infused intraorally with 0.1 M sucrose for 20 min at a rate of 1.1 ml/min. Latency to drip sucrose and intraoral intake (weight gain during the infusion) were measured. All rats received both a CCK and a vehicle injection, administered in a crossover design on 2 consecutive days.

2.9.2. Results

In contrast to that observed after GRP in Experiment 1, CCK potently suppressed both intraoral intake and latency to drip (see Table 1). The effect was large, with a suppression of intake by ≈70% and latency to drip by >90%.

2.10. Experiment III: GRP and independent sucrose intake

GRP failed to reduce intraoral intake of 0.1 M sucrose. The dose of GRP we used (28 μg/kg) has been previously shown to reliably suppress ad libitum intake from bottles [12]. To verify the efficacy of this dose of GRP in this particular group of rats, the rats from Experiments I and II were given GRP before a one-bottle test with the same 0.1 M sucrose used in the intraoral tests.

2.10.1. Procedure

Food and water were removed from the home cages. The rats from Experiment II ($n = 8$) were weighed, and then received an intraperitoneal injection of 28 μg/kg GRP or vehicle (bacteriostatic saline, 1 ml/kg). Five min later, bottles containing 0.1 M sucrose were placed on the home cages, and the rats were given ad libitum access to the sucrose for 20 min. The bottles were then removed and intakes were recorded. Food and water were returned to the animals. All rats received both a GRP and a vehicle injection, administered in a crossover design on 2 consecutive days.

2.10.2. Results

Baseline intake of sucrose during the one-bottle, independent intake test did not significantly differ from control intake observed during the previous intraoral test in Experiments I or II ($P > 0.7$). However, in contrast to that observed in the intraoral test, GRP significantly suppressed

intake of 0.1 M sucrose in the one-bottle test when compared with saline control (Table 1).

2.11. Experiment IV: GRP and intraoral milk intake

GRP failed to decrease intake of 0.1 M sucrose during a 20-min intraoral infusion. However, 0.1 M sucrose is neither maximally palatable to rats nor is it very calorically or osmotically dense. To test the possibility that GRP might decrease intraoral intake of a more palatable or concentrated solution, we tested the effects of GRP on intraoral intake of a palatable milk diet.

2.11.1. Procedure

A new group of rats ($n = 6$) received an intraperitoneal injection of 28 μg/kg GRP or vehicle (bacteriostatic saline, 1 ml/kg). Five min later, rats were placed in the test chamber and intraorally infused with milk for 20 min at a rate of 1.1 ml/min. Latency to drip milk and intraoral intake (weight gain during the infusion) were measured. All rats received both a GRP and a vehicle injection, administered in a cross-over design on 2 consecutive days.

2.11.2. Results

Consistent with the high palatability of the milk diet, rats consumed significantly more of the milk under control conditions during the intraoral test when compared with that observed with control intake of 0.1 M sucrose in Experiments I and II ($P < 0.001$, independent t tests, two-tailed). However, consistent with the results from Experiment I with intraoral intake of sucrose, GRP failed to suppress both intake and latency to drip during an intraoral infusion of milk (Table 1).

2.12. Experiment V: GRP and intraoral milk intake after preload

GRP failed to decrease intraoral intake of milk. The baseline intake of the palatable milk diet, however, was high (i.e. average intake was 20 g of a potential 22 g total during the 20-min intraoral tests). To ensure that a potential GRP-induced reduction of intraoral intake of milk was not masked by a ceiling effect in baseline intake, rats were tested after an intraoral preload of milk. Preloads reliably decrease intake in subsequent intake tests, and data indicate that preloads may potentiate the anorexic effects of GRP under some conditions [8]. Therefore, the combination of a preload and GRP might reduce intraoral intake.

2.12.1. Procedure

The same rats from Experiment IV ($n = 6$) were placed in the test chamber and intraorally infused with milk for 20 minutes at a rate of 1.1 ml/min. At the end of the infusion, rats were returned to their home cages without access to food. Twenty-five min after the end of the intraoral infusion, rats received an intraperitoneal injection of 28 μg/kg GRP or vehicle (bacteriostatic saline, 1 ml/kg). Five minutes later, rats were placed in the test chamber and again intraorally infused with milk for 20 min at a rate of 1.1 ml/min. Latency to drip milk and intraoral intake (weight gain during the infusion) were measured. All rats received both a GRP and a vehicle injection, administered in a crossover design on 2 consecutive days.

2.12.2. Results

Consistent with 20-min intraoral milk intake observed during control conditions in Experiment IV, rats consumed 19.5 ± 0.5 ml during the 20-min intraoral preload before saline injection and 19.6 ± 0.6 ml before the GRP test. As predicted, the preload of milk significantly ($P < 0.02$) reduced baseline intake during the 20-min intraoral infusion test to $\approx$70% of that observed in Experiment IV. However, despite the lower baseline intake, GRP failed to reduce both intake and latency to drip during an intraoral infusion of milk (Table 1).

2.13. Experiment VI: GRP and independent milk intake

To verify that GRP could decrease intake of milk when independently ingested from a sipper tube and bottle, the effects of GRP in a one-bottle test were evaluated using the rats from Experiments IV and V.

2.13.1. Procedure

Food and water were removed from the home cages. The rats from Experiments IV and V ($n = 6$) were weighed, and then received an ip injection of 28 μg/kg GRP or vehicle (bacteriostatic saline, 1 ml/kg). Five min later, bottles containing milk were placed on the home cages, and the rats were given ad libitum access to the milk for 20 min. The bottles were then removed and intakes were recorded. Food and water were returned to the animals. All rats received both a GRP and a vehicle injection, administered in a crossover design on two consecutive days.

2.13.2. Results

Consistent with previous reports, GRP significantly reduced intake of milk in a one-bottle test (Table 1).

3. Discussion

Using two nutrient solutions varying in both caloric density and palatability (0.1 M sucrose and milk), we have compared the ability of GRP to suppress intraoral versus independent intake in an attempt to dissociate the peptide's effects on appetitive and consummatory behaviors. As expected from the findings of numerous previous reports [12], GRP potently reduced independent intake of both sucrose and milk from a bottle. However, the same dose of GRP that

reduced independent intake had no significant effect on intraoral intake of either solution in the same groups of rats.

In contrast to our current findings, there is one report [4] that GRP reduced intraoral intake of 0.1 M sucrose in rats. The study examined the role of the hindbrain in the effects of bombesin and bombesin-like peptides (e.g. GRP) by using decerebrate rats. Because these animals lack the ability to independently ingest food, they received nutrients via oral gavage. Control animals, therefore, were also maintained solely by oral gavage throughout the study. It is unclear how daily oral gavage or the sustained absence of independent feeding might have altered the effects of GRP on intraoral intake.

It is clear, however, that GRP failed to affect intraoral intake in the present study, using both 0.1 M sucrose and milk in separate groups of rats; the same dose of GRP potently reduced independent intake from a bottle in the same animals. As a positive control for peptide-induced suppression of intraoral intake and to establish the sensitivity of our intraoral paradigm, we demonstrated that CCK potently reduced intraoral sucrose intake in the same group of rats that failed to show a GRP effect. Further, to eliminate the possibility that the absence of a GRP-induced suppression of intraoral milk intake was not the result of a ceiling effect in baseline intake, rats were tested after an intraoral preload of milk. Despite the lower baseline intake observed after a preload, GRP again failed to affect intraoral intake. This is a particularly strong demonstration of the inability of GRP to reduce intraoral intake, because data indicate that preloads potentiate the anorexic effects of GRP under some conditions [8].

Our current findings fit nicely with recent observations of the microstructural pattern of GRP's inhibitory effect on ad libitum feeding [9,10]. This work examined the effects of brief, meal-contingent infusion of GRP on the lick microstructure of spontaneous liquid (milk) meals in freely feeding rats. Doses of GRP from 2.5 to 20 nmol/kg potently decreased both meal size and duration. Examination of the microstructural parameters revealed significant reductions in the numbers of bursts and clusters of licks with no effect on other variables including burst and cluster size and initial and overall lick rate (licks/min). In other words, the pattern of eating during the shorter meals after GRP infusion was indistinguishable from that observed during a normal control meal. Thus, GRP's inhibitory effect on spontaneous meal size can be totally explained by a reduction in meal duration.

One hypothesis that can explain both this truncation in spontaneous meal size and the failure of GRP to affect intraoral intake is that GRP results in a failure to reinitiate consummatory behaviors (e.g. licking, chewing, and swallowing). Specifically, in the intraoral paradigm that isolates the consummatory aspects of ingestion independent of appetitive behaviors, reinitiation is not necessary for continuation of nutrient infusion. One would predict from this hypothesis, therefore, that GRP would have little effect on intraoral intake. The shortening in spontaneous meal size by GRP could result from a GRP-induced failure to reinitiate another burst of licking at some point during the meal. It is also possible that GRP promotes moving away from the food. This could also explain the failure of GRP to reduce intraoral intake, because moving away is not an option in the intraoral paradigm. In summary, both our current findings and previous results indicate that rather than directly altering consummatory behavior, GRP affects appetitive-related aspects of the feeding process to reduce food intake.

Acknowledgments

This study was supported by National Institutes of Health grants MH18390 (P.A.R.) and DC03198 (T.A.H.).

References

[1] Corp ES, Melville LD, Greenberg D, Gibbs J, Smith GP. Effect of fourth ventricular neuropeptide Y and peptide YY on ingestive and other behaviors. Am J Physiol 1990;259:R317–23.
[2] Craig W. Appetites and aversions as constituents of instincts. Biol Bull 1918;34:91–107.
[3] Ersparmer V. Discovery, isolation, and characterization of bombesin-like peptides. Ann NY Acad Sci 1988;547:3–9.
[4] Flynn F. Caudal brain stem systems mediate effects of bombesin-like peptides on intake in rats. Am J Physiol 1992;262:R39–R45.
[5] Grill HJ. Production and regulation of ingestive consummatory behavior in the chronic decerebrate rat. Brain Res Bull 1980;5:79–87.
[6] Grill HJ, Norgren R. Chronically decerebrate rats demonstrate satiation but not bait shyness. Science 1978;201:267–9.
[7] Houpt TA, Philopena JM, Wessel TC, Joh TH, Smith GP. Increased c-Fos expression in the rat nucleus of the solitary tract after conditioned taste aversion formation. Neurosci Lett 1994;172:1–5.
[8] Kirkham TC, Perez S, Gibbs J. Prefeeding potentiates anorectic actions of neuromedin B and gastrin releasing peptide. Physiol Behav 1995;58:1175–9.
[9] Rushing PA. Spontaneous and scheduled eating: microstructural and peptide effects. Appetite 1997;29:385.
[10] Rushing PA, Henderson R, Gibbs J. Meal-contingent infusion of gastrin-releasing peptide$_{1-27}$ reduces meal size and intermeal interval and increases the satiety ratio in spontaneously feeding rats (Abstract). Soc Neurosci Abstr 1996;22:456.
[11] Seeley RJ, Paune CJ, Woods SC. Neuropeptide Y fails to increase intraoral intake in rats. Am J Physiol 1995;268:R423–7.
[12] Smith GP, Gibbs J. The satiating effects of cholecystokinin and bombesin-like peptides. In: Smith GP, editor. Satiation: From Gut to Brain. New York: Oxford University Press, 1998:97–125.
[13] Wolgin DL, Oslan IA, Thompson GB. Effects of "anorexia" on appetitive and consummatory behavior. Behav Neurosci 1988;102:312–8.