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Human Satietin: Rapid Development of Tolerance and its Specificity to Feeding Behavior in Rats[1]

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MENDEL, V. E., R. R. BENITEZ AND T. A. TETZKE. *Human satietin: Rapid development of tolerance and its specificity to feeding behavior in rats.* PHARMACOL BIOCHEM BEHAV 31(1) 21–26, 1988.—Semipurified human satietin (SP hSAT) significantly ($p<0.05$) reduced food intake for each of five consecutive days when injected intraperitoneally (2 mg/kg/day). Tolerance developed rapidly during the second and third days of injection but food intake of those animals receiving the highest doses of Sp hSAT (1 and 2 mg/kg/day) remained significantly below ($p<0.05$) that of the saline-injected animals. Food intake of rats receiving doses of 0.5 and 0.25 mg/kg/day Sp hSAT was below that of the saline-treated group for the first two days of injection ($p<0.05$) but not thereafter. Water intake fell in all satietin-treated groups on the first day of injection, thereafter, it was similar to the saline-treated group. In a second study Sp hSAT (1, 0.5, 0.25 mg/kg/day) was continuously infused IP by Alzet pumps. All doses of Sp hSAT significantly reduced food intake below that of the saline-treated group for the entire 7 days of infusion ($p<0.05$); water was not affected except on the first day of infusion ($p>0.01$). In a third study, injection of purified human satietin (p hSAT) (2 mg/kg, IP) in fasted rats (24 hr) had no effect on water consumption either during fast or 24 hr postfast ($p>0.05$). In a fourth study p hSAT was continuously heated to 37°C. Aliquots were taken each day and injected (IP) into 4 naive rats (total of 24 rats) for a total of 6 days of heating (120 hr). The p hSAT remained fully active after 120 hr of heating to 37°C. We conclude that tolerance to satietin develops rapidly but that it can partially reduce daily food intake below saline-treated animals when injected intraperitoneally or continuously infused at doses of at least 1 mg/kg/day. Food intake was specifically reduced both by Sp hSAT and p hSAT while water intake changed only in response to changes in food intake. We, therefore, conclude that satietin specifically inhibits food intake.

Food intake Satiety Water intake

SATIETIN has been reported to be a powerful anorectic substance (3) which specifically inhibits food intake (4). Some controversy exists as to whether satietin specifically inhibits food intake in rats because Mendel and Bellinger (5) have reported a decrease in water intake and running wheel activity concomitant with reduced food intake. This study further examines that question.

With three exceptions (4,5) all studies of satietin have employed a single bolus injection of satietin. It occurred to us that if satietin is involved in induction of satiety that consecutive, daily injections of satietin might be expected to reduce daily food intake for as long as the injections are given. It is also possible that satietin might be involved in maintenance of satiety, thus, continuous infusion of satietin would reduce daily food intake. Support for these ideas is found in a preliminary study (5) where semipurified human satietin was injected daily for four consecutive days into the third ventricles of two rats. Food intake in both animals was progressively reduced from daily intakes of 40.6 and 31.5 to 2.3 and 7.3 g, respectively. When injections ceased, food intake returned to near normal over a 7-day period with no overshoot. Contrary to these results, intracerebroventricular injections given on alternate days quickly lost effectiveness and food intake, water intake and running wheel activity returned to normal after three injections (5). Thus, the present series of studies were designed to more critically examine the questions of whether continuous peripheral infusions or, consecutive injections of semipurified human satietin will result in a sustained reduction of food intake in the rat.

An additional objective was to further clarify the question of whether satietin specifically affects food intake without affecting water intake.

METHOD

Satietin Isolation Procedures

Batches of outdated human plasma (3 l each) were pur-

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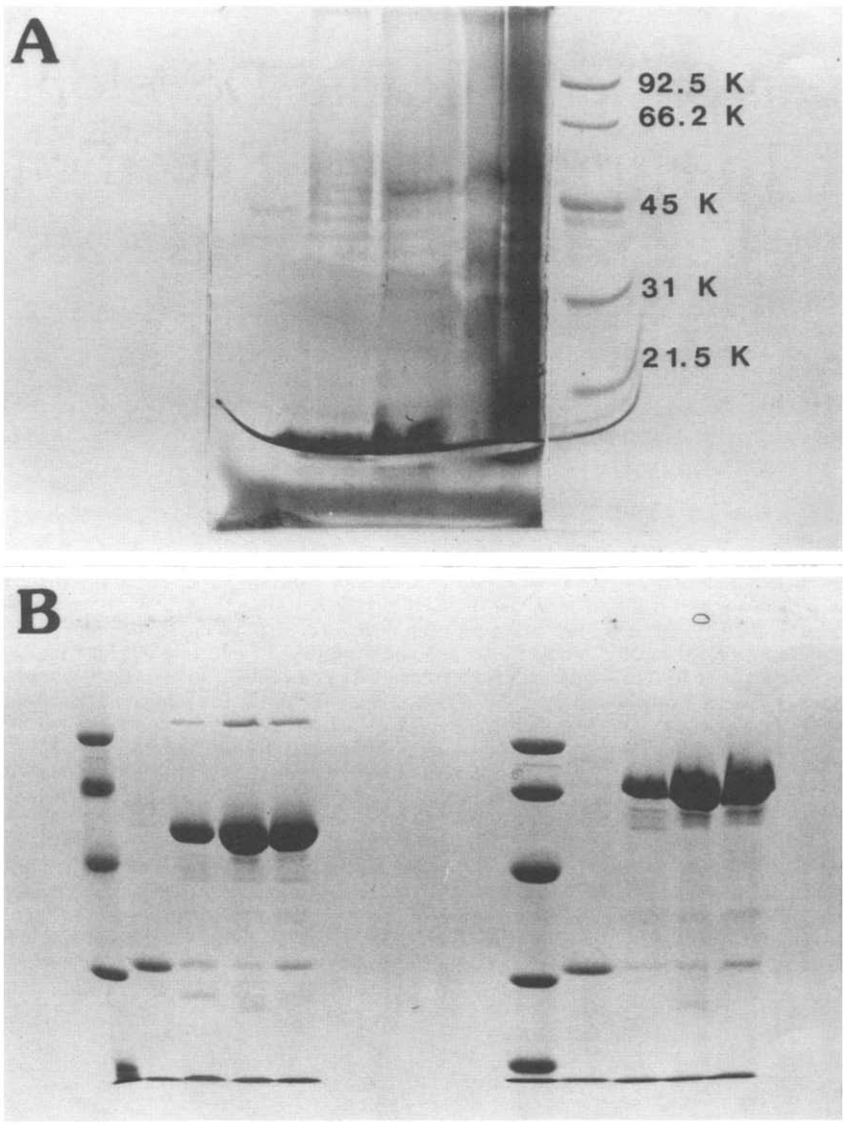

FIG. 1. (A) Semipurified human satietin on SDS-Page gel electrophoresis. Lane 1 (left), high molecular weight standards; lane 2, reduced 20 μg Sp hSAT; lane 3, reduced 60 μg SP hSAT; lane 4, nonreduced 60 μg Sp hSAT; lane 5, nonreduced 20 μg Sp hSAT; lane 6, low molecular weight standards; first stain, coomassie blue; second stain, silver stain. (B) Human satietin further purified on concanavilin-A column then placed on SDS-Page gel electrophoresis. Left panel, nonreduced; right panel, reduced. Left lane in both panels is low molecular weight standards identical to standards in A. Four separate samples are shown in lanes 2, 3, 4, 5, material from sample 2B was used in Experiment 3. Stained with coomassie blue, 20 μg each lane.

chased from the Sacramento Blood Bank from which a semipurified material was obtained according to the method of Nagy *et al.* (8). This method yields a heterogeneous material (Fig. 1A) which was used in Experiments 1 and 2. Semipurified human satietin (Sp hSAT) was further purified using a concanavalin-A (CON-A) column according to the method of Nagy *et al.* (7,8). This material, which we have identified as purified human satietin (p hSAT), was used in Experiments 3 and 4 (Fig. 1B). As can be seen, p hSAT is significantly more homogeneous than Sp hSAT but is still not without some heterogeneity.

Experiment 1

Thirty male Sprague-Dawley rats weighing 206.2±3.4 g were divided into 5 groups of six. The animals were individually housed in hanging wire cages which were equipped with feeders and apparatus to collect spillage. Light/dark ratio was 12:12 with lights out at 1100 hr. All weighing and injections took place between 0900 and 1100 hr. Rats were trained to intraperitoneal (IP) injections (0.5 ml) for four days with sterile saline. On the fifth day, injections of Sp hSAT (mg/kg/day) were given for 5 consecutive days as follows:

TABLE 1

EFFECT OF FIVE CONSECUTIVE INTRAPERITONEAL INJECTIONS OF Sp hSAT ON, FOOD AND WATER INTAKES, PERCENT BODY WEIGHT CHANGES AND, THE RATIO OF WATER INTAKE:FOOD INTAKE IN RATS

Group	Dose mg/kg/day	Treatment (days) Preinjected	1	2	3	4	5
				Food Intake			
I	2	23.6 ±0.47^{abc}	9.1 ±0.89^{e}	15.7 ±1.54^{d}	22.0 ±0.88^{d}	21.0 ±0.69^{c}	23.6 ±0.29^{c}
II	1	24.6 ±0.77^{abc}	16.6 ±2.94^{d}	16.8 ±1.30^{d}	22.2 ±1.53^{cd}	23.5 ±1.91^{bc}	24.3 ±0.80^{c}
III	0.5	25.8 ±0.54^{ab}	21.6 ±2.62^{bcd}	21.5 ±1.33^{c}	23.8 ±1.28^{bcd}	26.8 ±1.18^{ab}	27.8 ±0.81^{a}
IV	0.25	25.4 ±0.45^{ab}	19.8 ±1.18^{cd}	21.8 ±1.30^{bc}	25.1 ±0.81^{abcd}	29.5 ±1.29^{a}	26.9 ±0.66^{ab}
V	Saline	26.0 ±1.34^{ab}	27.7 ±0.72^{a}	25.1 ±1.48^{abc}	28.0 ±1.09^{a}	26.7 ±1.32^{ab}	27.5 ±1.73^{ab}
				Water Intake			
I		40.1 ±1.18^{a}	13.8 ±0.86^{d}	41.3 ±1.75^{a}	40.3 ±2.29^{a}	36.5 ±2.48^{b}	47.3 ±2.48^{a}
II		40.0 ±0.83^{a}	25.4 ±4.62^{bcd}	31.6 ±4.55^{ab}	40.2 ±4.04^{a}	46.0 ±3.85^{a}	45.6 ±3.15^{ab}
III		36.8 ±1.70^{ab}	29.8 ±5.43^{abc}	31.1 ±2.35^{b}	35.4 ±3.29^{a}	39.3 ±1.81^{ab}	40.1 ±2.20^{abc}
IV		36.5 ±1.51^{ab}	23.8 ±2.50^{cd}	33.2 ±2.64^{ab}	34.6 ±2.00^{a}	37.2 ±1.99^{b}	37.1 ±1.70^{c}
V		37.6 ±3.13^{ab}	36.2 ±2.65^{ab}	36.8 ±4.43^{ab}	40.6 ±5.24^{a}	36.5 ±3.27^{b}	38.8 ±1.80^{bc}
				Body Weight, %			
I		100^{bcde}	94.6 ±0.89^{c}	95.5 ±1.08^{d}	99.1 ±0.57^{e}	101.1 ±1.08^{c}	105.3 ±0.93^{a}
II		100^{bcde}	98.6 ±1.70^{b}	98.1 ±0.99^{c}	101.8 ±0.71^{cd}	104.7 ±0.67^{b}	
III		100^{bcde}	100.2 ±1.66^{b}	101.2 ±1.60^{b}	102.5 ±1.18^{bc}	105.5 ±1.55^{b}	
IV		100^{bcde}	99.7 ±1.16^{b}	101.4 ±0.85^{b}	104.0 ±0.97^{b}	106.9 ±0.81^{b}	
V		100^{bcde}	104.6 ±0.69^{b}	105.9 ±1.08^{a}	108.5 ±1.00^{a}	110.0 ±1.09^{a}	
				Water Intake:Food Intake Ratio			
I		1.70±0.06^{ab}	1.57±0.11^{bc}	2.74±0.23^{a}	1.83±0.08^{ab}	1.75±0.13^{abc}	2.01±0.10^{a}
II		1.89±0.16^{a}	1.58±0.18^{abc}	1.88±0.26^{b}	1.80±0.15^{abc}	2.00±0.24^{a}	1.88±0.11^{a}
III		1.43±0.04^{bc}	1.34±0.12^{bc}	1.46±0.12^{b}	1.46±0.08^{bcd}	1.48±0.08^{bcd}	1.44±0.05^{b}
IV		1.44±0.04^{bc}	1.20±0.07^{c}	1.51±0.05^{b}	1.37±0.06^{d}	1.26±0.06^{d}	1.38±0.06^{b}
V		1.47±0.13^{bc}	1.31±0.09^{bc}	1.46±0.14^{b}	1.45±0.18^{bcd}	1.37±0.12^{cd}	1.44±0.08^{b}

Values with different superscripts in each column are significantly different, $p<0.05$. Treatment values within each row were shown by ANOVA for repeated measures to be significantly different ($p<0.01$) from preinjections values.

Gr. I, 2 mg; Gr. II, 1 mg; Gr. III, 0.5 mg; Gr. IV, 0.25 mg and, Gr. V, sterile saline. Total volume of doses ranged from 0.43 to 0.5 ml. Food intake, water intake and body weights were measured daily.

Experiment 2

Thirty-seven male Sprague-Dawley rats weighing 251.0 ±8.1 g were fed Purina Lab Chow for seven days during which food intake, water intake and body weights were measured daily. Housing and lighting schedule were as in Experiment 1. On the eighth day (day 1 of treatment) during the last two hours of the light period (0900–1100 hr). Alzet pumps (Model 2001) containing Sp hSAT or sterile saline were implanted in the peritoneal cavity under chloral hydrate anesthesia. The animals were divided into five groups as follows: Gr. I, saline (n=7); Gr. II, 1 mg (n=8); Gr. III, 0.5 mg (n=7) and Gr. IV, 0.25 mg/kg/day Sp hSAT (n=8). On day 8 postimplantation, the pumps were removed from the peritoneal cavity and inspected for any remaining solution; the peritoneal cavities were also inspected for infections. All pumps were found to be empty and two rats had peritonitis (Gr. I and III); these rats were eliminated from the study.

Experiment 3

Because the satietin contained in the osmotic pumps partially lost its effectiveness in suppressing food intake over 7 days it was questioned whether the continuous exposure of satietin to a body temperature of 37°C would destroy its biological activity. Thus, twenty-four male Sprague-Dawley rats weighing 300.3±3.4 g were divided into 6 groups of four. Housing conditions and light schedule were as in Experiment 1. After five days of adjustment to the room and light schedule each animal received 0.5 ml sterile saline IP for five additional days before receiving a single IP injection of p hSAT. The schedule of satietin injections was as follows: Each day, thirty minutes before the dark period, four naive rats were injected IP with 2 mg/kg p hSAT which had been continuously maintained in a water bath at 37°C. Thus, each successive group of rats was injected with p hSAT that had been heated 24 hr longer than that injected into the previous group of rats (range of heating=0 to 120 hr). Food and water intakes and body weights were also measured for 2 days postinjection of p hSAT.

TABLE 2

EFFECT OF CONTINUOUS INTRAPERITONEAL INFUSION (ALZET PUMP) OF Sp hSAT ON, FOOD AND WATER INTAKES, PERCENT BODY WEIGHT CHANGES AND, THE RATIO OF WATER INTAKE:FOOD INTAKE IN RATS

Group	Dose (mg/kg/day)	Treatment (days)							
		Preimplant	1	2	3	4	5	6	7
					Food Intake				
I	Saline	28.0 ±0.24^{a}	19.0 ±2.39^{b}	24.1 ±1.33^{ab}	27.6 ±0.62^{a}	28.6 ±0.45^{a}	28.3 ±0.92^{a}	26.9 ±0.88^{a}	27.2 ±0.96^{a}
II	1 mg	25.4 ±0.80^{a}	10.6 ±1.98^{c}	19.1 ±0.83^{d}	23.3 ±1.18^{b}	23.5 ±0.82^{b}	23.5 ±0.63^{cd}	22.9 ±0.70^{b}	24.3 ±0.53^{b}
III	0.5 mg	23.9 ±0.64^{a}	11.5 ±2.24^{c}	20.0 ±2.72^{cd}	24.1 ±1.15^{b}	23.9 ±1.16^{b}	24.3 ±1.53^{cd}	24.0 ±0.67^{b}	23.8 ±0.68^{b}
IV	0.25 mg	23.4 ±0.56^{ab}	9.5 ±1.36^{c}	18.0 ±1.20^{d}	23.9 ±1.15^{b}	25.0 ±1.00^{b}	22.5 ±0.90^{d}	23.9 ±0.78^{b}	25.6 ±0.56^{ab}
					Water Intake				
I		37.8 ±1.29^{a}	28.0 ±2.83^{cb}	39.0 ±2.10^{a}	46.1 ±3.78^{a}	45.1 ±4.20^{a}	37.1 ±3.63^{ab}	40.8 ±4.88^{a}	41.7 ±2.66^{a}
II		33.6 ±1.20^{ab}	19.2 ±2.35^{d}	40.8 ±3.56^{a}	46.3 ±5.09^{a}	40.6 ±1.88^{ab}	40.9 ±2.14^{a}	31.0 ±3.48^{a}	38.1 ±1.69^{a}
III		32.9 ±1.58^{ab}	22.3 ±3.64^{cd}	36.6 ±5.01^{a}	43.2 ±2.58^{ab}	44.6 ±2.20^{a}	40.6 ±3.30^{a}	38.8 ±3.62^{a}	38.0 ±1.37^{a}
IV		35.7 ±0.86^{a}	20.9 ±1.89^{d}	33.9 ±2.39^{a}	40.5 ±3.64^{abc}	40.8 ±3.25^{ab}	33.9 ±1.90^{ab}	33.4 ±2.01^{a}	37.7 ±1.60^{a}
					Body Weight				
I		100^{a}	98.0 ±1.41^{a}	99.5 ±1.16^{a}	101.3 ±1.34^{a}	102.5 ±1.87^{a}	105.4 ±1.16^{a}	107.3 ±0.98^{a}	108.6 ±0.91^{a}
II		100^{a}	94.5 ±0.77^{b}	97.2 ±0.65^{ab}	99.1 ±0.73^{ab}	102.4 ±0.90^{a}	104.9 ±1.72^{a}	106.5 ±1.35^{a}	107.2 ±0.90^{a}
III		100^{a}	95.5 ±1.08^{b}	96.2 ±2.01^{b}	97.7 ±1.46^{b}	100.1 ±1.09^{a}	103.3 ±0.81^{a}	105.9 ±1.05^{a}	107.3 ±0.99^{a}
IV		100^{a}	95.7 ±0.70^{b}	97.7 ±0.89^{ab}	99.6 ±1.07^{ab}	101.7 ±0.58^{a}	105.2 ±0.98^{a}	106.9 ±0.77^{a}	108.4 ±0.83^{a}
					Water Intake:Food Intake Ratio				
I		1.36±0.04^{b}	1.54±0.08^{b}	1.65±0.12^{bc}	1.67±0.12^{abc}	1.58±0.15^{abc}	1.27±0.12^{b}	1.95±0.45^{a}	1.53±0.07^{a}
II		1.32±0.03^{b}	1.97±0.13^{ab}	2.19±0.25^{a}	2.02±0.24^{a}	1.78±0.13^{ab}	1.52±0.22^{ab}	1.18±0.23^{a}	1.58±0.08^{a}
III		1.34±0.06^{b}	2.36±0.47^{a}	1.85±0.25^{ab}	1.81±0.11^{ab}	1.90±0.12^{a}	1.74±0.22^{a}	1.61±0.14^{a}	1.60±0.09^{a}
IV		1.54±0.04^{b}	2.41±0.28^{a}	1.91±0.10^{ab}	1.69±0.12^{abc}	1.64±0.12^{abc}	1.52±0.11^{ab}	1.39±0.06^{a}	1.49±0.06^{a}

Values with different superscripts in each column are significantly different, $p<0.05$. Treatment values within each row were shown by ANOVA for repeated measures to be significantly different ($p<0.01$) from preimplantation values.

TABLE 3

EFFECT OF CONTINUOUS HEATING OF phSAT (37°C) ON FOOD INTAKE AND BODY WEIGHT FOR THREE DAYS FOLLOWING ONE INTRAPERITONEAL INJECTION OF PURIFIED HUMAN SATIETIN (2 mg/kg) WHEN COMPARED TO PREINJECTION VALUES

Hours of Heating phSAT	Food Intake (% of preinjection) Day Postinjection			Body Weight (% of preinjection) Day Postinjection		
	1	2	3	1	2	3
0	73.8	91.8	94.5	101.1	97.9	100.0
24	63.9	87.8	94.4	102.1	98.6	100.7
48	49.2	73.0	73.8	101.5	97.8	100.6
72	36.9	73.2	93.3	102.4	96.3	99.1
96	40.3	79.1	98.6	101.4	96.9	99.1
120	56.4	93.6	93.6	102.4	99.4	101.3

Experiment 4

Eighteen female Sprague-Dawley rats weighing 272.1 ±3.4 g were divided into 2 groups of nine. Housing conditions and light schedule were as in Experiment 1. After a few weeks of adjustment to the room, each rat was injected IP for 5 days with sterile saline; water intake was measured daily for the last 3 days. Food was then removed from both groups of animals at 1000 hr, Gr. I received 2 mg p hSAT/kg, IP while Gr. II received 0.5 ml sterile saline IP. Water bottles were weighed and attached to the cages at 1045 hr. On day 1 postinjection, water bottles were reweighed and water consumption calculated. At 1045 hr food was returned to all animals and water consumption measured an additional two days.

Statistics

Satietin-treated groups were compared to saline-treated groups by ANOVA and ANOVA for repeated measures (SAS). When significant treatment effects were found post hoc comparisons of means were made using Duncan's Multiple Range Test (SAS).

RESULTS

Experiment 1

Food intake was reduced from 23.6 to 9.1 g the first day of injection of Sp hSAT (Gr.I) and daily food intake remained below that of the saline-treated animals ($p<0.05$) after five consecutive days of injection (Table 1). Nevertheless, daily food intake of Gr. I had returned to within 2 g of the starting intake by the third day of injection. Reduction of food intake suggested dose dependence but the association was weak.

Water intake decreased in all Sp hSAT-treated groups on the first day of injection but had returned to baseline by the second day of injection (Table 1) where it remained throughout the injection period except on day 5 where it was significantly greater in Gr. I than in Gr. V ($p<0.01$).

Body weight of Gr. I was significantly reduced below that of Gr. II, III, IV and V (saline) from day 1 through day 4 ($p<0.05$, Table 1). An error was made when Gr. II–V were accidentally sacrificed before their day-5 body weights could be taken; the oversight was not realized until the bodies had been disposed of.

The ratio of water intake:food intake (Table 1) was significantly higher in Gr. I than in Grs. II, III, IV, V on day 2 of treatment and also on day 5 in Gr. I and III otherwise the ratio was the same in all groups; during the preinjection period all ratios were similar.

Experiment 2

Food intake of all satietin-treated groups fell significantly more ($p<0.01$) on the day of implantation than Gr. I (saline) and remained lower than that of Gr. I throughout the experimental period (Table 2) except on day 7 when Gr. IV was not significantly different than Gr. I.

Water intake of all groups fell as the result of surgery (Table 2) and increased on day 2 postsurgery to baseline where it remained or increased throughout the treatment period.

Body weights of all satietin-treated animals were significantly reduced ($p<0.05$) below those of Gr. I on day 1, no differences existed among Grs. II, III and IV, however (Table 2). By day 4 this small but significant difference had disappeared. Two days after implantation of Alzet pumps to the end of the experiment body weights of all animals increased, with the rate of increase being lowest in the satietin-treated groups [2.2 g/day vs. 2.7 (saline); 19% less].

Water intake:food intake ratios were increased in all groups on day 1 postimplantation ($p<0.01$). Thereafter, the ratios tended to remain elevated although in some instances it temporarily fell to, or below, baseline (Table 2).

Experiment 3

Food intake was consistently reduced by a single IP injection of p hSAT (range 26.2 to 63.1% reduction) despite the satietin being maintained at 37°C for 120 hr (Table 3). A clear residual effect on food intake is seen even after 3 days postinjection in all groups. However, body weight had returned to baseline by day 3 postinjection.

Experiment 4

Water intake for three days before fasting and satietin injection were similar (Gr. I, 34.2±3.8 ml/day vs. Gr. II, 33.0±1.3 ml/day; $p>0.05$). During the 24 hr fast Gr. I (2 mg/kg p hSAT) rats consumed 12.9±2.0 ml water while Gr. II (control) rats consumed 10.8±1.9 ml ($p>0.05$). During the first 24 hr postfast, water consumption was 44.1±2.0 and 48.3±2.1 ml in Gr. I and II, respectively ($p>0.05$).

Ratios of water intake:body weight were similar before fast (0.1284, Gr. I vs. 0.1189, Gr. II) and fell to low levels during fast (0.0485, Gr. I vs. 0.0389, Gr. II). During the 24 hr postfast both ratios rose similarly (0.1654, Gr. I vs. 0.1739, Gr. II). No statistical differences existed between groups at any of these times.

DISCUSSION

Use of human satietin in rodents prompts questions concerning homology of satietins from a different species (human) but until a suitably pure preparation of human satietin is available the risks of anaphylaxis and other undesirable reactions seem better taken with rodents than with primates. It is evident that pure satietins from both species are needed to determine homologies as well as biological effects in two species. Knoll (4) and Nagy (8) have referred to satietin purified by affinity chromatography (CON-A) as highly purified. The product that was used in Experiments 3 and 4

appears to be quite similar to their highly purified satietin based on a comparison of results of electrophoresis from the two laboratories.

Several points should be noted about the data of Experiments 1 and 2. First, food intake fell in all groups as a result of treatment with Sp hSAT and, in Experiment 1, consistently remained below the control group despite development of some tolerance to it. The trauma of surgery in Experiment 2 also decreased food intake as seen by the decrease of food intake by the saline group. A short-acting anesthetic may have been less inhibitory to food intake on the first day, however, there was a greater depression of food intake in the satietin-treated animals than in the saline-treated animals (Table 2). Second, increases in food intake during recovery from surgery (Experiment 2) of satietin-treated animals were nearly as great as that of saline-treated animals, suggesting the rapid development of tolerance. Because glycoproteins are often highly immunogenic it is possible that satietin's effect is reduced as antibody formation occurs.

Use of water-to-food intake ratios normalizes the data so that effects of body weight on food and water intake are eliminated. Use of these ratios indicates that throughout Experiment 1 food intake of Gr. I and II remained depressed relative to water intake (i.e., a higher ratio). In Experiment 2 the ratio tended to remain elevated in all groups throughout the experiment. The stability of the water intake:food intake ratio in these experiments suggests that satietin has a greater inhibitory effect on food than water intake. Thus, we suggest that animals ingesting more water relative to food are probably not experiencing nausea as it is well known that increasing the volume in the gut exacerbates nausea. Finally, water intake was similarly affected by saline and Sp hSAT in both Experiments 1 and 2. This suggests that Sp hSAT specifically inhibits food intake without affecting water intake. The results of Experiment 4 support this idea. Those data clearly show, without proving, that water intake is not affected by purified satietin when measured in the absence of food.

Knoll (3,4) has previously reported that satietin (both semipurified and purified) specifically inhibits food intake and our present data are in agreement with that observation. We have previously questioned whether satietin specifically inhibits food intake (5); however, in that experiment, we did not separate the effect of depressed food intake on water intake from the effect of satietin on water intake. It appears at this time that our earlier question was unfounded.

While it is known that tolerance to many pharmaceuticals occurs (2), and that it it not unusual for tolerance to develop rapidly (2); as was seen in Experiments 1 and 2, the mechanisms involved in the development of tolerance to satietin are unknown. This phenomena may be related to the relative impurity of our semipurified satietin but that is also unclear at this time.

The decrease in biological activity (tolerance) that was seen when osmotic pumps were placed in the body cavity was clearly not due to deterioration of the satietin molecule caused by prolonged exposure to body temperature. The rather wide range of reductions of food intake 26.2 to 63.1%, is difficult to understand. Certainly individual sensitivity to satietin is a possibility. An important component of the individual variation might be that satietin is not taken up from the peritoneal cavity at the same rate, or even during the same time period, in all individuals. Perhaps intravenous infusion would reduce the variability between animals.

We have previously reported that Sp hSAT is aversive in rats (1) but we have also reported that it may be a physiologically active anorectic substance because it inhibits food intake less after a 48 hr fast than it does after shorter fasts (1). Results of the present study question how important aversion might be because increases in food intake during the recovery period following surgery (Experiment 2) were nearly as great as that in the saline-treated group. It is possible that the doses used in Experiment 2 were too small to clearly answer the question concerning the importance of aversion. However, 1 mg/kg/day IP has previously been shown (4) (Experiment 1) to reduce food intake, thus, it seems unlikely that this dose was too small but that 0.5 and 0.25 mg/kg may have been.

In conclusion, the results reported herein strongly support the notion that satietin specifically inhibits food intake and is without effect on water intake. It is clear that tolerance to semipurified satietin develops rapidly; it is unknown whether higher doses will overcome the effects of tolerance.

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