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HPLC-Purified Bovine Satietin Suppresses Food Intake and Weight Without Causing Conditioned Taste Aversion

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BELLINGER, L. L., J. NAGY AND J. HAMILTON. *HPLC-purified bovine satietin suppresses food intake and weight without causing conditioned taste aversion.* PHARMACOL BIOCHEM BEHAV 47(3) 659–666, 1994.—Satietin (SAT) is a putative satiety agent found in a variety of species including man and the rat. In the present study, satietin was extracted from bovine plasma (b-SAT) and further high-pressure liquid chromatography (HPLC)-purified. Rats were given chronic third ventricle cannulas and patency was verified. In experiment 1, rats were divided into three groups and ICV infused with artificial cerebrospinal fluid (a-CSF) or b-SAT: group 1, a-CSF (n = 11); group 2, 20 μg/rat, b-SAT (n = 11); and group 3, 40 μg/rat, b-SAT (n = 9). Infusions were repeated thrice three days apart. Compared to a-CSF, the high b-SAT dose suppressed food intake for 24-h after each successive infusion. The low dose significantly decreased food intake only after the first infusion. Water intake was suppressed only after the first injection of the high dose. Body weight was decreased after the first and second infusions of both doses and following the third infusion of the high dose. In experiment 2, rats were trained to drink fluid for 1 h/day while food was ad lib. On day 1, both groups received no infusions and were given tap water. On day 2, the groups were ICV infused with a-CSF, but group 1 (n = 12) was given banana-flavored fluid (BFF) and group 2 (n = 12) almond-flavored fluid (AFF). On day 3, group 1 was again a-CSF-infused but given AFF, whereas group 2 received 40 μg/rat b-SAT and was given BFF. The b-SAT suppressed 1- and 24-h food intakes and BFF intake compared to group 1. Two days later during two-bottle testing both groups drank similar amounts of each FF. Thus, b-SAT was not aversive, as determined by this test, and this and the data from experiment 1 indicate b-SAT has the properties of a satiety agent.

RN 72026-83-6 Food intake Water intake Body weight Aversion Two-bottle test for aversion Satiety

SATIETIN (SAT) is a putative endogenous satiety agent that was discovered in Hungary in 1979 (17). This molecule was shown to be a glycoprotein (21–23) that suppresses food intake when given either ICV or peripherally (17,19,25,26). Satietin's effects on ingestion appear to be specific for food and not water intake (4,5,26,32). Single ICV infusions of SAT (3,5) into rats have been observed to cause significant attenuation of body weight for up to 14 days.

Satietin was first isolated from human plasma and most, but not all (3,4), biological testing has been performed using the human preparation. However, in 1980 anecdotal data were presented (18) that showed biologically active SAT could be isolated from cat, dog, guinea pig, mouse, rabbit, and rat sera. Later, rat SAT was isolated at the University of California at Davis and shown to suppress both food intake and body weight (3,4).

The SAT preparation extracted from human serum was originally thought (30) to be homogenous, but Mendel and Palieschekey (27) demonstrated it could be separated into two peaks by high-pressure liquid chromatography (HPLC). One peak was found to contain a biologically active molecule that could be SAT (5,8,27). The other peak was biologically inactive and contained albumin and α_1 glycoprotein (5,27).

The original semipurified human SAT (sph-SAT) prepara-

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tion (17,29,30) was found to be highly aversive when tested using the two-bottle taste aversive method (1). However, the biologically active preparation obtained by HPLC purification of sph-SAT appeared to be nonaversive using the two-bottle taste aversion method (6). Thus an impurity in the preparation was mostly likely producing the aversion response (7). Rat SAT was shown not to have aversive properties (3).

In the present study, SAT was isolated from bovine serum according to the original method of Nagy et al. (29,30) and then further purified by HPLC [(5,27); Nagy, in preparation). Satietin was isolated from bovine serum for a variety of reasons, which among others included the high cost of rat plasma and inherent concerns with using large volumes of human plasma. In this study, which has appeared in abstract form (8), the effects of dose, repeated ICV infusions, and aversiveness of bovine SAT (b-SAT) were studied.

METHODS

General

Male Sprague-Dawley rats (Harlan Industries, Houston) were individually caged in a light cycle (12-h light-dark, lights out at 1200 in experiment 1 and 1320 in experiment 2) and temperature-controlled (23°C) room. All animals were fed chow (Purina No. 5012) ad lib throughout the study.

At the time of surgery the animals (experiment 1, 38 rats, 273-325 g; experiment 2, 26 rats, 232-269 g) were anesthetized with ketamine (90 mg/kg body weight) and xylazine (9.1 mg/kg body weight). Using a Kopf stereotaxic instrument the rats' third ventricles were implanted [anterior-posterior (AP), 0.8 mm behind the bregma; depth, 3.0 mm above ear bar zero; lateral, on the midsagittal suture (31)] with a stainless steel (22 gauge) 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.

In experiment 1 two days following surgery correct cannula placement was determined by an increased drinking response following ICV infusion of sterile angiotensin II (Sigma, St. Louis, 150 ng/rat, 5 μl volume infused over 10 s). If a rat did not drink in response to ICV angiotensin II it was eliminated from the study. At study's end in experiments 1 and 2 the rats were ICV infused with 5 μl of india ink and cannula placement was verified histologically. Only those rats with correct cannula placements were used in the statistical analyses.

A semipurified bovine (spb) SAT preparation was obtained from bovine serum using the methods of Nagy et al. (29,30). As mentioned above, while this preparation was originally thought to be homogenous, Mendel and Paliescheskey (27) showed it could be further purified by HPLC. In the present study the spb-SAT preparation was further processed by reverse phase HPLC with a gradient elution using an acetonitrile-water-trifluoroacetic acid solvent system. In accordance with the findings of Mendel and Paliescheskey (27), two peaks were obtained. One was found to contain the anorectic molecule b-SAT and the other albumin (Nagy, in preparation).

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

Experiment 1

Five days after verifying cannula patency the rats were divided into three groups. Food intake, corrected for spillage gathered on pads below each cage, and water consumption, using Wahmann calibrated bottles with ball spouts, were measured daily starting the day prior to infusions and for 13 days thereafter. Body weight was measured, as indicated, for 15 days following infusion.

At 1100 group 1 was infused ICV with 10 μl of sterile artificial cerebrospinal fluid [a-CSF (28)]. Groups 2 and 3 were ICV infused (10 μl) with 20 or 40 μg/rat of b-SAT, respectively. The b-SAT was dissolved in a-CSF and sterilized by passing through a 0.22-μm filter (Gelman Sciences, Ann Arbor, MI). Three and six days later the injections were repeated.

Experiment 2

Five days prior to surgery, and for the remainder of the study, the animals received food ad lib, but were presented with water only between 1210 and 1310 (1,14) in calibrated drinking bottles. Three days following surgery the rats were divided into two 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 between 1110 and 1140. At 1210, group 1 was presented with a novel solution (1,14) of water flavored with banana extract (0.5% v/v, McCormick and Co Inc., Hunt Valley, MD) and group 2 was offered a novel solution of water flavored with almond extract (0.5% v/v). Flavored water was presented approximately 30 min following infusion because it takes approximately 60 min for the manifestation of SAT's food intake suppression effects. Fluid and food consumption were recorded as noted above. On day 3 at 1110 each rat of group 1 was again ICV infused with a-CSF (10 μl) but at 1210 presented with almond-flavored water. At this same time, group 2 was ICV infused (10 μl) with sterile b-SAT (40 μg/rat) and offered banana-flavored water. On day 4 all groups received water. Two-bottle testing was not conducted on this day because single SAT infusions have been reported to suppress food intake for up to 36 h (17,21,23). Therefore, on day 5 the rats were presented with both almond- and banana-flavored solutions at 1210 (1). If a rat did not sample both bottles within 15 s of fluid presentation the rat 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

Two rats died shortly after surgery, four other rats did not drink in response to angiotensin II infusion, and one rat became ill during the course of the study. Data from all these animals were eliminated prior to statistical analyses; group sizes were group 1, $n = 11$; group 2, $n = 11$; and group 3, $n = 9$.

Both groups of rats infused with b-SAT showed a significant attenuation of food intake when compared to the a-CSF-infused controls, $F(2, 389) = 6.30$, $p < 0.001$ (Fig. 1). The a-CSF infusions did not significantly alter that group's food ingestion. The food intake of the group treated with 20 μg/rat of b-SAT was significantly suppressed for one day after the first treatment. Food consumption of the rats receiving the 40-μg/rat dose was significantly decreased for two days. Both experimental groups' food ingestion had returned to control levels by day 3. The second b-SAT treatment again re-

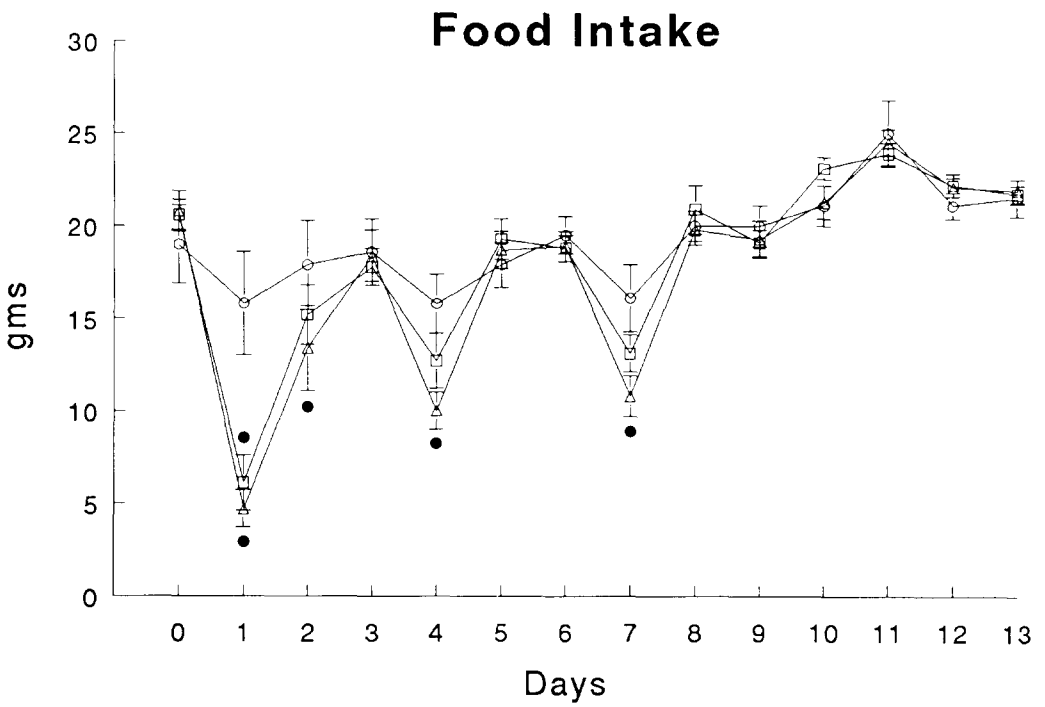

FIG. 1. Food intake of rats ICV infused with artificial cerebrospinal fluid (a-CSF, n = 11, ○), 20 μg/rat bovine satietin (b-SAT, n = 11, □), or 40 μg/rat b-SAT (n = 9, △) at the start of days 1, 3, and 6. Means ± SE. ● $p < 0.05$.

duced the rats' intake, but significance was only reached for the group treated with 40 μg/rat of b-SAT. The Duncan's test showed the magnitude of the food intake suppression was less ($p < 0.01$) after the second infusion than after the first. Food consumption of both treatment groups had returned to control levels on day 5 and remained there on day 6. After the third infusion the food intake of the group getting 40 μg/rat of the b-SAT was again significantly depressed, while the consumption of the group infused with 20 μg/rat of b-SAT was nonsignificantly attenuated. The intake of both groups was back to control levels on day 8 and remained there for the rest of the experiment. At no time was there a rebound overconsumption of food by the b-SAT-treated groups.

Water ingestion (Fig. 2) of the three groups was similar prior to treatment, but differed significantly after infusion, $F(2, 391) = 6.49$, $p < 0.002$. Following the first treatment, water ingestion of both treatment groups was attenuated, but significance was only reached in the group receiving b-SAT at 40 μg/rat. There appeared to be a rebound overconsumption of water by both experimental groups on day 3, but significance was not reached. Water intake was not lowered after the second or third infusion but instead was increased significantly in both treatment groups on days 5 and 6.

Body weights prior to first infusion were similar in the three groups: group 1, 279.5 ± 5.80 g; group 2, 283.5 ± 5.08 g; and group 3, 276.4 ± 6.18 g. After the first infusion, body weights (Fig. 3), expressed as a change from baseline, were significantly, $F(2, 392) = 21.73$, $p < 0.001$, and comparably suppressed following both doses of b-SAT. On days 2 and 3 body weights of the two experimental groups had only partially recovered to control levels. Body weights of both treatment groups were again significantly decreased following the second infusion. The weight difference between the b-SAT groups and the a-CSF-treated rats increased after the second dose when compared to what occurred following the first infusion. The body weights of the two treatment groups recovered somewhat on days 5 and 6. After the third treatment only the body weight of the group receiving the larger of the two doses was again significantly suppressed. The initial body weight changes after each infusion can be accounted for by changes in ingestion. Interestingly, the body weights of both treatment groups still had not asymptotically reached the controls by day 15. Finally, it should be noted that in the control group the infusion of a-CSF or the infusion procedures themselves nonsignificantly decreased the rat's food ingestion and somewhat attenuated their growth.

Experiment 2

Data from two rats that died during surgery were eliminated, leaving for statistical analyses group sizes of n = 12 (group 1) and n = 12 (group 2). On day 1 the body weights of the two groups were comparable: group 1, 227.4 ± 3.50 g; group 2, 228.5 ± 2.78 g.

Food consumption (Fig. 4) during the hour of water presentation was similar in both groups during baseline measurement (day 1) and on day 2 after the groups received a-CSF. Twenty-four-hour intakes (Fig. 5) were also comparable between the groups on days 1 and 2.

On day 3 the 1-h food ingestion (Fig. 4) of the b-SAT-treated group was significantly suppressed ($p < 0.01$) when compared to the a-CSF-infused group. The 1-h consumption of the experimental group, but not of the control animals, was also decreased ($p < 0.01$) after drug treatment when

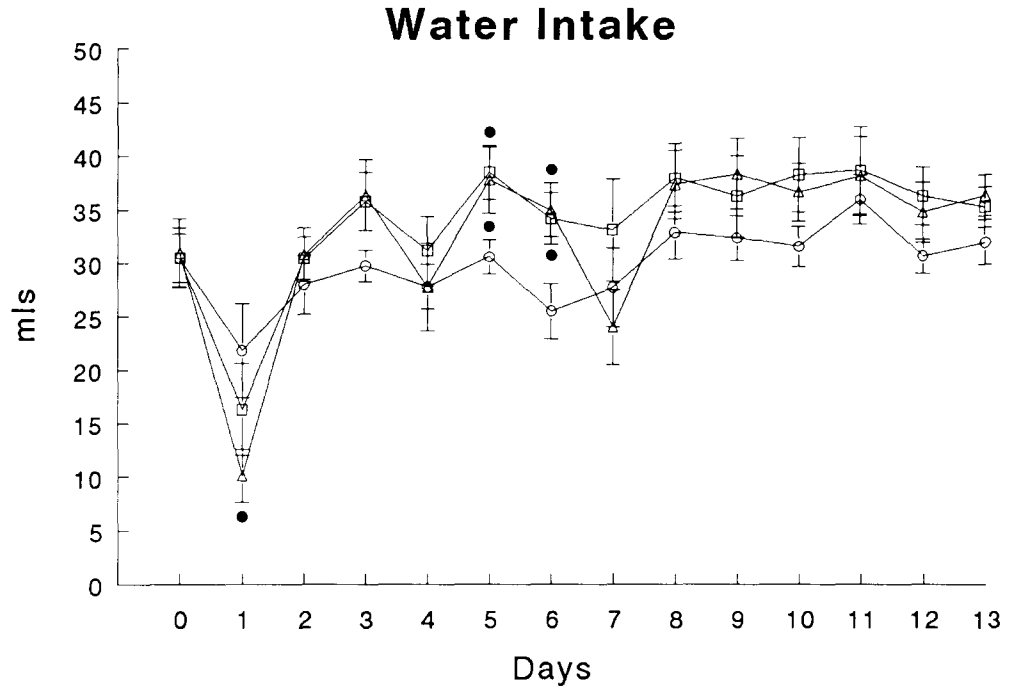

FIG. 2. Water intake of rats ICV infused with artificial cerebrospinal fluid (a-CSF, $n = 11$, ○), 20 μg/rat bovine satietin (b-SAT, $n = 11$, □), or 40 μg/rat b-SAT ($n = 9$, △) at the start of days 1, 3, and 6. Means ± SE. ● $p < 0.05$.

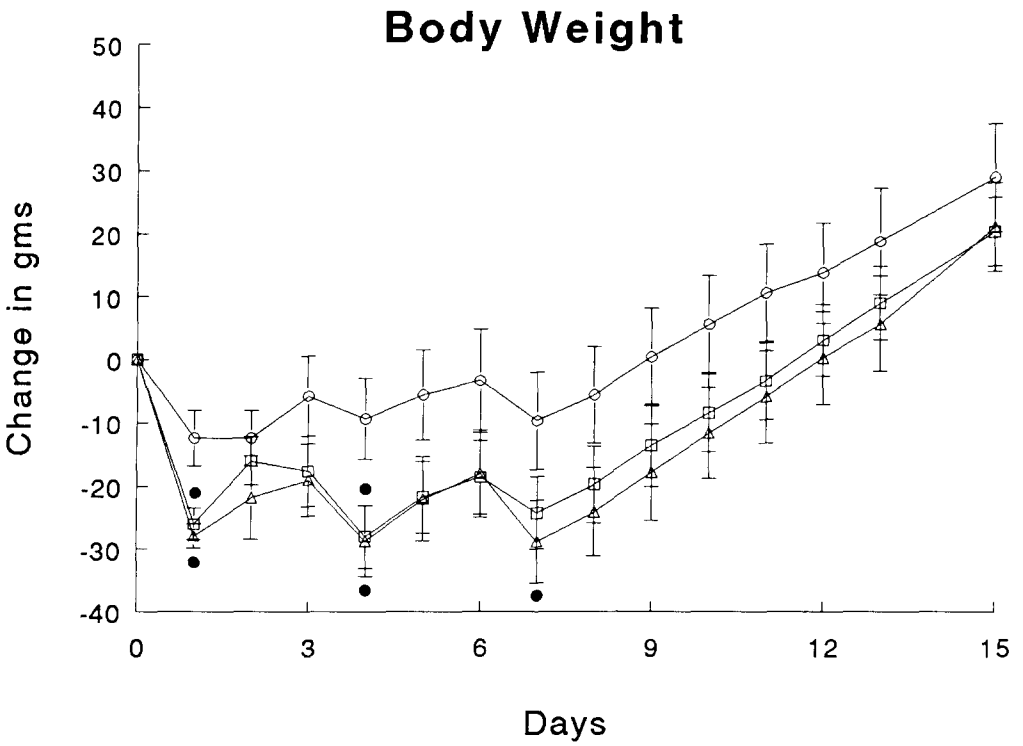

FIG. 3. Body weight change days 1-15 postinfusion of rats ICV infused with artificial cerebrospinal fluid (a-CSF, $n = 11$, ○), 20 μg/rat bovine satietin (b-SAT, $n = 11$, □), or 40 μg/rat b-SAT ($n = 9$, △) at the start of days 1, 3, and 6. Means ± SE. ● $p < 0.05$.

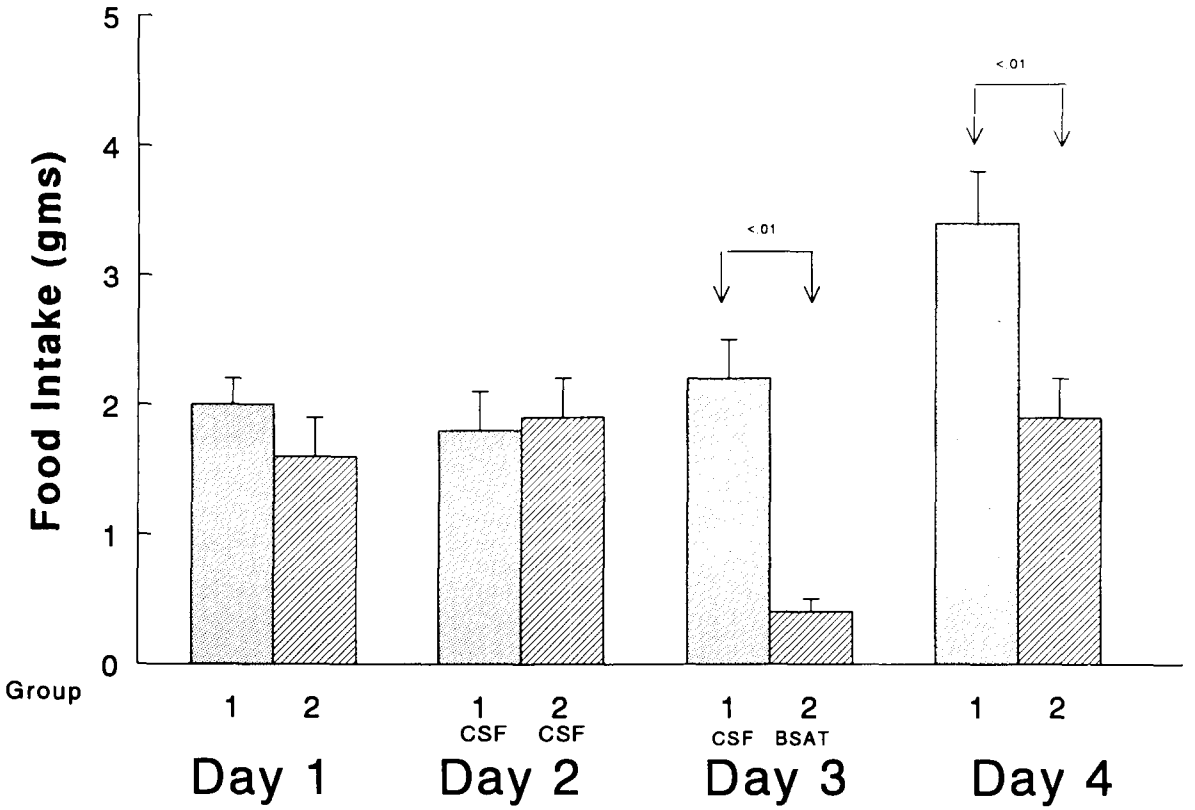

FIG. 4. One-hour food intake during the period of fluid presentation in group 1 (n = 11) and group 2 (n = 11). On day 1 there was no treatment; on day 2 all animals were ICV infused with artificial cerebrospinal fluid (CSF); on day 3 the rats of group 1 were ICV infused with CSF, whereas group 2 was infused with 40 μg/rat of bovine satietin (b-SAT); and on day 4 there was no treatment. Means ± SE.

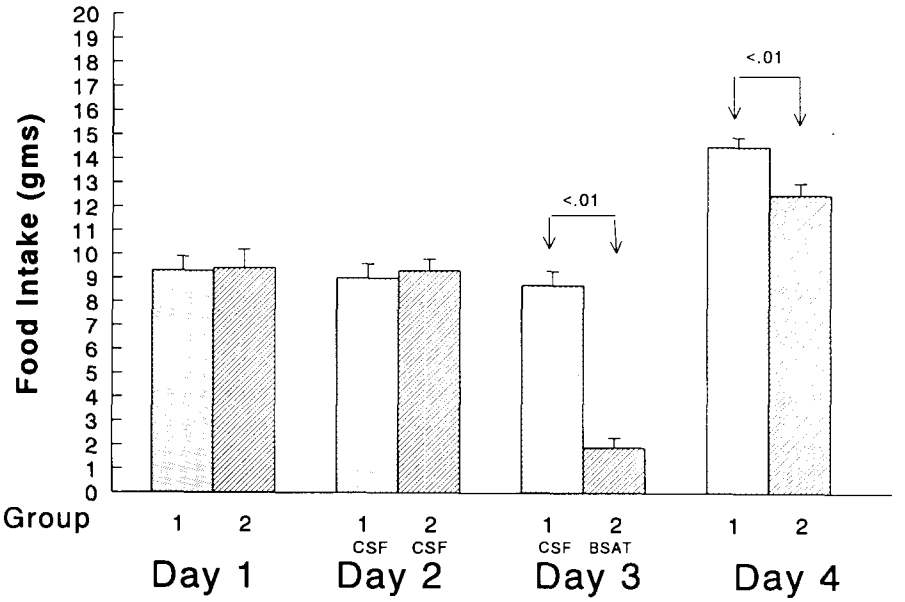

FIG. 5. Twenty-four-hour intakes in group 1 (n = 11) and group 2 (n = 11). On day 1 there was no treatment; on day 2 all animals were ICV infused with artificial cerebrospinal fluid (CSF); on day 3 the rats of group 1 were ICV infused with artificial CSF, whereas group 2 was infused with 40 μg/rat of bovine satietin (b-SAT); and on day 4 there was no treatment. Means ± SE.

compared to intake on day 2. Twenty-four-hour food intake (Fig. 5) of group 2 was also lowered ($p < 0.01$) when compared to the control group. The intake of the experimental group, but not of the control group, was also depressed ($p < 0.01$) on day 3 when contrasted to intake on day 2.

On day 4, 1- and 24-h food ingestions of group 2 were still suppressed when compared to the control group (Figs. 4 and 5). On the two-bottle test day (day 5) the 1- and 24-h food consumptions of the groups were again alike.

As shown in Fig. 6, baseline water ingestion (day 1) was similar in the two groups. After receiving a-CSF infusion on day 2, flavored water consumption of the groups was also comparable. Following drug infusion (day 3), flavored water ingestion of the b-SAT-infused group was significantly decreased, but this experimental group still consumed a significant amount of fluid on day 3. On day 4 water intake of the b-SAT group was still attenuated.

Figure 7 shows that in the two-bottle choice test the controls and the rats receiving b-SAT consumed similar quantities of both flavored solutions. Thus b-SAT was not aversive to these animals as measured by these procedures.

DISCUSSION

In the present study the 40- and 20-μg/rat doses differed in their ability to significantly suppress food consumption when compared to the controls. On the first day postinfusion the larger dose of b-SAT decreased food intake slightly more than the smaller dose. However, when compared to the controls on the next day, food consumption was significantly lower only in the group receiving the large dose. Following the second and third treatments, only the group receiving b-SAT at 40 μg/rat showed a significant suppression of food intake. These data also demonstrate that b-SAT given at 40 μg/rat, in repetitive treatments spaced three days apart, was effective in significantly lowering food ingestion.

Knoll (20) reported that no tolerance to sph-SAT capacity to lower food intake occurred when ICV treatments were spaced five days apart. On the other hand, Mendel et al. (26) showed tolerance to sph-SAT's ability to lower food consumption fully developed when it was ICV infused every other day. Furthermore, tolerance to sph-SAT capacity to suppress food ingestion developed on the third day after starting a continuous ICV infusion (2). Similarly, tolerance to ICV infused rat SAT also occurred when it was administered daily, but it was less so when the agent was given every fourth day (4). Some tolerance to b-SAT ability to suppress food intake, but not body weight (see below), developed following the second b-SAT treatment. Nevertheless, the present data demonstrate the high dose of the b-SAT was still effective in suppressing food ingestion after the third treatment when infusions were spaced three days apart. It should be remembered that the above SAT preparations (2,4,20,26) were in fact only semipure, whereas the b-SAT used in this study was further purified using HPLC. This may have diminished the buildup of tolerance to b-SAT's ability to suppress feedings. It will be necessary to repeat the tolerance studies using HPLC-purified human and rat SAT to resolve this issue.

Interestingly, while some tolerance to the capacity of b-SAT to decrease food intake developed following the second

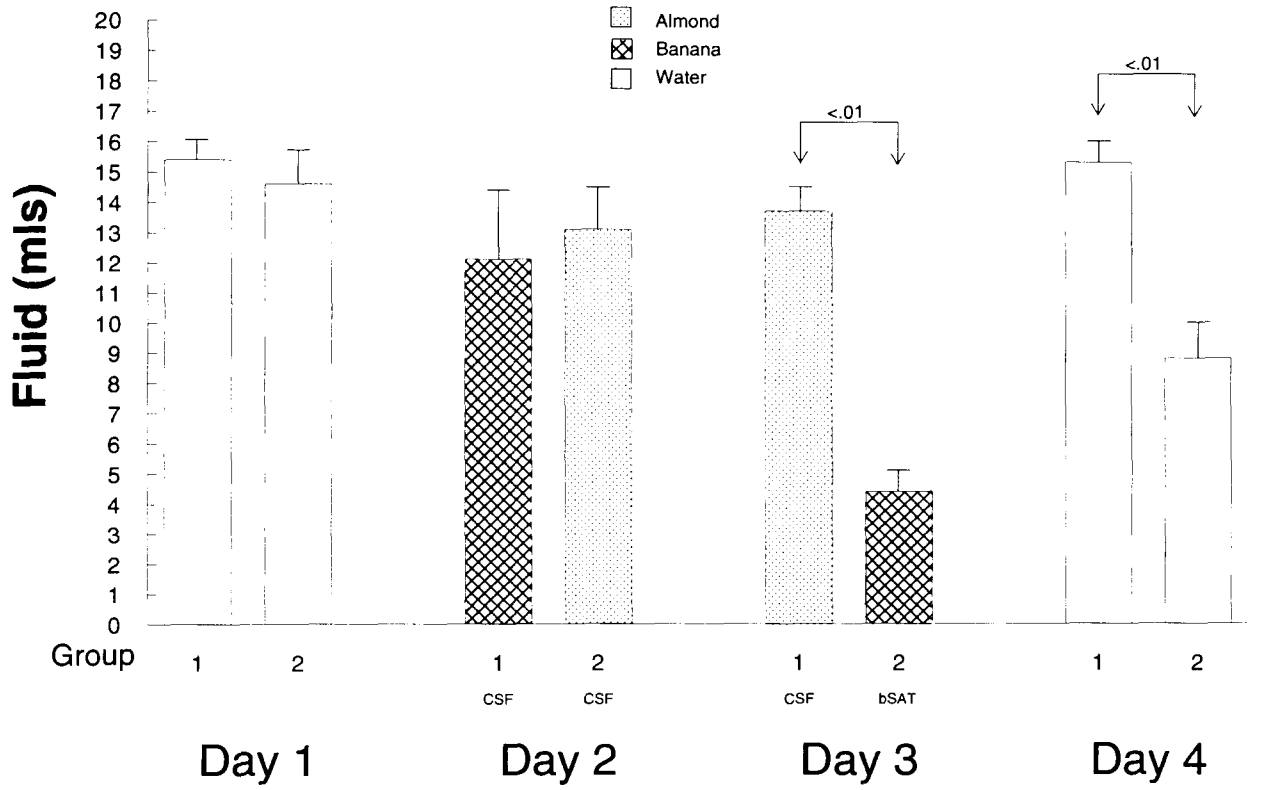

FIG. 6. One-hour fluid intake. On days 1 and 4 both groups were given water, and on days 2 and 3 the groups were given almond- or banana-flavored water as indicated. CSF = artificial cerebrospinal fluid, b-SAT = bovine satietin.

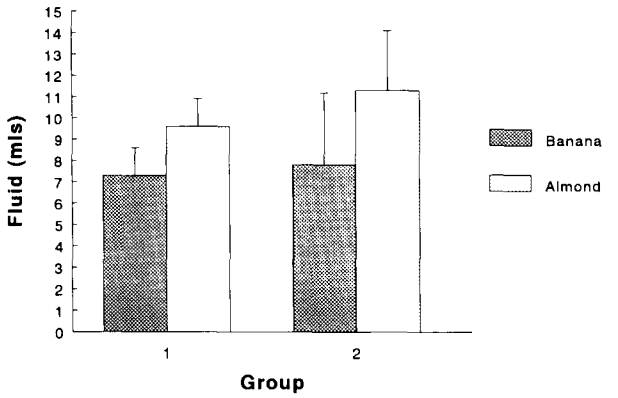

FIG. 7. Fluid intake on day 5 when all groups were given a choice between almond- and banana-flavored water.

dose, its effectiveness to suppress body weight remained intact. Previously, using a rat SAT preparation (4) it was noted that tolerance to its capacity to lower food consumption developed after the third ICV dose, yet it still caused a significant suppression in body weight. Why body weight was significantly attenuated in this case (4) with only a slight depression of food ingestion is uncertain. It probably did not depend on water intake or activity changes, since water consumption of the treated rats was normal throughout the study and activity (wheel running) was, if anything, depressed (4).

In the present study, body weight rebounded between the spaced infusions. However, the body weight difference between the experimental and control groups increased slightly after the second and third treatments when compared to the difference found following the first infusion. Previously, using rat SAT (3,4), sph-SAT (5), and HPLC-purified human SAT (7) it was found that body weight remained significantly suppressed for 3 to 14 days after ICV infusion(s) were terminated. In the present study, only a trend for a prolonged attenuation of body weight was seen after b-SAT treatment; however, the b-SAT dose employed was less than used previously with rat and human SAT. These early studies (3,4,5,7) suggested that the SAT treatment may be altering the normal metabolic compensatory response that occurs during and following food deprivation (11,16). Normally these compensatory changes in metabolism allow intact rats to limit weight loss during periods of food restriction or permit them to quickly regain lost body weight even when food intake is only allowed to return to prerestriction levels (9,12,16,24).

Studies by Knoll's group (32) and our laboratories (4,5,26) have suggested that sph-SAT, HPLC-purified h-SAT, and rat SAT may act specifically to lower food and not water consumption. These studies showed that, depending on experimental design, SAT either decreased food intake without affecting water intake (4,5,32) or apparently decreased water intake commensurate with a decrease in food consumption (5,26,32). This latter finding is well established (33). In keeping with this, the water consumption of the b-SAT-treated groups of experiment 1 was only suppressed after the first infusion. Interestingly, water consumption was slightly but significantly elevated after the second but not the third infusion. This exact pattern after ICV infusion of human and rat SAT has not previously been observed. Therefore, the reason for this after ICV infusion of b-SAT is unclear. It is probably not a compensation for their lowered water intake after the first infusion, since this overconsumption did not occur until four days later.

In a previous study (1), giving sph-SAT preparations ICV (in doses that ranged from 25 to 100 μg/rat) was found to be highly aversive to rats using the two-bottle test method. It was thought at that time (1) that this might either be due to a species difference or an impurity in the preparation. Later, it was demonstrated (3) that rat SAT (100 μg/rat) given ICV appeared not to be aversive using the two-bottle taste test method. Subsequently (6), it was observed that when the sph-SAT was further purified by HPLC the biologically active component no longer appeared to be aversive. Nor was this biologically active sample as potent as the parent sph-SAT preparation in suppressing food intake and body weight (5-7). The conclusion was drawn that part of sph-SAT's ability to suppress food intake and body weight was derived from an aversive substance that was lost during HPLC purification (6). These data also indicated that a possible species difference in the SAT molecule was not responsible for the aversive nature of the sph-SAT preparation. The present study using HPLC-purified bovine preparation further demonstrates that b-SAT can suppress food intake and body weight in the rat and this seemingly cannot be attributed to b-SAT causing a nonspecific suppression of feeding. However, a word of caution needs to be interjected as to whether the two-bottle taste test method can precisely ascertain whether a substance is aversive or nonaversive (13,15). There is no completely satisfactory method to determine satiety versus aversion, and the two are not mutually exclusive (10).

Finally, the b-SAT preparation has been recently administered peripherally (1 mg/kg) and found to be effective in suppressing both food intake and body weight (Hamilton and Cabanac, submitted). These findings are complementary to previous studies (17,19,25,26) which showed that sph-SAT was effective in attenuating food intake and body weight when administered peripherally to rats in a similar dose range. This adds support to the original hypothesis that SAT is first formed peripherally and then acts centrally to control food intake.

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