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Differential Mechanisms of Feeding Modulation Induced by Amino Sugars in Rats (42701)

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Abstract. The present study examined and compared the effects of *N*-acetylglucosamine and 1-deoxy-*N*-acetylglucosamine on feeding behavior with those of glucosamine and 1-deoxyglucosamine. Infusion of 12 μmole *N*-acetylglucosamine and 24 μmole 1-deoxy-*N*-acetylglucosamine into the rat third cerebroventricle did not affect the feeding behavior. However, oral administration of 1200 μmole *N*-acetylglucosamine elicited feeding and 2400 μmole 1-deoxy-*N*-acetylglucosamine markedly suppressed feeding. These effects were abolished by truncal vagotomy. Both glucosamine and 1-deoxyglucosamine affected feeding by intra-third cerebroventricular and oral administration. These findings indicate that *N*-acetyl amino sugars modulate feeding behavior peripherally through the vagal afferent nerve.

We have previously demonstrated that glucosamine, an endogenous glucose analog, elicits feeding, and its deoxy form, 1-deoxyglucosamine, markedly suppresses feeding, and that these amino sugars are related to physiological feeding behavior (1–3). Glucosamine is known to be rapidly converted to UDP-*N*-acetylglucosamine via *N*-acetylglucosamine 6-phosphate which in turn is converted to hyaluronic acids and other glucoproteins in the rat liver (4, 5). Moreover, *N*-acetylglucosamine is degradated from hyaluronic acids, which are natural constituents of polyglycans in the rat (5, 6). Thus, the *N*-acetyl derivatives are intermediates of amino sugars in rat plasma (4, 5). Nothing, however, is known about their role in feeding modulation. The present study was initiated to investigate and compare the effects of *N*-acetylglucosamine on feeding elicitation after cerebroventricle or oral administration with those of glucosamine. We also attempted to clarify the effects of 1-deoxy-*N*-acetylglucosamine on feeding, because we previously demonstrated that 1-deoxyglucosamine markedly suppresses feeding (1, 3).

Materials and Methods. *Animals.* Mature male Wistar King A rats, 260–310 g, were used. They were housed in a sound-proof room which was illuminated daily from 0800 to 2000 hr (12/12-hr light–dark cycle) and maintained at 21 ± 1°C with humidity at 45 ± 5%. The rats were allowed free access to standard pellet rat chow (Kanae Inc., Japan, mean pellet weight, 48.6 ± 0.7 mg), and tap water (mean droplet volume, 33.0 ± 0.7 μl). Before each experiment, all rats were handled for 5 min daily for 5 successive days to equilibrate their arousal levels. Body weight was measured at 1030 to 1100 hr.

Apparatus. Each rat was housed in a 30 × 25 × 25-cm testing chamber equipped with a pellet-sensing eatometer, a drinkometer and photo-sensing counters to measure ambulatory activity (1, 7). The number of food pellets and water droplets consumed and the activity, measured by counting crossings of 12 photobeams points 12 mm above the floor, were automatically recorded on a minicomputer. This system has been described in detail elsewhere (7).

Reagents. Glucosamine (2-amino-2-deoxy-D-glucose; Sigma), *N*-acetylglucosamine (2-acetamido-2-deoxy-D-glucose; Sigma), 1-deoxyglucosamine (2-amino-1,5-anhydro-2-deoxy-D-glucitol; Chugai Pharmaceutical), 1-deoxy-*N*-acetylglucosamine (2-acetamido-1,5-anhydro-2-deoxy-D-glucitol; Chugai), D-mannose (Sigma) and 1-deoxymannose (1,5-anhydro-D-mannitol; Chugai) were dissolved in distilled water, and were adjusted to 7.0–7.5 pH with 0.1 *N* hydrochloric acid.

Surgery. After being anesthetized with sodium pentobarbital (50 mg/kg, ip), each rat was positioned and fixed in a stereotaxic in-

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strument. A stainless steel cannula (29 gauge, 18 mm long) was inserted into the third cerebroventricle to a depth of 7.8 mm from the cortical surface at a point on the midline 6.0 mm anterior to ear bar zero (2, 8). Vagotomy was accomplished by gently lifting the esophagus just below the liver and diaphragm, and cutting the mesentery and all visible vagal fibers within 2 cm of the esophagus (9). For this procedure and the succeeding 24-hr ingestive procedures, transection of vagal fibers on both sides was microscopically verified after the experiment in each rat (10). These operations were carried out at least 1 week before testing. At testing time, food intake and body weight were ascertained as having returned to normal.

Procedure: (1) One-hour ingestive and ambulatory behaviors after administration of glucosamine and N-acetylglucosamine at 1100 hr. To observe the feeding elicitation, test solutions were administered at 1100 hr. Ingestive patterns were recorded for 2 days before and 1 day after administration. Then, the incidence of feeding (elicited/tested), latency from the first meal, meal duration, incidence of drinking, and ambulatory activity were analyzed for 1 hr after administration. Ambulation was determined by the difference in 1-hr photobeam crossing readings before and after administration.

Incidence data were evaluated by the Fisher exact probability test and latency and duration data were evaluated by the Welch test.

(i) Injection into the third cerebroventricle. Rats were divided into four groups of six on the basis of preinfusion food consumption. Under unanesthetized and unrestrained conditions, 10 μl of 1.2 *M* glucosamine or *N*-acetylglucosamine was infused into the third cerebroventricle at a rate of 1.0 μl/min (2, 7). Each rat was infused a total dose of 12 μmole test solution. Mannose at a dose of 1.2 *M* was used as the osmotic control and 0.15 *M* saline was used as the mechanical control. Dye was infused through the same cannula after the experiment to verify the cannula position in the third ventricle.

(ii) Oral administration. All rats were administered 1 ml distilled water via a gastric cannula for 1 week, and adaptation to the gastric cannula was evaluated by the baseline data, which was defined as the daily average amount of food intake based on those recorded two days immediately before treatment. Matched on the basis of food consumption, 40 rats were divided into four groups of 10, and an additional 20 rats were divided into two groups with 10 undergoing bilateral truncal vagotomy. One milliliter of 1.2 *M* glucosamine or *N*-acetylglucosamine was orally administered via a gastric cannula. The total dose of the test solution was 1200 μmole, 100 times larger than that infused into the third ventricle. The same dose of test solution was orally administered to the rats with truncal vagotomy. Distilled water and 1.2 *M* mannose solution were used as mechanical and osmotic controls, respectively.

(2) Twenty-four hour ingestive and ambulatory behavior after administration of 1-deoxyglucosamine and 1-deoxy-N-acetylglucosamine at 1930 hr. To test feeding suppression, the solutions were administered at 1930 hr. Daily behavioral patterns were recorded 2 days before and 3 days after administration. Measurement of a baseline level was the same as for the first experiment.

Analyses of 24-hr cumulative food and water intake and ambulation were performed. Values are expressed as differences from the corresponding baseline levels. Statistical evaluation of the data was carried out by analysis of variance with replication.

(i) Injection into the third cerebroventricle. Rats were divided into four groups of four on the basis of preinfusion data. Ten microliters of 2.4 *M* 1-deoxyglucosamine or 1-deoxy-*N*-acetylglucosamine were infused into the rat third cerebroventricle. The total dose infused of each test solution was 24 μmole. Saline at a concentration of 0.15 *M* was used as the mechanical control, and 2.4 *M* 1-deoxymannose was used as the osmotic control since it has been reported to have no effect on feeding (10). Other experimental procedures were the same as experiment *(1)-(i).*

(ii) Oral administration. Rats were divided into four groups of four on the basis of preinfusion data, 1-deoxy-*N*-acetylglucosamine, 1-deoxyglucosamine, 1-deoxymannose, and distilled water. Four additional rats with bilateral truncal vagotomy were administered 1-deoxy-*N*-acetylglucosamine. One milliliter of 2.4 *M* test solution was orally

TABLE I. ONE-HOUR PERIOD RESPONSE OF FEEDING TO INFUSION OF 12 μmole TEST SOLUTIONS OR 1.5 μmole SALINE INTO THE THIRD CEREBROVENTRICLE AT 1100 hr

	Feeding				
	Incidence	Latency (min)	Duration (min)	Drinking	Ambulation
GlcN	5/6*	9.4 ± 3.6	13.6 ± 4.1	0/6	(++)
GlcNAc	0/6	ND	ND	0/6	(−)
Mannose	0/6	ND	ND	0/6	(−)
Saline	0/6	ND	ND	0/6	(−)

Note. GlcN, glucosamine. GlcNAc, *N*-acetylglucosamine. Values, mean ± SE. In this table and in Table II, ambulation was expressed as the difference between photobeam crossings before and after infusion: −40 to 40 = (−), 40 to 80 = (±), 80 to 160 = (+), over 160 = (++).

* $P < 0.05$, compared to the corresponding control.

administered via a gastric cannula. The total dose of the test solution was 2400 μmole. The other experimental procedures were the same as experiment *(1)-(ii)*.

Results. *(1) Feeding elicitation after administration of glucosamine and N-acetylglucosamine at 1100 hr.* Table I shows the incidence of feeding and drinking, latency from the first meal and meal duration, and 1-hr ambulatory activity after infusion of 12 μmole glucosamine, *N*-acetylglucosamine, and mannose or 1.5 μmole saline into the third cerebroventricle. The incidence of feeding induced by glucosamine, 83% in five of six rats, was significantly higher than the controls ($P < 0.05$). A short latency to the first meal (9.4 ± 3.4 min from the start of infusion) and a long meal duration (13.6 ± 4.1 min) were observed. This feeding was not accompanied by periprandial drinking, but a concomitant increase in ambulation was observed. In contrast to glucosamine, the rats administered an equimolar infusion of *N*-acetylglucosamine did not eat or drink during the test period and ambulation was not affected.

As shown in Table II, oral administration of glucosamine induced feeding in 6 of 10 rats tested, which was significant compared to the controls ($P < 0.01$). There was no difference in feeding incidence between the ventricular and oral groups. However, rats in oral group ate the first meal with longer latency ($P < 0.05$) and shorter duration ($P < 0.05$) than the rats administered by infusion into the third cerebroventricle. Oral administration of *N*-acetylglucosamine significantly induced feeding compared to the controls ($P < 0.05$), but the latency and meal duration were not different from those ad-

TABLE II. INGESTIVE BEHAVIOR DURING A 1-hr PERIOD AFTER ORAL ADMINISTRATON OF 1200 μmole TEST SOLUTIONS OR DISTILLED WATER AT 1100 hr

	Feeding				
	Incidence	Latency (min)	Duration (min)	Drinking	Ambulation
GlcN	6/10**	33.2 ± 5.3	1.7 ± 0.4	ND	(++)
GlcN + Vgx	3/10	32.6 ± 5.8	1.8 ± 0.3	ND	(+)
GlcNAc	4/10*	25.2 ± 9.6	1.2 ± 0.4	ND	(++)
GlcNAc + Vgx	0/10***	ND	ND	ND	(−)
Mannose	0/10	ND	ND	ND	(−)
Distilled water	0/10	ND	ND	ND	(−)

Note. GlcN, glucosamine. GlcNAc, *N*-acetylglucosamine. Vgx: bilateral truncal vagotomy. Values, mean ± SE.

* $P < 0.05$, ** $P < 0.01$, compared to the corresponding mannose and distilled water controls.

*** $P < 0.01$, compared to the corresponding groups without vagotomy.

ministered glucosamine. No periprandial drinking was observed after oral administration of glucosamine or *N*-acetylglucosamine. A concomitant increase of ambulation was observed in both groups. Equimolar mannose and equivolume distilled water did not affect behavior. As shown in Table II, feeding elicitation by *N*-acetylglucosamine was completely abolished by bilateral truncal vagotomy ($P < 0.05$), and accelerated ambulation also disappeared. Truncal vagotomy attenuated the incidence of feeding induced by glucosamine, but this was not significant compared to the corresponding group without vagotomy ($P < 0.1$). Ambulation was also attenuated.

(2) Feeding suppression after administration of 1-deoxyglucosamine and 1-deoxy-N-acetylglucosamine at 1930 hr. Figure 1 shows the changes in food intake and body weight during the three experimental days after intra-third ventricle infusion of 1-deoxyglucosamine and 1-deoxy-*N*-acetylglucosamine. Compared to the saline control, the food intake and body weight were decreased by 1-deoxyglucosamine throughout the whole experimental period (food intake, $F(1,22) = 76.1$; body weight, $F(1,22) = 60.4$; $P < 0.01$ in each). In contrast, 1-deoxy-*N*-acetylglucosamine had no effect on food intake or body weight. Neither daily food intake nor body weight was affected by 1-deoxymannose which was used as the osmotic control.

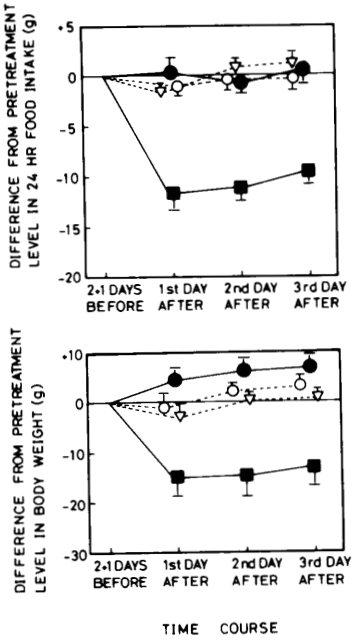

FIG. 1. Changes in level of food intake and body weight after infusion of 24 μmole test solution and 1.5 μmole saline into the third cerebroventricle at 1930 hr. Baseline level (2 + 1 days before) is the daily average calculated from the measurements 2 days before infusion. Values, mean ± SE. (●) 1-Deoxy-*N*-acetylglucosamine, (■) 1-deoxyglucosamine, (▽) 1-deoxymannose, (○) saline.

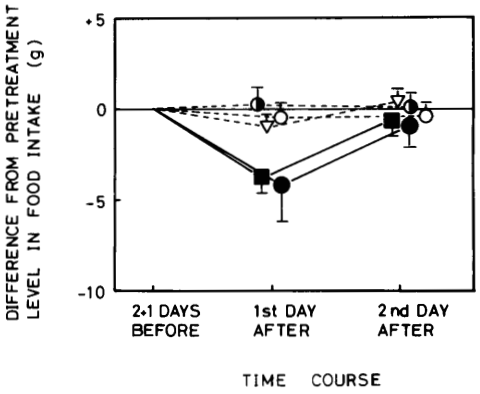

FIG. 2. Changes in food intake during the first and second day following oral administration of 2400 μmole test solution and 1 ml distilled water at 1930 hr. Baseline level (2 + 1 days before) is the daily average calculated from 2 days before infusion. Values, mean ± SE. (●) 1-Deoxy-*N*-acetylglucosamine, (◑) 1-deoxy-*N*-acetylglucosamine administered into the bilateral truncal vagotomized rat, (■) 1-deoxyglucosamine, (▽) 1-deoxymannose, (○) distilled water.

Changes in food consumption after oral administration of 1-deoxy-*N*-acetylglucosamine and 1-deoxyglucosamine are shown in Fig. 2. Neither the distilled water used as the mechanical control nor the 1-deoxymannose as the osmotic control affected the food intake. Food intake after oral administration of 1-deoxyglucosamine or 1-deoxy-*N*-acetylglucosamine was significantly suppressed compared to the 1-deoxymannose control (1-deoxyglucosamine, $F(1,22) = 4.8$; 1-deoxy-*N*-acetylglucosamine, $F(1,22) = 7.6$; $P < 0.05$ in each); however, this suppression returned to the control values on the second day after administration.

The effect of 1-deoxy-*N*-acetylglucosamine on feeding after bilateral truncal vagotomy is also shown in Fig. 2. The suppres-

sive effect was completely abolished by vagotomy ($F(1,22) = 7.0$, $P < 0.05$), and then recovered to the control levels ($F(1,22) < 1$, N.S.).

Typical feeding, drinking, and ambulation patterns of one rat after oral administration of 2400 μmole 1-deoxy-*N*-acetylglucosamine is shown in Fig. 3. Oral administration of 1-deoxy-*N*-acetylglucosamine had no effect on drinking ($F(1,22) < 1$, N.S.) or ambulation ($F(1,22) < 1$, N.S.), but it did decrease food intake (-4.7 ± 0.5 g, $F(1,22) = 7.6$; $P < 0.05$). This feeding suppression was the result of a decrease in meal size ($P < 0.05$). In addition, the suppressive effect promptly disappeared within 24 hr and the food intake returned to the pretreatment level of the first day.

Discussion. The present experiments demonstrated that *N*-acetylglucosamine induced feeding and 1-deoxy-*N*-acetylglucosamine markedly suppressed feeding after oral administration, but not after infusion into the third cerebroventricle. The effects after oral administration were abolished by bilateral truncal vagotomy. Previously reported electrophysiological findings have shown that chemosensitive units responding to glucose or other chemical substances exist in the liver and in the gastrointestinal tract (9, 12–14). These chemosensitive units are thought to be involved in the peripheral control of feeding behavior through the influence of afferent vagal nerve activity (14). The pathway of these visceral signals from hepatic and intestinal chemosensitive units was found immunohistochemically and electrophysiologically to reach upper brain structures, such as the nucleus of the solitary tract, the parabrachial nucleus and the lateral hypothalamic area (16–19). On the other hand, the feeding modulation induced by the peripheral administration of glucose (9), 2-deoxyglucose (12), or several peptides such as cholecystokinin (CCK) (20), somatostatin (21), and glucagon (22) was abolished or attenuated by vagotomy. These results suggest that chemical information received by peripheral receptive sites was transmitted to the central nervous system through the afferent vagal nerve, which ultimately then controls food intake.

N-Acetyl derivatives failed to affect feeding when directly infused into the central nervous system, but did modulate feeding peripherally. At least two possible reasons for their ineffectiveness can be considered. First, *N*-acetyl derivatives, particularly *N*-acetylglucosamine is mainly metabolized in the liver and the spleen (5, 23, 24), but is not readily metabolized in the brain (24). It is also demonstrated that *N*-acetylglucosamine

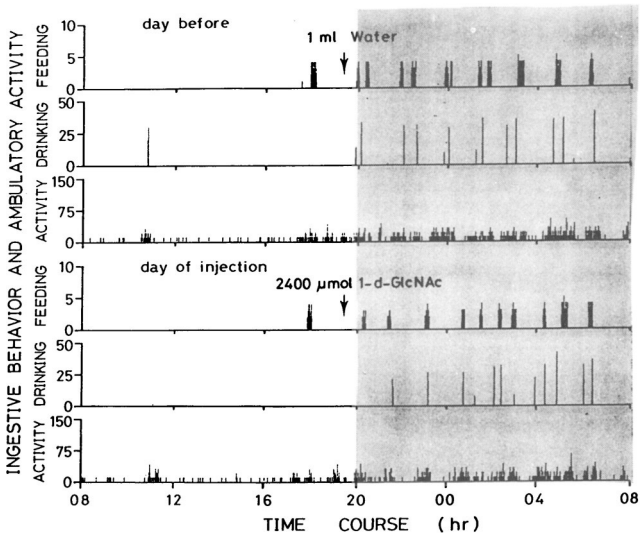

FIG. 3. Eating, drinking, and ambulation patterns of one rat 36 hr before and 12 hr after oral administration of 1-deoxy-*N*-acetylglucosamine (2400 μmole) at 1930 hr. Shaded, dark period.

is only slightly deaminated or phosphorylated in the central nervous system due to the absence of a deaminating enzyme system in the brain (25). These metabolism inadequacies in the brain might explain why the metabolic affinity for *N*-acetyl derivatives was less in the brain than in the peripheral organs.

Second, we have previously demonstrated that modulation of feeding behavior and changes in hypothalamic neuronal activities produced by glucose analogs depend on the substituents at carbon 2 of the glucopyranose ring (1, 2, 11, 26). Substitution of a hydroxyl group with hindering groups at C-2 attenuated the effect of glucose analogs on feeding. Consequently, replacement with *N*-acetyl groups can be expected to attenuate the effect on feeding, because *N*-acetyl groups at C-2 are spatially different from the hydroxyl group. Thus, *N*-acetylglucosamine and 1-deoxy-*N*-acetylglucosamine, with an *N*-acetyl group at C-2, may not have direct effects on hypothalamic neurons which relate to food intake.

The amino sugars used in the present study modulated feeding in a different manner. *N*-acetyl derivatives had only peripheral actions, while amino sugars, not substituted with an *N*-acetyl group, had both central and peripheral actions. Amino sugars deoxidized at C-1 showed an inhibitory effect on feeding, while those without deoxidation showed a reciprocal effect. The relationship of the chemical structure of these amino sugars and the behavioral responses are useful for further analysis of chemosensitive neuronal mechanisms with regard to feeding modulation.

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