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HPLC-Purified Human Satietin Does Not Produce Conditioned Taste Aversion in Rats[1]

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BELLINGER, L. L. AND V. E. MENDEL. *HPLC-purified human satietin does not produce conditioned taste aversion in rats.* PHARMACOL BIOCHEM BEHAV **39**(1) 161–165, 1991.—Human satietin is thought to be an endogenous glycoprotein that can suppress food intake and body weight. However, it was also found to be aversive when rats infused intracerebroventricularly (ICV) with human satietin were subjected to a two-bottle taste aversion test. More recently, the human satietin previously thought homogenous was separated by HPLC into two Peaks, denoted as A and B. In the present study, male Sprague-Dawley rats were fitted with chronic third ventricle cannulas and presented with fluid for 1 h/day, while food was given ad lib. After training, the rats were ICV infused with either artificial cerebrospinal fluid, Peak A or Peak B of human satietin. Peak B significantly reduced short-term and 24-h food intake, whereas their fluid intake was nonsignificantly attenuated. Peak A had no affect on either food or fluid intake on the day it was administered. When the rats were given the two-bottle taste aversion test neither compound was found to be aversive. These data suggest that Peak B may contain satietin(s) which could be a candidate for an endogenous satiety agent.

Food and water consumption Two-bottle taste aversion test Endogenous satiety agent Chemical number 72026-83-6

SATIETIN, a putative satiety agent, is found in a variety of species including man and the rat (3, 4, 10–15). Human satietin given either intracerebroventricularly (ICV) or peripherally has been reported to cause a dose-dependent suppression of food consumption for up to 36 h after a single administration [(10, 12, 13), but see (16)]. Similarly, rat satietin infused ICV has also been shown to be effective in suppressing food intake of rats and their body weight may remain attenuated for several days following a single dose (3,4).

It is, nevertheless, imperative to determine whether any proposed satiety agent is working through physiological or nonspecific mechanisms. Accordingly, it must be clearly demonstrated that satietin is not suppressing food ingestion by making the animal ill or causing it to experience malaise.

One must also be cognizant of the purity of any compound when evaluating its behavioral effects. All previous behavioral satietin studies except one (5) have used satietin that was extracted by the methods of Knoll (10) and Nagy, Kalasz and Knoll (22,23). It has recently been demonstrated by Mendel and Paliescheskey (19) using HPLC that the human satietin (h-SAT) preparation previously thought to be homogenous (22,23) was in fact only semi-pure (sph-SAT). These investigators found sph-SAT could be separated on HPLC into two fractions that they called Peak A and Peak B (5,19). Peak A contained alpha-1-glycoprotein and albumin, whereas Peak B contained an unknown compound that could be satietin (5). Thus all early studies have used only sph-SAT or rat satietin (r-SAT), which was also prepared using earlier methods (22,23) making it likely that it also was not a pure preparation.

Nevertheless, when r-SAT was tested in a sensitive two-bottle taste aversion paradigm (8), it was found not to be aversive (3). Thus the r-SAT preparation has one of the characteristics to suggest that it could be a possible physiological regulator. On the other hand, the testing of sph-SAT to ascertain whether it reduces food ingestion of rats by nonspecific means has produced equivocal results (1, 2, 16–18). Two-bottle taste aversion testing of sph-SAT given ICV demonstrated that the preparation was highly aversive (1). It was suggested (1) that a species difference or impurities in the sph-SAT preparation may have caused the aversion. Running wheel activity is apparently reduced after ICV infusion of sph-SAT (4,16), whereas water consumption does not appear to be directly affected (5, 17, 26). Mendel and Castonguay (18) showed that peripherally injected sph-SAT affected meal patterns in a manner that suggested it was nonaversive.

The present study investigated whether ICV infusions of

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Peak A and Peak B derived from sph-SAT by HPLC separation could attenuate ingestion in rats and by using a two bottle taste aversion test determine if this occurs by nonspecific means.

GENERAL METHOD

Male Sprague-Dawley rats (Harlan Industries, Madison, WI) were individually caged in a light-cycle (L:D 12:12 h, lights out at 1130 h) and temperature-controlled (23°C) room. They were given chow (Purina No. 5001) and tap water ad lib for two weeks prior to ventricular cannulation.

At the time of surgery 39 animals (320–400 g body weight) were anesthetized with pentobarbital sodium (4.2 mg/100 g body weight). Using a Kopf stereotaxic instrument the 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 (25)] 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.

The starting material (15 mg) used in the studies was sph-SAT, which was extracted at U.C. Davis according to the method of Nagy et al. (22,23). The sph-SAT was further purified using a weak anion exchange Synchronpak AX300 HPLC (Rainin Sci. Inst. Inc., Woburn, MA) and employing 0.02 M tris acetate, pH 7.8 and a linear gradient of 0 to 60%, 1 M sodium acetate (5). A total of 12.5 mg (83%) of the starting material was recovered. The sph-SAT separated into only two peaks (denoted as A and B) which were then desalted on a Bio Gel P-2 column (5).

It was found that 10.7% of the recovered starting sph-SAT was in Peak A and the rest (89.3%) in Peak B. In previous studies, we have ICV infused up to 100 μg/rat of sph-SAT (1,16). In the present study based on the above separation percentages, Groups 2 and 3 (see below) were given doses that if added would approximate the previously used 100 μg/rat amount of sph-SAT. That is, the animals of Group 2 were ICV infused with 11 μg/rat of Peak A and Group B with 90 μg/rat of Peak B.

Experiment Method

The first week following surgery, the animals continued to receive food ad lib but were presented with water only between 1030 and 1130 h (1) in Wahmann calibrated drinking bottles equipped with ball spouts. At the end of this period, food intake, corrected for spillage, was measured for the hour that water was present, and for 24 h; water consumption was also recorded. Bottle positioning (right or left side of cage front) was randomized here and throughout the study to avoid a place preference.

Four days later, the rats were randomly divided into three equal groups and baseline 1-h water and food consumption and 24-h food intake were 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 (the solution was passed through 0.22 micron filter, Gelman Sciences, Ann Arbor, MI) artificial cerebrospinal fluid [(a-CSF)] (21) at 1000 h. At 1030 h, Group 1 [320±7.4 g body weight (b.wt.)] was presented with a novel solution (1) of water flavored with almond extract (0.5% v/v, "French's," Durkee-French Foods, Wayne, NJ). Groups 2 (317±7.0 g b.wt.) and 3 (319±5.3 g b.wt.) were offered a novel solution of banana (0.5% v/v) flavored water. Fluid and food consumption were recorded as noted above. On Day 3 at 1000 h each rat of Group 1 was again ICV infused with a-CSF (10 μl) but at 1030 h presented with banana-flavored water. At this same time, Group 2 and Group 3 were ICV infused (10 μl) with sterile Peak A (11 μg/rat) or Peak B (90 μg/rat), respectively, dissolved in a-CSF. Both groups were offered almond flavored water at 1030 h. 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 (10, 12, 13). Therefore, on Day 5 the rats were presented with both almond- and banana-flavored solutions at 1030 h (1). If a rat did not sample both bottles within 15 seconds of fluid presentation, the rat was forced to drink from the untouched bottle for 10 seconds by briefly removing the other bottle.

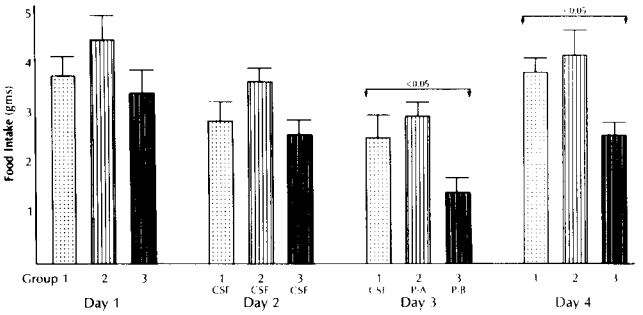

FIG. 1. One-hour food intake during the period of fluid presentation in Group 1 (n=8), Group 2 (n=11) and Group 3 (n=10). On Day 1 no treatment; on Day 2 all animals were ICV infused with artificial cerebrospinal fluid (CSF); on Day 3 the rats of Group 1 were ICV infused with CSF, whereas Group 2 and 3 were infused with 11 and 90 μg/rat of Peak A (P-A) and B (P-B), respectively; and Day 4 no treatment. Means ± SE.

The animals were then returned to ad lib water intake for several days. Correct cannula placement was demonstrated by a drinking response (3) following ICV infusion of sterile angiotensin II (Sigma, 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.

Data were analyzed using ANOVA, Duncan's Multiple Range Test and Student's paired *t*-test.

RESULTS

Two rats did not drink in response to angiotensin II infusions and five rats became sick during the course of the study. Three additional rats (one from each group) did not eat at least 1.0 g following a-CSF infusion on Day 2. All the above animals were eliminated, which left n=8 in Group 1, n=11 in Group 2 and n=10 in Group 3 for data analyses.

Food consumption (Fig. 1) during the hour of water presentation was similar in all groups during baseline measurement (Day 1): $F(2,26)=1.59$, ns, and on Day 2 after they received a-CSF, $F(2,26)=2.93$, ns. Twenty-four-hour intakes (Fig. 2) were also comparable among the groups on Day 1, $F(2,26)=1.40$, ns and Day 2, $F(2,26)=0.45$, ns.

After drug infusion on Day 3 the 1-h food consumptions differed, $F(2,26)=5.34$, $p<0.01$, among the groups (Fig. 1). The intake of Group 3, that was infused with Peak B, was suppressed ($p<0.05$) compared to the control rats (Group 1) which received a-CSF, whereas the food consumption of Group 2 infused with Peak A was similar to Group 1. The food intake of Group 3 on Day 3 was suppressed ($p<0.01$) compared to that

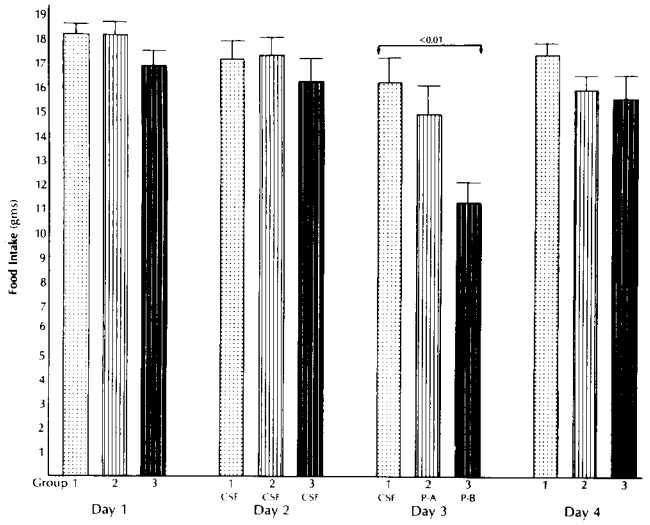

FIG. 2. Twenty-four-hour intakes, see the legend of Fig. 1 for explanation of abbreviations.

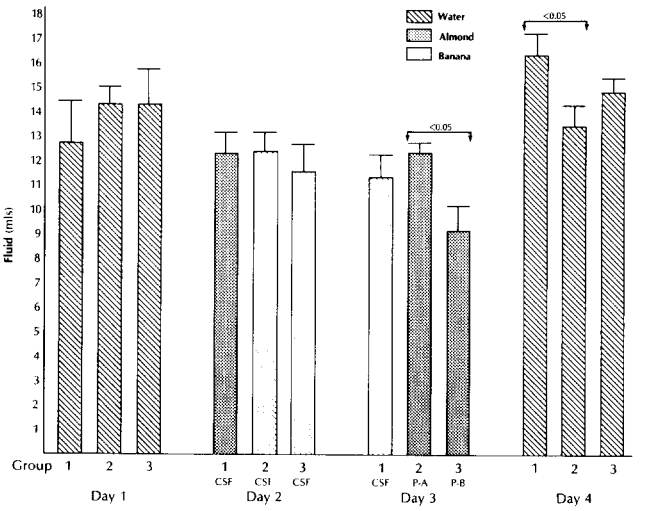

FIG. 3. One-hour fluid intake. On Days 1 and 4 all groups were given water, and on Days 2 and 3 the groups were given almond- or banana-flavored water as indicated. See the legend of Fig. 1 for explanation of abbreviations.

group's consumption on Day 2; whereas the Day 3 food ingestion of Groups 1 and 2 was similar to that eaten by these groups on Day 2. On Day 3 the 24-h food intake (Fig. 2) of the groups differed, $F(2,26)=5.73$, $p<0.01$, but only the ingestion of Group 3 (Peak B) was decreased ($p<0.01$) with respect to the control group. The 1-h food intakes (Fig. 1) of the groups on Day 4 were also different, $F(2,26)=4.83$, $p<0.02$. Further analysis showed that the food consumption of Group 3 was less ($p<0.05$) than the controls. Interestingly, 24-h intakes (Fig. 2) were comparable, $F(2,26)=1.86$, ns, among the groups. During two-bottle testing (Day 5), 1-h food consumptions (Group 1, 3.8 ± 0.9 g; Group 2, 4.2 ± 0.6 g; and Group 3, 3.4 ± 0.4 g) were again similar, $F(2,26)=0.77$, ns, among the three groups.

As shown in Fig. 3, baseline water ingestion (Day 1) was similar, $F(2,26)=0.50$, ns, in the three groups. After receiving a-CSF infusions on Day 2, flavored-water consumption of the three groups was also comparable, $F(2,26)=0.34$, ns. However, after receiving drug infusion (Day 3) flavored-water ingestion of the three groups differed, $F(2,26)=4.22$, $p<0.03$. The flavored-water intake of Group 3, which received Peak B, was significantly ($p<0.05$) reduced compared to Group 2 and attenuated with regard to Group 1; these latter groups had similar intakes. On Day 4, water consumption of all the groups was greater than their ingestion of the flavored water on the previous day, and the intake of Group 3 was again similar to the control group, but the drinking of Group 2 was now suppressed ($p<0.05$) compared to Group 1.

Figure 4 shows that in the two-bottle choice test there was no difference in flavored water preference by any group. Therefore, no ICV-infused substance produced aversion as measured by this procedure.

DISCUSSION

Loading sph-SAT on HPLC resulted in its separation into two peaks (denoted A and B) which is in accordance with previous data (5,19). The present study also confirms the finding (5) that Peak B given ICV has the ability to suppress 24-h food consumption of rats. Furthermore, the onset of Peak B's satiety effect is rapid as shown by its significantly reducing food intake during the first hour after food presentation. Food consumption remained suppressed the rest of the day and was also significantly decreased during the initial hour of food intake measurement the following day. These data are consistent with previous experiments using sph-SAT or r-SAT (1–5, 10–18), in which food consumption was suppressed rapidly and for up to 36 h following a single treatment.

Marked two-bottle taste test aversion was shown by rats receiving ICV infusions of 25–100 μg/rat of sph-SAT (1) but no aversion was noted after ICV infusion of 90 μg/rat of Peak B. These data are consistent with the hypothesis that Peak B may contain satietin(s), a possible physiological satiety agent(s). Still, this latter statement should be approached with caution since there is no totally acceptable method to distinguish whether a compound inhibits feeding by a process of satiety or aversion (7,9). Furthermore, behavioral satiety and aversion are

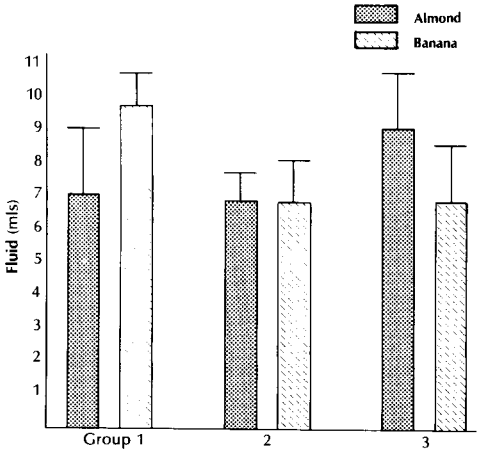

FIG. 4. This shows fluid intake on Day 5 when all groups were given a choice between almond- and banana-flavored water. See the legend of Fig. 1 for explanation of abbreviations.

not mutually exclusive (6).

Peak A, when infused ICV, did not suppress short-term or 24-h food ingestion. In a previous study (5) it was found that Peak A contained both alpha-1-glycoprotein and albumin. In that investigation (5), when a large dose (50 μg/rat) of Peak A was given ICV to rats, it reduced both their food and water consumption. In the present study, it was found that when sph-SAT was purified on HPLC, only 10.7% of the recovered material was in Peak A. As noted above, the previous maximal dose of sph-SAT we have infused ICV during two-bottle taste aversion testing was 100 μg/rat (1). Accordingly, for the present study, the 11 μg/rat dose of Peak A was utilized because it represented the amount of Peak A that would be in 100 μg of the sph-SAT preparation. This 11 μg/rat dose of Peak A and the 90 μg/rat dose of Peak B were in fact slight overdoses because recovery of the HPLC was not 100 percent. Nevertheless, this 11 μg/rat dose did not suppress either food or water consumption on the day of testing. Therefore, the 50 μg/rat dose of Peak A infused ICV in the former study (5) was an overdose that probably nonspecifically reduced food and water ingestion.

Notably, the ICV infusion of Peak A also did not produce aversion in the rats. Thus neither Peak A nor Peak B infused ICV produced aversion, whereas the parent molecule (sph-SAT), when similarly infused, is highly aversive in rather small (25 μg/rat) doses (1). The reasons for this discrepancy are not clear, but one possibility is that Peaks A and B interact somehow to be aversive. Alternatively, since not all the starting material (sph-SAT) was recovered after HPLC separation, it is conceivable that a small fraction, which caused the aversion, was lost. Thirdly, HPLC separation could possibly have changed the molecules making them nonaversive.

In addition to considering the aversive properties of Peaks A and B, further consideration needs to be given to the effectiveness of the dose utilized in suppressing food consumption. In a comparable study (1), a 100 μg/rat dose of sph-SAT infused ICV was much more effective in decreasing food ingestion than the 90 μg/rat dose of Peak B utilized in the present study. This potency difference between sph-SAT and Peak B can also be seen under ad lib water conditions (5). Thus, during HPLC separation of sph-SAT, some of its food intake suppression activity is lost. It could be that components found in Peak A have the ability to enhance the satiety activity of the material in Peak B. Alternately, satietin is proposed to be a member of the alpha-1-glycoprotein (14,15) family. These classes of proteins readily attach to other plasma protein and thus can serve as carrier molecules (20,24). Accordingly, it is conceivable that Peak B contains both a large carrier molecule and a smaller biologically active molecule. It is, therefore, hypothetically possible that during HPLC separation potency could be diminished if some of the small biologically active molecules separated from the carrier component and were lost. Thirdly, as noted above, Peak A and B do not produce taste aversion, whereas sph-SAT is highly aversive. Thus part of sph-SAT's greater ability to suppress food ingestion might be due to it making the animal somewhat ill. Therefore, as we have previously suggested (1), sph-SAT may decrease food consumption by a mixture of specific and nonspecific (aversive) mechanisms. Lastly, HPLC separation might have physically altered the molecule which reduced its capacity to promote satiety.

When compared to the control rats, water intake was attenuated nonsignificantly in Group 3 (Peak B) and not at all in Group 2 (Peak A) on the first day of drug testing (Day 3). Earlier studies by Knoll's group (26) and our laboratories (4, 5, 17) have indicated that sph-SAT and Peak B specifically lowers food and not water consumption. The attenuation of water ingestion in the current study and previous studies using sph-SAT or Peak B probably is just a reflection of the close interrelationship of food intake and water consumption (27).

In summary, the present data indicate that Peak B, which was separated from sph-SAT by HPLC, has the ability to suppress food ingestion by apparently nonaversive mechanism(s). Therefore, Peak B may contain satietin(s) the proposed endogenous satiety agent.

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