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Effects of Components Derived From HPLC Purification of Human Satietin on Ingestion, Body Weight, and Taste Aversion in the Rat

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BELLINGER, L. L. AND V. E. MENDEL. *Effects of components derived from HPLC purification of human satietin on ingestion, body weight, and taste aversion in the rat.* PHARMACOL BIOCHEM BEHAV 47(2) 255-263, 1994. – The putative satiety agent human satietin (h-SAT) once thought to be homogenous has been separated by high-performance liquid chromatography (HPLC) into components designated peak A (P-A, 53%/w) and Peak B (P-B, 47%/w); P-B contains a putative satiety agent. In Experiment 1, male Sprague-Dawley rats were divided into six (n = 9-11) groups (Grps) and ICV infused: Grp 1, artificial cerebrospinal fluid (a-CSF), 10 μl/rat; Grp 2, albumin (ALB), 53 μg/rat; Grp 3, semipurified (sp) h-SAT (parent compound), 100 μg/rat; Grp 4, P-A, 53 μg/rat; Grp 5, P-B, 47 μg/rat; and Grp 6, P-A+B, 53 + 47 μg/rat, respectively. Compared to Grp 1, food intake, the first day postinfusion, was suppressed in Grp 3 ($p < 0.01$) and equally attenuated ($p < 0.06$) in Grps 5 and 6. Body weight remained suppressed ($p < 0.05$) in Grps 3, 5, and 6 for 3 days and in Grps 3 and 6 ($p < 0.05$) for an additional 3 days; Grps 2 and 4 did not differ from Grp 1. These data show P-B suppresses food intake comparably to P-A+B and causes a prolonged weight loss. In Experiment 2, sph-SAT and a recombination of P-A and P-B was tested for aversiveness using the two-bottle test. Both sph-SAT and P-A + B significantly suppressed food intake, but only sph-SAT was found to be aversive. These data show that most likely during HPLC processing of sph-SAT an aversive component was lost.

Human satietin RN : 72026-83-6 HPLC Food intake Water intake Ingestion Body weight Feeding behavior ICV infusion Aversiveness

IN 1979, the discovery of a putative satiety agent, extracted from human plasma, was reported (16); this compound was called human satietin (h-SAT). When h-SAT was infused ICV or peripherally, it caused a dose-dependent suppression of food consumption in rats for up to 36 h after a single administration [(15,17,18), but see (21)]. Later, satietin was extracted from rat plasma (3,4) and bovine plasma (7) and it also was found to be effective in suppressing the food intake of rats. Interestingly, the body weights of treated animals remained attenuated for several days following a single dose (3-5).

Human SAT prepared by Nagy, Knoll, and coworkers (15,27,28) was originally reported to be homogenous; however, Mendel and Palieschesky (24) using high-performance liquid choromatography (HPLC) separated h-SAT into two fractions: peak A (P-A) and peak B (P-B). Therefore, the starting h-SAT material was in fact only semipure (sph-SAT). Peak A contained α_1-glycoprotein and albumin [(ALB) (5)] and was not effective in suppressing food consumption or body weight when administered ICV to rats (6). Peak B contained an unknown substance(s) that suppressed food intake and body weight of rats when given ICV (5,6). It is therefore possible that P-B contains satietin. However, P-B was not as effective as its parent sph-SAT preparation in suppressing feeding and body weight (5); therefore, activity was seemingly lost during HPLC separation.

Earlier, the original sph-SAT compound, when given ICV, was found to be highly aversive in a two-bottle taste test (1) and when given by continuous ICV infusion (2). Rat satietin, when similarly tested, was not found to be aversive (3). Later, HPLC-prepared P-A and P-B were tested for aversiveness,

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and both were found to be nonaversive (6); therefore, the aversiveness of sph-SAT was lost during HPLC purification.

As noted above, P-A was found not to be effective in suppressing food intake or body weight (6). It is, however, possible that if P-A were given simultaneously with P-B they might produce an interactive effect and cause a greater suppression of ingestion and body weight than giving P-B alone. Alternatively, it could be that the ability of sph-SAT to suppress food intake and body weight to a greater degree than P-B was partly due to the loss of an aversive agent during HPLC purification. Finally, it is possible that some of the active molecule was lost during HPLC separation of sph-SAT. In the present study, we tested these possibilities by comparing ingestion, body weight gains, and aversiveness of compounds in rats treated with sph-SAT to animals receiving P-A, P-B, or P-A + B.

METHOD

General

Male Sprague–Dawley rats (Harlan Industries, Madison, WI) were individually caged in a light cycle- (12 L : 12 D, lights out at 1400 h in Experiment 1 and 1130 h in Experiment 2) and temperature-controlled (23°C) room. They were given chow (Purina 5001) and tapwater ad lib for 4 days prior to surgery.

At the time of surgery (Experiment 1, 67 rats, 249–298 g body wt.; Experiment 2, 30 rats, 299–343 g body wt.), rats were anesthetized with ketamine (90 mg/kg body wt.) and xylazine (9.1 mg/kg body wt.). Using a Kopf stereotaxic instrument (Kopf, Tujunga, CA), rats' third ventricles were implanted [AP, 0.8 mm behind the bregma; depth, 3.0 mm above ear bar zero; lateral, on the midsagittal suture (30)] with a stainless steel (22 ga) guide cannula (Plastic Products, Roanoke, VA). The cannula was held in place with four stainless steel screws and dental cement and then occluded with an obturator.

Three days following surgery, correct cannula placement was determined by an increased drinking response following ICV infusion of sterile angiotensin II (Sigma Chemical Co., St. Louis, MO, 150 ng/rat, 5 μl volume infused over 10 s). If a rat did not drink in response of ICV angiotensin II, it was eliminated from the study. Also, at experiment's end rats were ICV infused with 5 μl India Ink and cannula placement verified histologically. Only those rats with correct cannula placements were used in the statistical analysis.

The starting material used in the studies was sph-SAT, which was extracted at University of California, Davis, according to the method of Nagy et al. (27,28). The sph-SAT was further purified using a weak anion exchange Synchronpak AX300 HPLC (Rainin Scientific Instruments, Inc., Woburn, MA) and employing 0.02 M Tris acetate, pH 7.8, and a linear gradient of 0–60%, 1 M sodium acetate (5,6). The sph-SAT separated into only two detectable peaks (denoted as A and B) that were then desalted on a BioGel P-2 column. It was found that 53%, by weight, of the recovered sph-SAT was in P-A and 47% by weight in P-B.

Data were analyzed using analysis of variance (ANOVA), Duncan's multiple-range test, and Student's *t*-test.

Experiment 1

In previous studies, up to 100 μg/rat sph-SAT (1,5) was infused ICV to suppress food intake. In the present study, 10 days after surgery food was removed at 0930 h and rats were divided into six groups (Grps): Grp 1 received artificial cerebrospinal fluid [a-CSF, (26)]; Grp 2 received 100 μg/rat human ALB (Sigma); Grp 3 received 100 μg/rat sph-SAT; Grp 4 received 53 μg/rat P-A; Grp 5 received 47 μg/rat P-B; Grp 6 received a combination of P-A (53 μg/rat) and P-B (47 μg/rat) in a single infusion. All substances were dissolved in a-CSF and sterilized by passing through a 0.22-μm filter (Gelman Sciences, Ann Arbor, MI). ICV infusions (10 μl/rat) occurred between 1135–1310 h and food was returned at 1400 h. Food intake, corrected for spillage (gathered on pads below the cages), and water consumption, using Wahmann calibrated bottles with ball spouts, were recorded daily for 14 days. Body weights were recorded as noted.

Experiment 2

Five days following surgery, correct cannula placement was demonstrated with angiotensin II as denoted in the General Method section. For the next 6 days, animals with correct cannula placement received food ad lib but were presented with water only between 1015 and 1115 h (1) in Wahmann calibrated drinking bottles equipped with ball spouts. At the end of this period, rats were divided into three groups. Food intake, corrected for spillage, was measured for the hour that water was present and for a 24-h total; water consumption was also recorded (day 1). This measuring regimen continued until experiment's end.

The next day (day 2), all rats were ICV infused (10 μl) with sterile a-CSF at 0900 h. At 1015 h, Grp 1 (289.1 ± 5.3 g body wt.) and Grp 3 (290.0 ± 5.8 g body wt.) were presented with a novel solution (1,12) of water flavored with banana extract (0.5% v/v, McCormick and Co., Inc., Hunt Valley, MD). Group 2 (289.0 ± 4.6 g body wt.) was offered a novel solu-

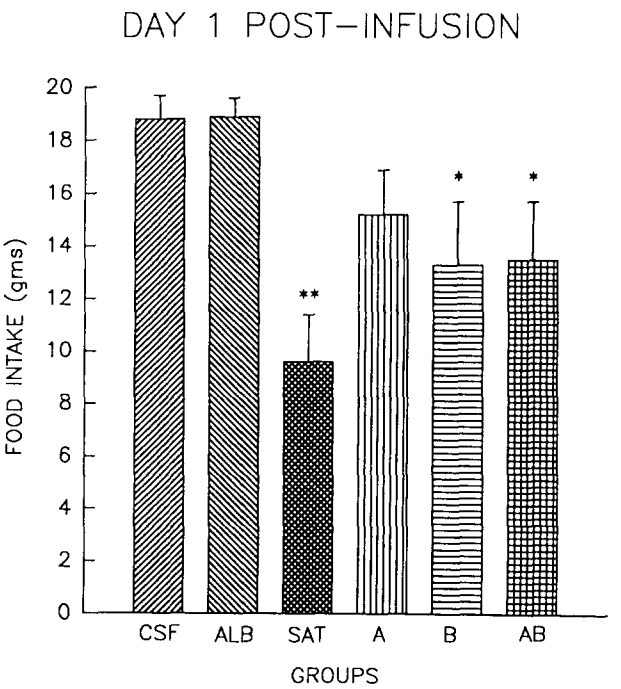

FIG. 1. Twenty-four-hour food intake first day postinfusion. Infusate: CSF, artificial cerebrospinal fluid (n = 9); ALB, albumin (n = 9); SAT, satietin (n = 9); A, Peak A (n = 11); B, Peak B (n = 10); and AB, Peaks A and B (n = 11). $^{**}p < 0.01$; $^{*}p < 0.06$.

TABLE 1

WATER CONSUMPTION (ml) THE DAY PRIOR TO INFUSION AND 2 DAYS THEREAFTER

Day		a-CSF (n = 9)	ALB (n = 9)	$p<$*	sph-SAT (n = 9)	$p<$*	P-A (n = 11)	$p<$*	P-B (n = 10)	$p<$*	P-A + B (n = 11)	$p<$*
−1		30.8 ± 3.5	31.4 ± 1.8	n.s.	28.1 ± 1.8	n.s.	32.3 ± 3.2	n.s.	31.5 ± 2.5	n.s.	30.6 ± 3.1	n.s.
+1		27.4 ± 5.6	22.0 ± 2.7	n.s.	15.0 ± 2.5	n.s.	20.5 ± 3.6	n.s.	18.4 ± 3.1	n.s.	16.2 ± 3.3	n.s.
	$p<$†	n.s.	n.s.		<0.05		<0.05		<0.05		<0.05	
+2		27.2 ± 3.6	25.9 ± 4.4	n.s.	27.4 ± 2.5	n.s.	26.3 ± 2.4	n.s.	31.8 ± 3.3	n.s.	27.6 ± 2.6	n.s.
	$p<$†	n.s.	n.s.		n.s.		n.s.		n.s.		n.s.	

a-CSF, artificial cerebrospinal fluid; sph-SAT, human satietin; P-A, Peak A; P-B, Peak B; P-A + B, Peaks A and B; see text for dosage. n.s., nonsignificant.
*$p<$ comparison made with a-CSF.
†$p<$ comparisons made with day −1.

tion of water flavored with almond extract (0.5% v/v). Fluid and food consumption were recorded as noted above. On day 3, at 0900 h each rat of Grp 1 was again ICV infused with a-CSF (10 μl) but at 1015 h presented with almond-flavored water. At this same time, Grp 2 and Grp 3 were ICV infused (10 μl) with sterile sph-SAT (100 μg/rat) or P-A + B (100 μg/rat), respectively. Group 2 was offered banana-flavored water and Grp 3 was presented with an almond-flavored solution. On day 4, all groups received water. Two-bottle testing was not conducted on this day because single satietin infusions have been reported to suppress food intake for up to 36 h (15,17,18). Therefore, on day 5 rats were presented with both almond- and banana-flavored solutions at 1015 h (1). If a rat did not sample both bottles within 15 s of fluid presentation, it was forced to drink from the untouched bottle for 10 s by briefly removing the other bottle. Bottle positioning (right or left side of cage front) was randomized throughout the study to avoid a place preference.

RESULTS

Experiment 1

One rat died shortly after surgery, two other rats did not drink in response to angiotensin II infusion, and five rats became ill during the course of the study. Data from all these animals were eliminated prior to statistical analyses; group sizes were: Grp 1, $n = 9$; Grp 2, $n = 9$; Grp 3, $n = 9$; Grp 4, $n = 11$; Grp 5, $n = 10$; and Grp 6, $n = 11$.

The food consumption of the groups was comparable, $F(5, 53) = 0.41$, n.s., the day prior to infusion: Grp 1, 22.8 ± 2.0 g; Grp 2, 21.9 ± 0.8 g; Grp 3, 21.4 ± 0.9 g; Grp 4, 22.3 ± 0.5 g; Grp 5, 21.3 ± 1.0 g; and Grp 6, 21.2 ± 0.5 g. The food intake of the groups differed, $F(5, 53) = 3.57$, $p < 0.01$, on the first day postinfusion (Fig. 1). Compared to the intake of Grp 1 (a-CSF), animals receiving sph-SAT (Grp 3) showed the greatest depression ($p < 0.01$) of ingestion. The groups infused with P-B (Grp 5) or a combination of P-A + B (Grp 6) also showed attenuated food consumption that approached significance ($p < 0.06$) when contrasted to Grp 1. The two groups that received ALB (Grp 2) or P-A (Grp 4) did not differ significantly in their intake compared to Grp 1. Food consumption of the six groups was alike, $F(5, 53) = 0.23$, n.s., on the second day postinfusion and for the remainder of the study.

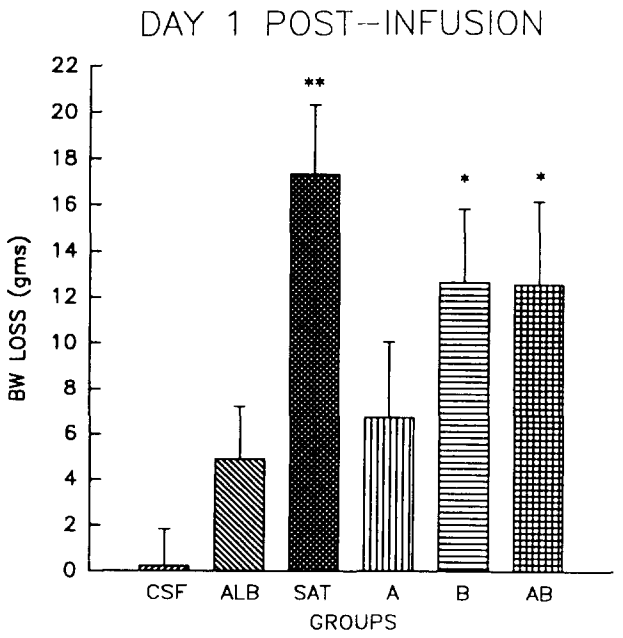

FIG. 2. Body change (loss) first day postinfusion. See legend of Fig. 1 for explanation of abbreviations.

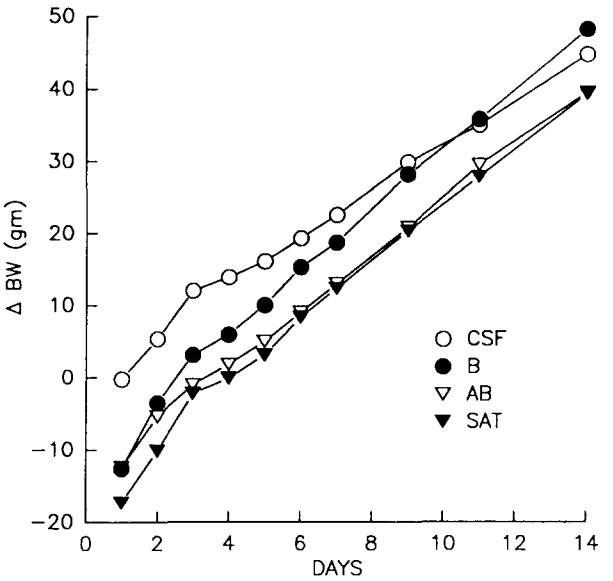

FIG. 3. Body weight change days 1–14 postinfusion. Significance comparison with cerebrospinal fluid (CSF): day 1, B and AB $p < 0.05$, SAT $p < 0.01$; days 2–3, B and AB $p < 0.05$, SAT $p < 0.01$; days 4–6, AB and SAT $p < 0.05$. Peak A and albumin are not graphed because they never differed statistically from CSF groups. See legend of Fig. 1 for explanation of abbreviations.

The water intake of the groups was similar the day prior to infusion (Table 1). Following infusion, there was a significant day effect, $F(2, 159) = 19.98$, $p < 0.01$, but not a group effect, $F(5, 159) = 0.98$, n.s. Water ingestion of all groups was attenuated somewhat after infusion, with significance ($p < 0.05$) being reached for the sph-SAT, P-A, P-B, and P-A + B groups (Table 1). By the second day after infusion, water consumption had returned to baseline for all groups. Water intake did not vary significantly among the groups over the remainder of the study.

At the time of infusion, the groups had similar, $F(5, 53) = 0.07$, n.s., body weights: Grp 1, 286.7 ± 5.4 g; Grp 2, 288.2 ± 5.7 g; Grp 3, 283.2 ± 6.1 g; Grp 4, 287.2 ± 9.3 g; Grp 5, 288.6 ± 8.7 g; and Grp 6, 287.5 ± 5.5 g. On the first day following infusion, the body weights, expressed as a change in body weight, of the groups receiving ALB and P-A were comparable to the a-CSF control group (Fig. 2). Compared to the a-CSF control group, the body weight of the groups receiving P-B, P-A + B, or sph-SAT were significantly, $F(5, 53) = 3.51$, $p < 0.01$, and similarly suppressed for the first 3 days following surgery (Fig. 3). However, over the next 3 days (days 3–6) the groups receiving P-A + B and sph-SAT continued to show a significant weight difference from the control groups. Over these 3 days, the group receiving P-B showed an intermediate weight loss that was not significantly different from the control group. The body weights

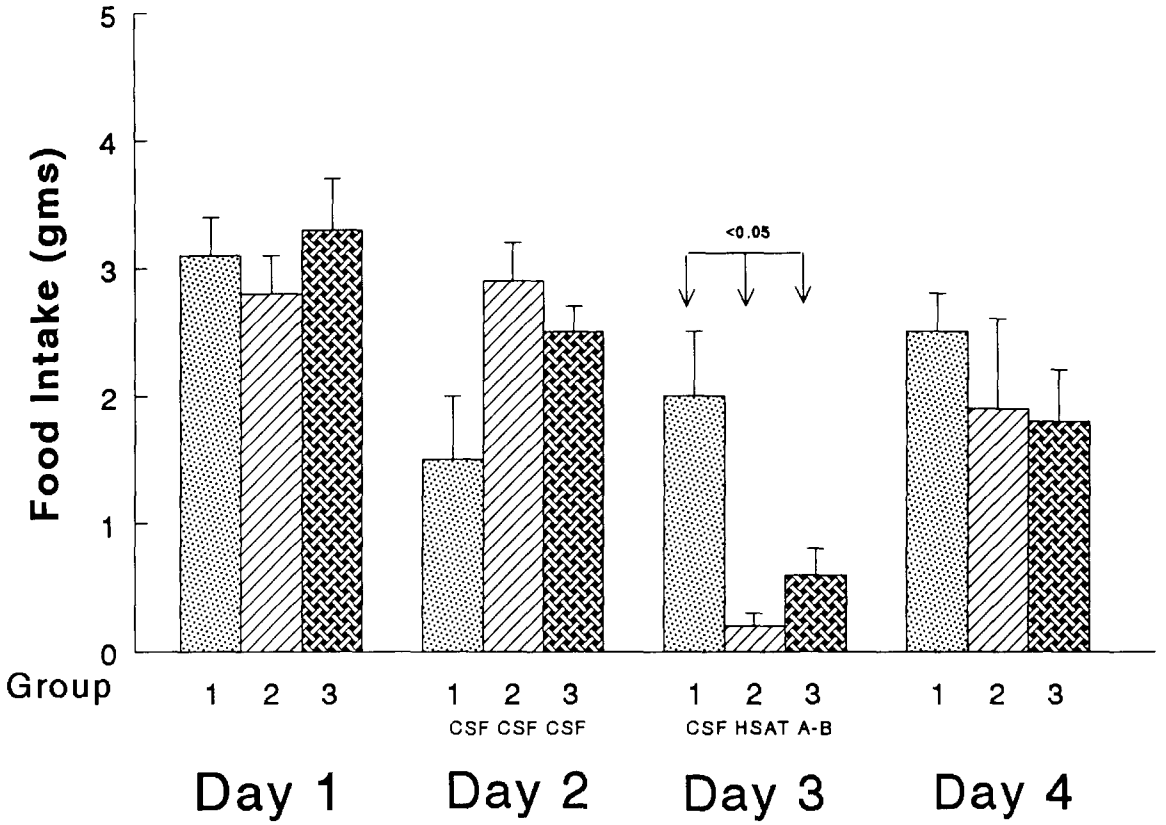

FIG. 4. One-hour food intake during the period of fluid presentation in group 1 (n = 8), group 2, (n = 6), and group 3 (n = 8). Day 1, no treatment; day 2, all animals were ICV infused with artificial cerebrospinal fluid (a-CSF); day 3, rats of group 1 were ICV infused with CSF whereas groups 2 and 3 were infused with 100 μg/rat h-(satietin) SAT or Peak A + B, respectively; day 4, no treatment. Means ± SE.

in groups receiving ALB and P-A did not significantly differ at any time from the a-CSF control group.

Experiment 2

Two rats died during surgery, two rats did not drink following angiotensin II infusion, and four rats became ill during the study. Data from these animals were eliminated, leaving for statistical analyses group sizes of: Grp 1, n = 8; Grp 2, n = 6; and Grp 3, n = 8.

Food consumption (Fig. 4) during the hour of water presentation was similar in all groups during baseline measurement (day 1), $F(2, 29) = 0.55$, n.s., and on day 2, $F(2, 19) = 3.39$, n.s., after all groups received a-CSF. Twenty-four-hour intakes (Fig. 5) were also comparable among the groups on day 1, $F(2, 19) = 1.02$, n.s., and day 2, $F(2, 19) = 0.45$, n.s.

After drug infusion on day 3, the 1-h food consumption differed, $F(2, 19) = 6.82$, $p < 0.01$, among the groups (Fig. 4). The intakes of Grp 2 (sph-SAT) and Grp 3 (P-A + B) were significantly suppressed compared to the control group that received a-CSF. The 1-h consumption of the two experimental groups, but not the control animals, was also attenuated ($p < 0.01$) after drug treatment when compared to their intake on day 2. Twenty-four-hour food consumption (Fig. 5) of Grps 2 and 3 was also suppressed, $F(2, 19) = 7.14$, $p < 0.01$, when compared to the control group. The 24-h intake of the two experimental groups but not the control group was also attenuated ($p < 0.01$) on day 3 when contrasted to their intakes on day 2.

On day 4, 1- and 24-h food ingestion of the three groups was again similar, $F(2, 19) = 0.66$, n.s., and $F(2, 19) = 2.36$, n.s., respectively. On the two-bottle test day (day 5), the 1-h food consumptions of the groups were also alike, $F(1, 19) = 0.05$, n.s.

As shown in Fig. 6, baseline water ingestion (day 1) was similar, $F(2, 19) = 0.78$, n.s., in the three groups. After receiving a-CSF infusion on day 2, flavored-water consumption of the groups was also comparable, $F(2, 19) = 0.68$, n.s. Following drug infusion (day 3), flavored-water ingestion of the three groups did not differ significantly, $F(2, 19) = 2.36$, n.s. However, the fluid intake of the two experimental groups, but not the control group, was attenuated ($p < 0.01$) after drug treatment when contrasted to their intakes on day 2. Nevertheless, the experimental groups consumed a significant amount of fluid on day 3. On day 4, water intakes of the three groups were similar, $F(2, 19) = 0.95$, n.s.

Figure 7 shows that in the two-bottle choice test controls (Grp 1) and rats receiving P-A + B (Grp 3) consumed similar quantities of both flavored solutions. Thus, P-A + B was not aversive to these animals as measured by these procedures. This contrasted sph-SAT-treated animals (Grp 3), which consumed significantly less of the banana-flavored solution that was paired with sph-SAT infusion, as compared to the almond-flavored fluid paired with a-CSF infusion. The re-

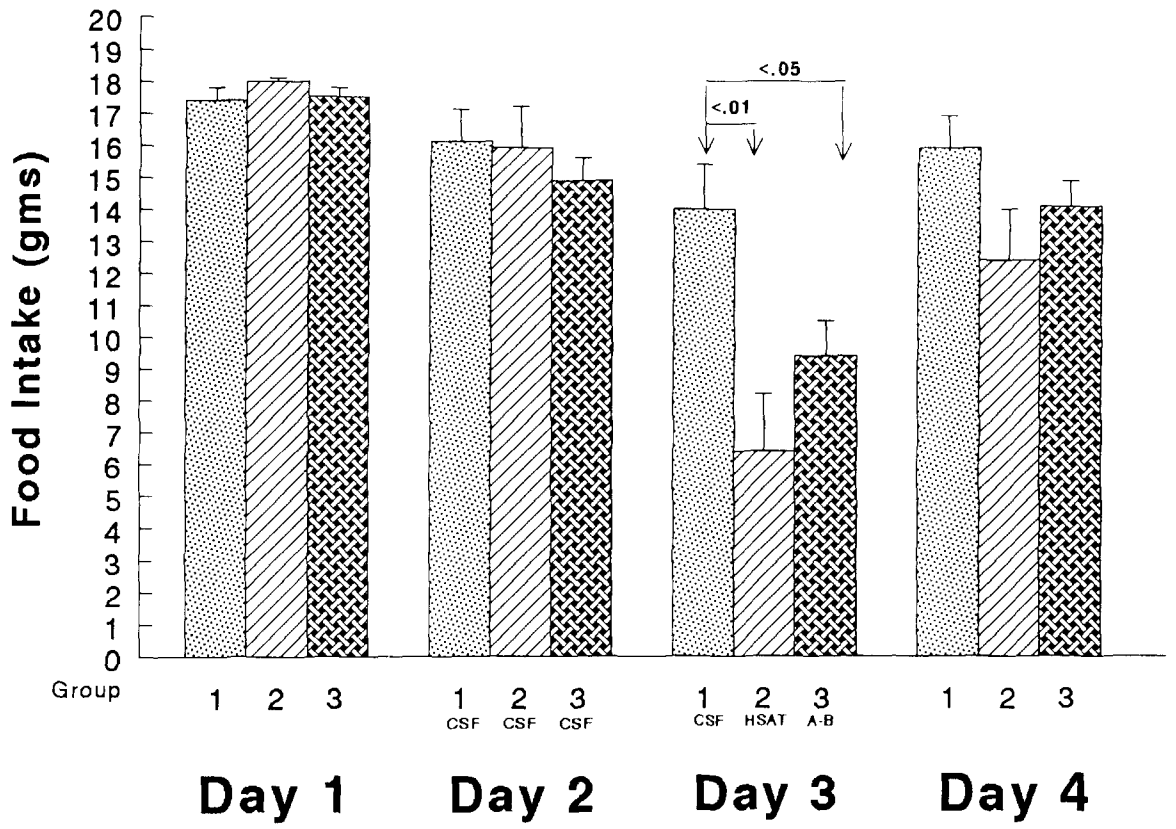

FIG. 5. Twenty-four-hour intakes; see the legend of Fig. 4 for explanation of abbreviations.

sponses of sph-SAT-treated animals indicate that they found the sph-SAT aversive.

DISCUSSION

Previously (5), we noted that sph-SAT could be separated by HPLC into P-A and P-B. We further found that 50 μg/rat P-B infused ICV into rats was not as effective in suppressing food intake and body weight as 50 μg/rat of the parent sph-SAT preparation. One possibility (5) was that P-B interacted with P-A to cause a more potent effect. In the present study, the parent sph-SAT preparation was also separated by HPLC into P-A (53% by weight) and P-B (47% by weight). The ICV infusion of 100 μg/rat sph-SAT caused the largest depression of food consumption. Rats receiving P-B (47 μg/rat) or P-A + B (53 μg + 47 μg/rat, respectively/rat) showed a comparable attenuation of feeding that was slightly less than that caused by sph-SAT. Albumin and P-A did not significantly suppress food consumption when compared to the a-CSF-infused group. Therefore, recombining P-A with P-B was no more effective in suppressing food intake than P-B alone. These data speak against a possible interaction (5) of P-A and P-B in reducing food intake to the level found after giving sph-SAT.

Earlier, we demonstrated that the sph-SAT preparation was highly aversive [25–100 μg/rat; (1)] when given ICV but apparently not aversive when given peripherally (23). Satietin prepared from rat plasma (3,4) and given ICV also suppressed food intake and caused a long-term decrease in rats' body weight. However, the rat satietin preparation given ICV was not aversive to animals as determined by the two-bottle test (3). We originally thought (1) the difference between the rat and sph-SAT might have been due either to a species difference or impurities in the sph-SAT preparation. Later (6), we found neither P-A or P-B was aversive using the two-bottle test. This demonstrated that a species difference in the active molecule(s) was not responsible for the aversive nature of the sph-SAT preparation. It also suggested, as we had originally entertained (1), that either there were impurities in the sph-SAT preparation that were lost during HPLC separation or, as we had also speculated (6), that P-A and P-B interacted in some way to cause the aversion. The present study speaks against the interaction hypothesis as P-A + B was not aversive while the parent sph-SAT preparation was aversive. As noted above, the food intake attenuation potency of the P-A + B was not as great as the sph-SAT. Thus, this difference in potency may in fact be derived from an aversive agent, an impurity, that was lost during HPLC separation of the sph-SAT into P-A and P-B. In accord with this possibility is the finding that while recovery following the HPLC procedure is usually greater than 80% some material is lost. Therefore, the sph-SAT preparation may lower food intake by both specific and nonspecific means. Finally, although remote, it is possible that the HPLC procedure could have changed the active molecule(s)'s physical structure such that its aversive nature was lost.

A statement of caution needs to be included as to whether the two-bottle test accurately determines aversion or nonaver-

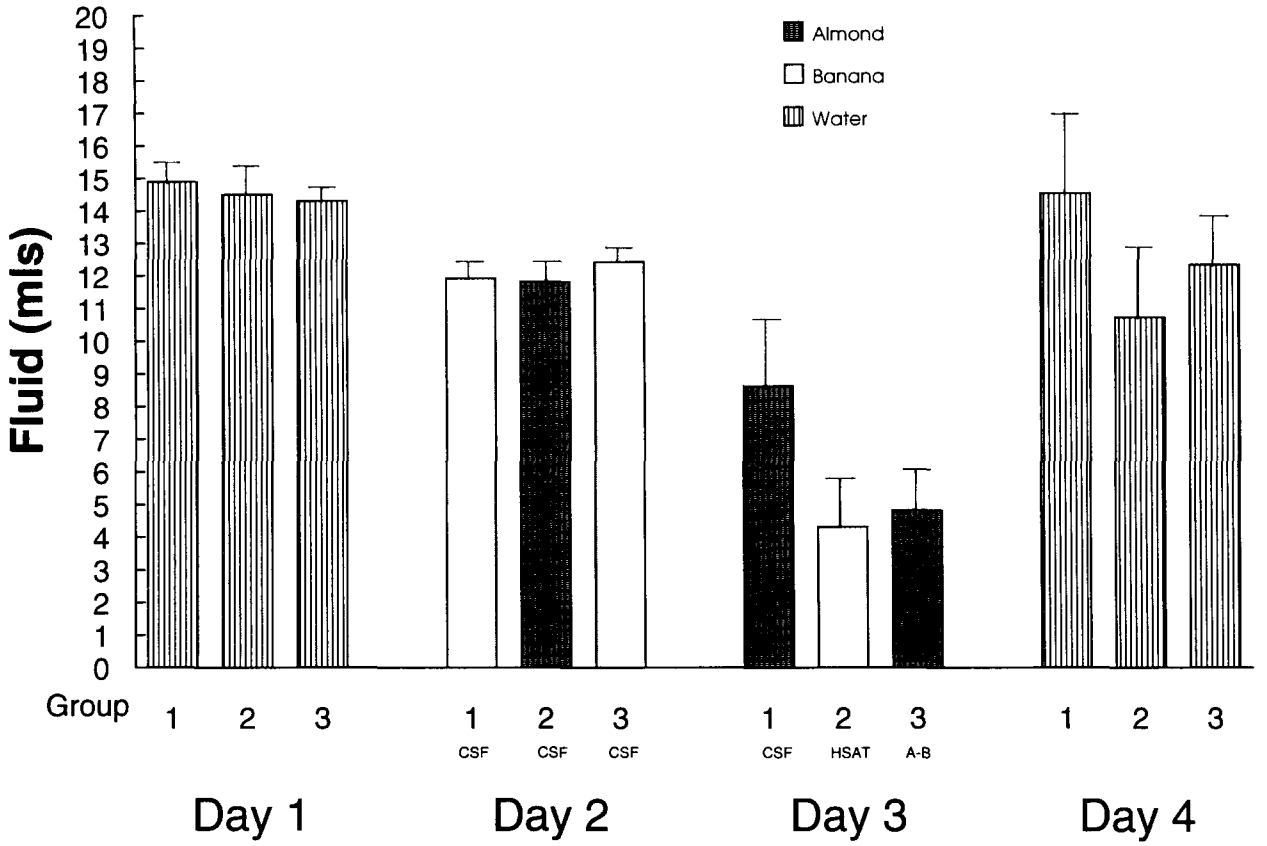

FIG. 6. One-hour fluid intake. On days 1 and 4, all groups were given water and on days 2 and 3 groups were given almond- or banana-flavored water as indicated. See the legend of Fig. 4 for explanation of abbreviations.

sion. There is no one totally accepted method to determine satiety vs. aversion (11,13) and the two are not mutually exclusive (9).

Water consumption was significantly decreased for 24 h in Experiment 1 following treatment with sph-SAT, P-A, P-B, and P-A + B. Water intake was nonsignificantly attenuated following treatment with a-CSF and ALB. In Experiment 2, water consumption was also attenuated following treatment with sph-SAT and P-A + B. Studies by Knolls' group (31) and our laboratories (4,5,22) suggest that sph-SAT, rat SAT, and P-B specifically lower food and not water consumption. The decrease in water consumption after treatment probably is just a reflection of the close interrelationship of food and water ingestion (32).

Single ICV infusion of rat satietin (3) or sph-SAT (5) have been shown to significantly reduce body weight of rats for 7 and 14 days, respectively. In the present study, changes in body weight the first day postinfusion paralleled food consumption in that treatment with sph-SAT caused the greatest weight loss. The significant weight loss in the group receiving P-B was identical to that observed in the group given P-A + B. These data also indicate that an interaction of P-A + B does not cause a weight loss comparable to that observed with the sph-SAT on the first day postinfusion. Over the next 2 days, groups receiving sph-SAT, P-B, and P-A + B had significantly reduced body weight gains compared to a-CSF-treated rats. Interestingly, over the subsequent 3 days (days 3-6) groups receiving sph-SAT and P-A + B continued to show significant attenuated body weight gains compared to controls. The P-B group showed a nonsignificant intermediate weight loss when contrasted to a-CSF-treated rats. Groups receiving P-A and ALB did not show a significant decrease in body weight at any time throughout the study. If the noted body weight differences are real, it may suggest some interaction of P-A with P-B to cause the greater effect on body weight gain. These studies will need to be repeated to confirm that an interaction effect does exist.

During food restriction, rats use a variety of strategies to limit weight loss. They may increase efficiency of food utilization and/or decrease their basal metabolic needs (10,14) so as to maintain growth or limit weight loss. When rats are returned to ad lib food intake following a short-term moderate food restriction, they regain the lost body weight in a day or two (8). In the present study, the food consumption of groups treated with sph-SAT, P-B, and P-A + B was moderately depressed the first day posttreatment. The food intake of these groups had returned to normal by day 2, yet their body weight remained significantly attenuated for 3-6 days following infusion. We had already observed these long-term suppressions of body weight in face or normal food intake in rats given single injections of rat satietin (3) or sph-SAT (5). The present data and earlier findings (3-5) suggest that satietin may be altering the normal metabolic compensatory responses that occur during and following food deprivation such that rats maintain a prolonged weight loss.

Finally, satietin is thought to be a member of the α_1-

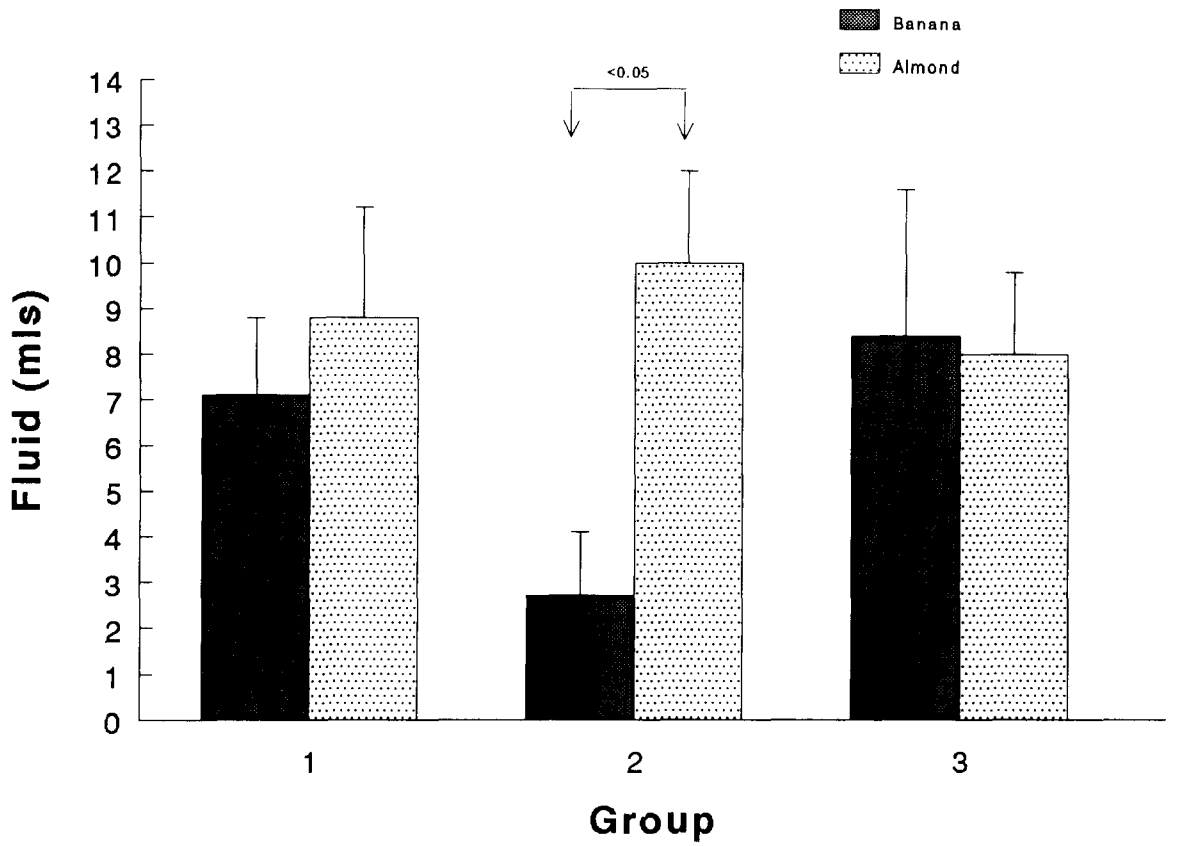

FIG. 7. Fluid intake on day 5, when all groups were given a choice between almond- and banana-flavored water. See the legend of Fig. 4 for explanation of abbreviations.

glycoprotein family (19,20), which serve as carriers for a number of hormones (25,29). As we have previously noted (5), it is theoretically possible that some biological activity could have been lost during HPLC purification of sph-SAT if P-B contained both a carrier molecule and a small active molecule. Some of the latter could have been lost during the separation procedure. Thus, it should be kept in mind that the ultimate physiological relevance of satietin will only be ascertained when testing is performed with satietin of known structure and purity. We hope to resolve these issues with use of polyclonal and monoclonal antibodies; these studies are underway.

ACKNOWLEDGEMENTS

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