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The Effect of Intracerebroventricularly Infused Satietin on Conditioned Taste Aversion and Feeding in Rats Fasted Different Lengths[1]

LARRY L. BELLINGER* AND VERNE E. MENDEL†

**Department of Physiology, Baylor College of Dentistry, 3302 Gaston Avenue, Dallas, TX 75246*
†Department of Animal Physiology and Animal Science and Food Intake Laboratory
University of California, Davis, CA 95616

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BELLINGER, L. L. AND V. E. MENDEL. *The effect of intracerebroventricularly infused satietin on conditioned taste aversion and feeding in rats fasted different lengths.* PHARMACOL BIOCHEM BEHAV **23**(4) 559–566, 1985.—Satietin is a glycoprotein (50,000–70,000 daltons MW) found in human serum (>2 μg/ml) that is reported to be a strong anorexigenic agent when infused (10–100 μg/rat) intracerebroventricularly (ICV) into rats. The initial three experiments presented here explored whether satietin suppresses food intake by making the animals ill or causing them to experience malaise. A two-bottle taste aversion paradigm was used for this testing. In all experiments the rats were fitted with chronic third ventricle cannulas. After recovery from surgery the rats were trained for at least 6 days to drink their water in one hour a day, 1100–1200-hr (LD12:12-hr, lights out 12:00-hr). In Experiment 1 and 3 satietin (100 or 25 μg/rat) or vehicle was infused ICV 30 minutes prior to exposure to a novel neutral preference fluid flavor (banana or almond flavoring in water). Three days later the rats were given a choice of the two flavors to consume; this was repeated the next day. In both experiments satietin treated rats showed strong aversion to the flavor paired with satietin infusion, while saline infused controls showed no aversion. A similar paradigm was used during the second experiment, except satietin or vehicle infusion was paired with a highly preferred saccharin-water solution. Three days later the rats were given a choice between water and the saccharin-water solution. The satietin (50 μg/rat) treated rats exhibited a marked aversion to the saccharin-water solution. These data suggest that satietin may be an aversive substance. In Experiment 4, rats were ICV infused with satietin (25 μg/rat) or vehicle 30 minutes prior to refeeding after a 12-hr or 46-hr fast. Satietin was less effective in suppressing feeding after a 46-hr fast than after a 12-hr fast. This has been suggested to indicate that a substance has some properties of being a satiety agent. The validity of this latter assumption is discussed in the text. Taken as a whole these data suggest that caution should be used in interpreting satietin's ability to suppress food intake as being physiological in nature.

Food intake Water intake Putative satiety glycoprotein Conditioned aversion Fasting Human

IN the years 1978 and 1979 the existence of a new endogenous anorexigenic agent was reported [10,11]. This substance was extracted by filtration [10, 11, 16] from human serum, concentrated by gelchromatographic techniques and found to produce anorexia when administered peripherally or centrally. This new compound was named satietin by its discoverer [11]. Satietin has also been isolated from a number of animal species including rodent, lagomorph, ungulate, avian, and other species [14]. In humans, concentrations of satietin in excess of 2 μg/ml have been found [16]. Chemically, satietin is reported to be a 50,000–70,000 dalton MW glycoprotein, containing 14–15% amino acids and 70–75% carbohydrates, with an isoelectric point of 7.0 [16,21]. The biological activity of satietin survives digestion with proteases and boiling [16]. Polyacrylamide gel electrophoresis has shown satietin to be a homogenous product [12,21].

Interestingly, satietin suppressed feeding in animals that were refed after 96 hours of fast, a time when other known satiety agents are no longer effective in reducing food intake of animals [9, 12–15]. The onset of food suppression after intracerebroventricular (ICV) infusion of satietin was observed to occur within 30 minutes and continues for 24 to 36 hours [14,16] in a dose dependent manner (10–100 μg/rat). Of note, rats seemingly failed to overeat after the food suppression caused by satietin had abated [16].

To demonstrate that suspected physiological anorexigenic agents do not suppress feeding by making the animal sick or causing malaise is essential. The aversive affects of satietin were studied [23] in a one-bottle taste-aversion test. While that study indicated that satietin was not an aversive agent it was flawed by design problems. For example, the animals of that study had access to water for only 15 minutes per

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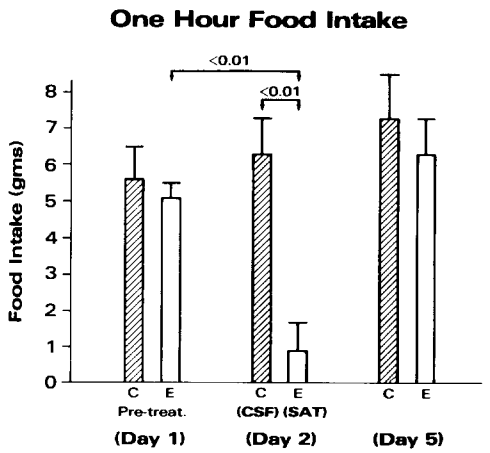

FIG. 1. Experiment 1, one hour food intake during the period of fluid presentation in control (C, n = 8) and experimental (E, n = 5) rats. Rats were ICV infused on day 2 with 100 μg/rat of satietin (SAT) or artificial cerebrospinal fluid (SCF) 30 minutes prior to fluid availability.

day and were not sufficiently well trained (two days training) to drink sufficient quantities of fluid prior to introduction of the novel flavored (saccharin) solution which was paired with satietin. After this pairing, on the next day, the rats were again given saccharin, but because of insufficient previous fluid intake due to a lack of training, the animals were most likely dehydrated and very thirsty. Since only one test bottle was provided, from which the thirsty, previously satietin treated animals could drink, the possibility exists that they may have consumed the test solution even if they preceive it to be aversive. A two-bottle, taste-aversion paradigm, using well trained animals, has been shown to be a more sensitive method for testing whether a compound is aversive [4] than is a one-bottle test.

The present experiments address these issues by testing the aversiveness of satietin or vehicle paired with solutions of either two neutral-preference food flavorings (i.e., banana and almond extract) or saccharin in a two-bottle, choice test. The latter agent was chosen because saccharin is highly palatable to rats and therefore a preferred solution. A two-bottle, taste-aversion test using neutral flavors (i.e., banana and almond), was also employed in one experiment to test a highly purified satietin extract generously provided by Dr. Knoll.

Recently it has been proposed that if an agent is truly an aversive substance then prolongation of fast from 12 to 48 hours should not reduce the ability of the drug to inhibit feeding. Conversely, if the agent is a true satiety substance then increasing lengths of fast should reduce the effectiveness of the substance to suppress feeding. We report here the ability of satietin to suppress feeding after different lengths of fast.

GENERAL METHOD

Male Sprague Dawley rats from the U.C. Davis Animal Science small animal colony or from Bantin and Kingman, Freemont, CA were utilized in the experiments. Animals were individually caged in a light cycled (L:D 12:12-hr, lights out at 12:00-hr) and temperature controlled (22°C) room. They were given chow (Purina No. 5001) and tap water ad lib for 7 to 10 days prior to ventricular cannulation.

At the time of surgery the rats were anesthetized with chloral hydrate (Sigma, 40 mg/100 g BW). Using a Kopf stereotaxic instrument, the rats' third ventricle was implanted (AP, 0.8 mm behind the bregma; depth, 3.0 mm above ear bar zero; lateral, on the midsagittal suture) with a stainless steel (22 gauge) guide cannula (Plastic Products Co., Roanoke, VA). The cannula was held in place with stainless steel screws and dental cement and then occluded with an obturator. Several days later, cannula placement was verified by a drinking response to intracerebroventricularly (ICV) infused Angiotensin II (Sigma, 100 ng/rat, 10 μl volume, infusion over 10 sec). Infusions were made with a Hamilton syringe connected to an injector cannula (Plastic Products) with tubing filled with sterile saline. If a rat did not drink in response to ICV Angiotensin II it was eliminated from the study. Also, in the initial studies at experiments end, the rats were ICV infused with 5 μl of India Ink and cannula placement verified histologically with frozen sections.

Data were analyzed by ANOVA, ANOVA for repeated measurements, Duncan's multiple range test and Student's-*t* test (paired and unpaired).

Experiment 1

Human satietin was purified according to the method of Knoll [11] and Nagy *et al.* [20]. At the time of surgery, the BW of the control group (383.1 ± 14.9 g) was similar to that of the experimental group (375.8 ± 16.1 g). Twelve days after surgery the rats were given water for only one hour a day (11:00–12:00-hr) while chow was presented ad lib. Food intake was measured, and corrected for spillage during the hour of water presentation and for a 24 hour total. Water consumption was measured by weighing the bottles which were equipped with ball spouts, prior to and, after presentation to the rats. After six days on this regimen the control group (n = 8) was offered water flavored with almond extract (0.5% v/v Schilling, McCormick and Co. Inc.) while the experimental group (n = 5) received banana (0.5% v/v) flavored water. The next day the animals were again given tap water. On the following day the rats were ICV infused (10 μl over 10 sec) with sterile, filtered a-CSF [19] or, human satietin (100 μg/rat) dissolved in a-CSF. Infusions were made 30 minutes prior to presentation of flavored fluid. Three days later (single satietin infusions have been reported to suppress food intake for up to 36 hours), the rats were given a choice between almond and banana flavored water. 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 removing the other bottle. The two-bottle test was repeated the following day (extinction trial). During this and all other two bottle tests the position of the bottles on the cages was randomized to avoid position preference.

Experiment 2

At the time of surgery the control group's (n = 8) BW was 273.8 ± 10.3 g, while BW of the experimental group (n = 8) was 265.4 ± 7.0 g. Seven days post-operation the rats were given water for only one hour a day (11:00–12:00 hr) and food offered ad lib, consumption of both were measured as in Experiment 1. Nine days after this regimen had started the rats were infused ICV (10 μl) with sterile saline or, human satietin (50 μg/rat) dissolved in saline, 30 minutes prior to fluid presentation (water with saccharin 0.125%). Three days later the animals were given a choice between

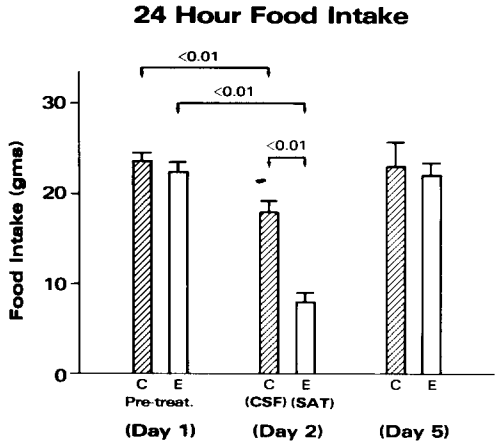

FIG. 2. Experiment 1, 24 hour food intake, see the legend of Fig. 1 for explanation of abbreviations.

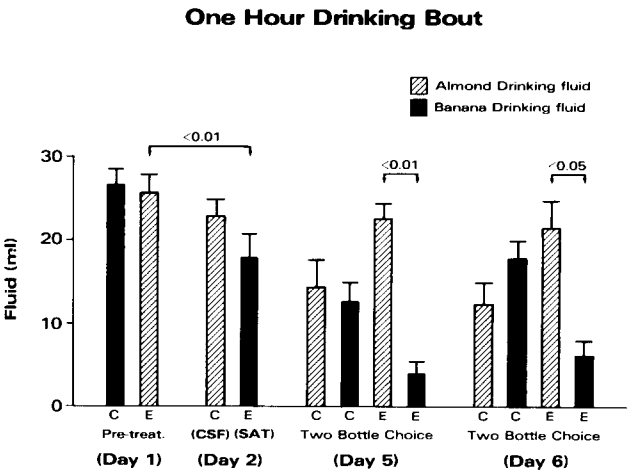

FIG. 3. Experiment 1, one hour fluid consumption, on days 5 and 6 the rats were given a choice between almond and banana flavored fluid. See the legend of Fig. 1 for explanation of abbreviations.

tap water and the saccharin flavored water using the methods described in Experiment 1. The two bottle choice was repeated the following day (extinction).

Experiment 3

The control group (n = 12) weighed 273.3 ± 2.3 g and the experimental group (n = 10) weighed 270.4 ± 3.0 g at surgery. Following a ten-day post-operative period the rats were given water for one hour daily (11:00–12:00 hr) while food was presented ad lib, consumption of both was measured as in Experiment 1. After 10 days on this regimen the rats were ICV (10 μl) with either 25 μg/rat of highly purified [21] human satietin (a gift from Dr. J. Knoll), which was dissolved in a-CSF, or a-CSF. Infusions were made 30 minutes prior to novel flavor presentation (controls, 0.5% v/v banana extract flavored water or experimental rats, 0.5% v/v almond flavored water). Three days later rats were given a choice between almond and banana flavored water as described in Experiment 1. The following day the procedure was repeated (extinction).

Experiment 4

The control group weighed 283.9 ± 4.6 g and the experimental group 278.6 ± 4.3 g at the time of surgery. Eight days post-surgery one half of the rats (controls, n = 9 and experimental, n = 9) were started on a 46 hour fast beginning at 1400 hr. Water was available ad lib. The remaining rats (both groups n = 9) were subjected to a 12 hour fast beginning at 24:00-hr. Initiation of the two fast periods was staggered, such that refeeding occurred at the same time for all four groups. Rats from each fast period were infused ICV (10 μl) with sterile saline or 25 μg/rat of highly purified [21] human satietin 30 minutes prior to refeeding. Food intake (corrected for spillage) was recorded for one hour (12:00–13:00-hr) and for a 24 hour total. Daily food consumption and BW were then recorded for an additional three days.

RESULTS

All rats that drank in response to Angiotensin II infusion were found to have dye in their ventricles. In several cases, rats that did not drink after Angiotensin II were subsequently infused with satietin. In no instance did satietin suppress the food intake of these rats; therefore, satietin apparently must enter the ventricles to effectively influence feeding. Finally, the majority of animals testing positive for Angiotensin II drinking were also observed to leak CSF during the infusion procedures.

Experiment 1

Both groups of rats had similar food intake prior to infusion of satietin or a-CSF (Figs. 1 and 2). Satietin suppressed ($p<0.01$) both the 1-hr and 24-hr food consumption of the experimental group when compared to their own pretreatment values or the controls receiving a-CSF. The controls receiving a-CSF showed no change in 1-hr food consumption relative to the pretreatment period; their 24-hr food intake was, however, slightly lowered after a-CSF. On the day of the two-bottle testing, food consumption of the two groups was again similar.

Prior to infusion of satietin, water intake of the two groups was similar (Control, 27.8 ± 2.1 vs. Experimental, 31.0 ± 2.1, n.s.). Initial exposure to novel flavored water (Fig. 3, day 1) did not significantly affect fluid consumption in either group when compared to their previous water intake (Fig. 3, day 1). Satietin infusion significantly reduced ($p<0.01$) consumption of flavored fluid (Fig. 3) when compared to the initial exposure to a novel flavor but infusion of a-CSF did not (Fig. 3, day 2). When contrasted with the rats receiving a-CSF, satietin infused rats (Fig. 3, day 2) exhibited a small but nonsignificant reduction in fluid intake.

Two-bottle testing (Fig. 3, day 5 and 6) revealed that the controls had an equal preference for the almond and banana flavored drinking fluid. The rats in which banana flavored water was paired was satietin infusion showed striking ($p<0.01$) aversion to that flavor (Fig. 3, day 5). Their preference ($p<0.05$) for the almond flavored fluid was still evident on retesting one day later (Fig. 3, day 6).

Experiment 2

During pretreatment, 1-hr and 24-hr food intakes of both control and experimental groups were similar (Figs. 4 and

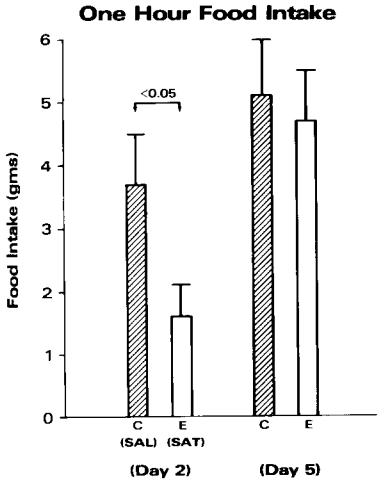

FIG. 4. Experiment 2, one hour food intake during the period of fluid presentation in control (C, n = 8) and experimental (E, n = 8) rats. Rats were ICV infused on day 2 with 50 μg/rat of satietin (SAT) or saline (SAL) 30 minutes prior to fluid availability.

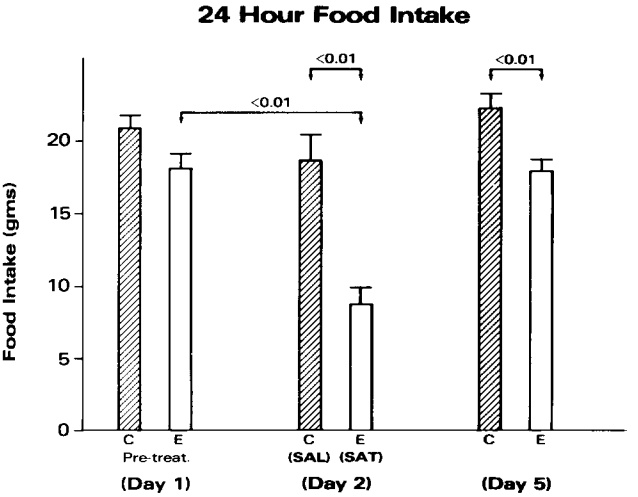

FIG. 5. Experiment 2, 24 hour food intake, see the legend of Fig. 4 for explanation of abbreviations.

5). Ventricular infusion of satietin, however, reduced both 1-hr and 24-hr food consumption relative to the controls receiving ICV infusions of saline (Figs. 4 and 5). While the 24-hr food intake of the group receiving satietin was less ($p<0.02$) than their consumption during the pretreatment period the intake of the controls receiving saline was not significantly reduced. On the day of two-bottle testing the 24-hr food consumption of the satietin-infused group had recovered to pretreatment levels, but still remained somewhat below the controls.

Prior to ICV infusions, water consumption of the two groups was similar (Fig. 6, day 1). Satietin infusion suppressed the group's consumption of saccarin flavored water in comparison to their own pretreatment ingestion of water and, that of the control group (ICV infused with saline); saline infusion did not alter the control group's drinking (Fig. 6, day 2).

During two-bottle testing (Fig. 6, day 5) the controls preferred saccharin-water solution over water; therefore ICV infused saline was not aversive to these rats. Preference for the saccharin-water solution by the control animals was also evident on the second day of testing (Fig. 6, day 6). The rats in which ICV infused satietin was paired with saccharin-water solution exhibited a clear aversion ($p<0.001$) to this normally preferred sweet solution (Fig. 6, day 5); the aversion ($p<0.01$) remained unchanged during the second day of testing (Fig. 6, day 6).

Experiment 3

One hour or 24-hr (Figs. 7 and 8) food consumption did not differ significantly in the two groups preceeding the ICV infusion of satietin or a-CSF. As seen previously, ICV satietin infusion reduced ($p<0.001$) 1-hr and 24-hr food intake of the rats when compared to their pretreatment values or to the control rats receiving a-CSF. The ICV infusion of a-CSF caused no change in consumption of food when compared to pretreatment food intake. On the day of two-bottle testing the two groups did not vary significantly in their 1-hr or 24-hr food intakes.

Prior to treatment, the water consumption (Fig. 9, day 1) of the two groups was similar. Satietin infusion reduced ($p<0.01$) fluid intake when compared to their pretreatment consumption of water or to that of the controls receiving a-CSF (Fig. 9). The fluid intake of the controls after receiving a-CSF was similar to pretreatment levels (Fig. 9).

Two-bottle testing (Fig. 9, day 5) revealed that the control group initially preferred the banana drinking fluid (i.e., the fluid paired with a-CSF infusion), while on the next day of testing (Fig. 9, day 6) they showed equal preference for the two flavors. Thus, no aversion was shown to the novel fluid paired with a-CSF infusion. In contrast (Fig. 9, day 5), the satietin treated rats exhibited marked aversion ($p<0.001$) to the almond flavored drinking fluid (i.e., the fluid paired with satietin infusion), a strong aversion ($p<0.001$) remained on the second day of testing (Fig. 9, day 6).

Experiment 4

During the first hour of refeeding (Fig. 10) the food intake of the four groups varied significantly, $F(3,35) = 5.50$, $p<0.01$. Satietin infusion, following 12 hours of fast, caused a nonsignificant reduction in grams of food consumed during the first hour of refeeding when these satietin infused rats were compared to their saline-infused controls. A similar trend was noted after 46 hours of fast. However, when food consumed during the first hour was corrected for slight differences (Fig. 13) in body weight (g eaten/100 g BW) a difference was found, $F(3,32) = 9.80$, $p<0.001$, with both satietin treated groups eating less ($p<0.05$) than their respective controls (12-hr fast: 0.33 ± 0.12 vs. 0.77 ± 0.17; 46-hr fast: 1.12 ± 0.23 vs. 1.70 ± 0.20). Prolongation of fast, in the saline infused controls, increased the amount eaten during the initial hour of refeeding ($p<0.05$). Interestingly, satietin was less effective ($p<0.01$) in suppressing food consumption during the first hour of refeeding when the fast was lengthened from 12 to 46 hours (Fig. 10).

There was also a significant group effect, $F(3,152) = 11.97$, $p<0.01$, and, group × days effect,

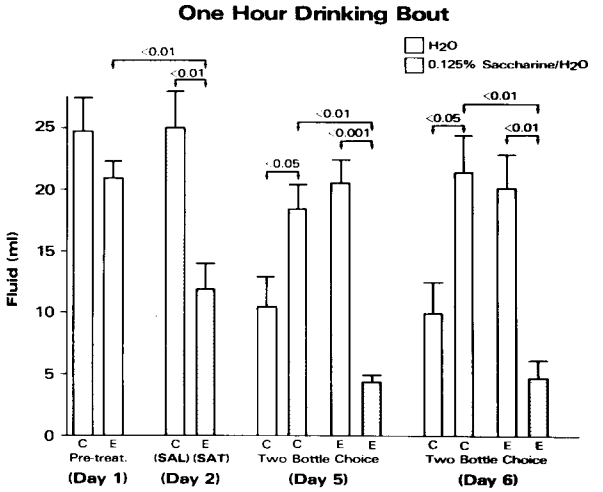

FIG. 6. Experiment 2, one hour fluid consumption, on days 5 and 6 the rats were given a choice between a 0.125% saccharin/water solution and tap water. See the legend of Fig. 4 for explanation of abbreviations.

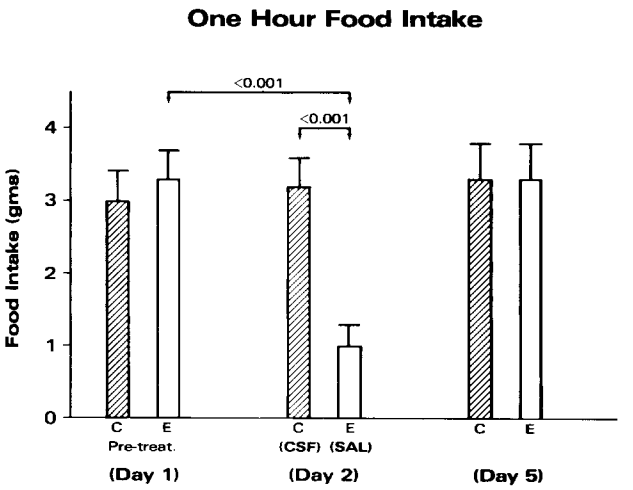

FIG. 7. Experiment 3, one hour food intake during the period of fluid presentation in control (C, n = 12) and experimental (E, n = 10) rats. Rats were ICV infused on day 2 with 25 μg/rat of highly purified satietin (SAT) or artificial cerebrospinal fluid (CSF) 30 minutes prior to fluid presentation.

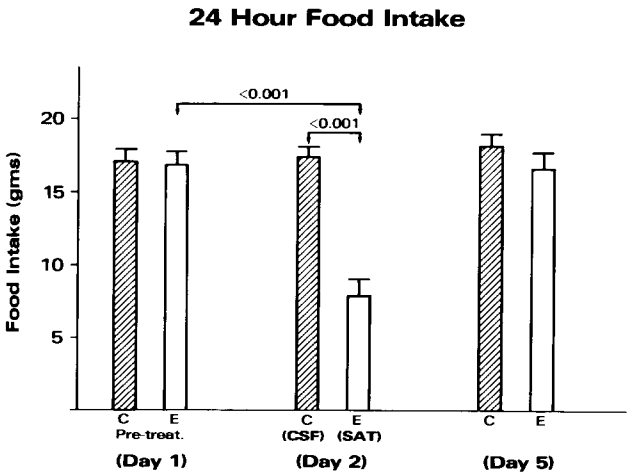

FIG. 8. Experiment 3, 24 hour food intake, see the legend of Fig. 7 for explanation of abbreviations.

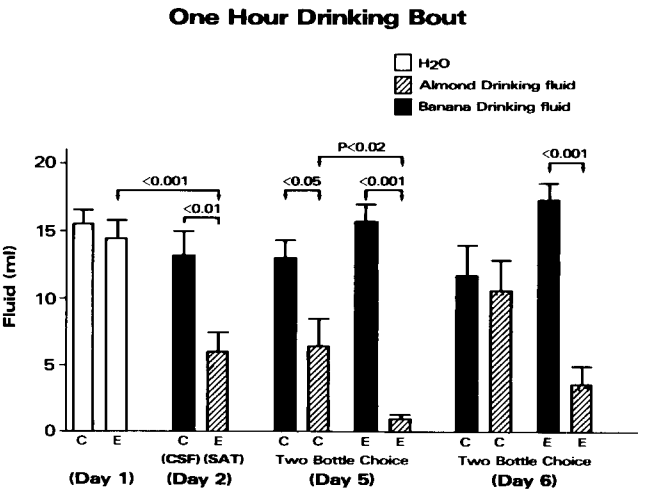

FIG. 9. Experiment 3, one hour fluid consumption, on days 5 and 6 the rats were given a choice between almond and banana flavored fluid. See the legend of Fig. 7 for explanation of abbreviations.

$F(12,152) = 2.36$, $p<0.03$, on 24-hr food intake (Fig. 11). Both groups receiving satietin ate less ($p<0.05$) than their saline infused counterparts over the first day of refeeding. On the second day of refeeding only the short-term fasted, satietin-treated group, when compared to its control group, had a reduced ($p<0.05$) food consumption, thereafter, intakes of all groups were similar.

A group effect, $F(3,35) = 14.69$, $p<0.01$, with regard to body weight changes (Fig. 12) was also noted on the first day of refeeding. Both saline control groups gained weight on refeeding, with the 46-hr fasted group gaining ($p<0.05$) the most on day one. As expected, the 12-hr fasted, satietin-treated group had a weight loss ($p<0.01$), but interestingly the 46-hr fasted, satietin-treated group had a weight gain. The latter group's weight gain, on the first day of refeeding, was ($p<0.01$) different from that of the short-term-fasted, satietin-treated group, but not reliably dissimilar from its saline control (Fig. 12).

There was both a group, $F(3,152) = 58.2$, $p<0.01$, and days effect, $F(4,152) = 17.7$, $p<0.01$, on daily body weight (Fig. 13). At the time of refeeding, the weight of both 46-hr fasted groups was less ($p<0.01$) than their short-term-fasted counterparts. Once refeeding began, all groups, except the 12-hr fasted, satietin-treated group, immediately gained weight. After four days of refeeding the two 46-hr fasted groups still weighed less ($p<0.05$) than their short-term-fasted counterparts. While differences occurred in weight gains on the first day of refeeding there was no time when the satietin treated rats weighed significantly less than their saline infused controls.

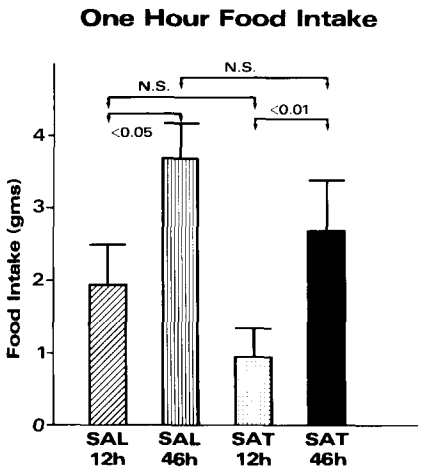

FIG. 10. Experiment 4, food intake during the first hour of refeeding in 12 hour (hr) or 46 hr fasted rats ICV infused with saline (SAL) or 25 μg/rat of highly purified satietin (SAT) 30 minutes prior to refeeding. All groups n = 9, N.S. = not significant.

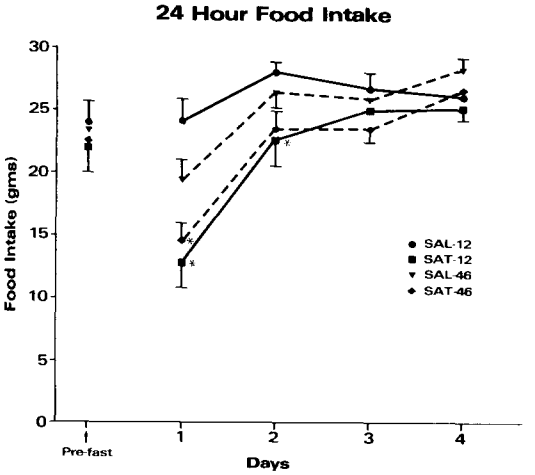

FIG. 11. Experiment 4, 24 hour food intake prior to fast and during the first four days of refeeding. See the legend of Fig. 10 for explanation of abbreviations.

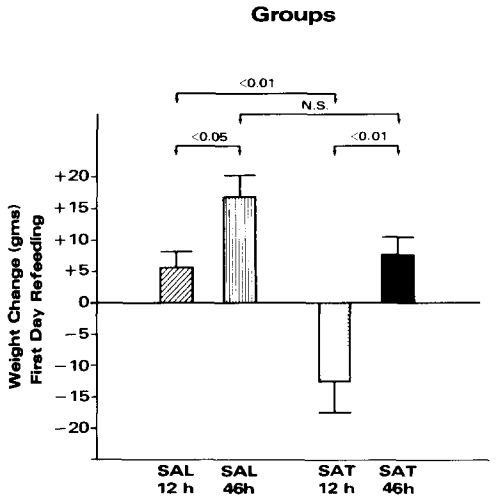

FIG. 12. Experiment 4, body weight change during the first day of refeeding after ICV infusion of satietin or saline. See Fig. 10 for explanation of abbreviations.

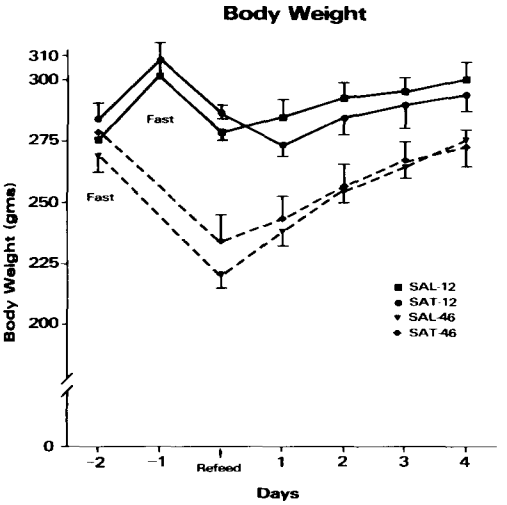

FIG. 13. Experiment 4, body weight prior to start of either a 12 and 46 hr fast, at time of ICV infusion of saline or satietin prior to refeeding and for four days after refeeding. See Fig. 10 for explanation of abbreviations.

GENERAL DISCUSSION

Satietin has been shown to be a potent, long-lasting anorectic agent in rats when infused ICV [16]. While most agents known to reduce food intake are no longer effective after the test animals are subjected to a 96-hr fast, satietin infused ICV does suppress feeding. Thus, a possible role for satietin in the regulation of feeding behavior has been proposed [11,16]. With regard to mechanism of action, satietin is hypothesized [16] to be a rate-limiting, plasma borne, satiety agent that functions as a negative feedback link from the gut to the brain [11, 13, 16]. Satietin is hypothesized to reside in the blood as an inactive molecule that is converted into an active form by "signals" arising from the gut as food enters it [16]. The activated satietin is then suggested to bind specific brain receptors that cause an inhibition of feeding [16]. Emptying of the gut is proposed to cause a gradual decrease of active satietin, with less brain receptor binding in turn and, ultimately, reduced satiety [16].

The experiments of the present study demonstrate that satietin infused ICV over a dose range of 25–100 μg/rat (5×10^{-10} to 2×10^{-9} moles) markedly suppresses food consumption in non-deprived rats. Food consumption was reduced after satietin infusion both during the first hour of feeding and during the next 24 hours. The magnitude of food intake suppression observed in our experiments after ICV infusion of satietin was similar to that reported earlier [16].

In addition to suppressing feeding behavior, all doses

of satietin significantly reduced fluid intake. It should be noted that the rats only had access to fluid for one hour a day, thus, were very thirsty at the time of testing; satietin, therefore, did not specifically suppress only feeding behavior. Thus, it is unclear as to whether satietin's action is physiological or causes a general disruption of behavior.

For satietin to be a physiological controller of feeding behavior it must be demonstrated that it does not produce its effect on food intake through non-specific mechanisms (e.g., sickness, malaise). As noted above, the ability of satietin to produce conditioned aversion was first investigated using a one-bottle taste-aversion paradigm [23]. However, this procedure has been criticized on methodological grounds of being insensitive [4]. These authors [4] suggest that a two-bottle taste-aversion paradigm is a more sensitive indicator of a compound's ability to cause sickness or malaise.

In our first experiment we presented well trained rats with two novel and neutral flavors (i.e., the rats do not prefer one flavor over the other), one of which was paired with ICV infusions of satietin and the other with an infusion of a-CSF. The data clearly showed that satietin produced a strong aversion to the flavored fluid that was paired with its infusion. These findings indicate that rats receiving satietin perceived it as being aversive, thus, their reduction in food and water consumption may be attributed to satietin induced nausea and/or malaise. The infusion procedure or vehicle were in themselves not responsible for the observed findings because control rats receiving a-CSF showed no conditioned taste aversion.

Because the two-bottle, taste-aversion test is highly sensitive [4] it may even show a substance to be aversive whose ability to produce nausea and/or malaise is slight. Therefore, we chose to use this method again, but paired satietin infusion to novel exposure of a saccharin solution that is highly preferred by rats [4]; we also reduced the dose of satietin infused by one half. Again the data demonstrated that satietin was highly aversive.

The third experiment was a repeat of our first experiment and was carried out with highly purified satietin kindly supplied by Dr. J. Knoll. Even though we only used 50 percent of the dose used in Experiment 2, the data clearly indicates that satietin is aversive.

The above findings, taken as a whole, suggest that satietin may reduce food and water consumption in rats because the compound produced sickness and/or malaise in the animals.

There is, however, no totally accepted method (including the two-bottle, taste-aversion test) to distinguish whether a compound inhibits feeding by a process of satiety or aversion ([3,5] see [2] for discussion). This problem has resulted in controversy in the literature over the physiological nature of recently investigated putative satiety peptides such as cholecystokinin and bombesin [1, 4, 6, 7, 17, 24, 25]. Some experimental procedures indicate the above two compounds are satiety agents [7, 17, 24], while other methodologies suggest the two peptides affect feeding because they are aversive [4, 6, 25]. Interpretation of data to determine whether an agent suppresses food intake by physiological or non-specific mechanisms is further complicated by the finding that behaviorally satiety and aversion are not mutually exclusive [2].

In an attempt to overcome some of these difficulties a hypothesis was set forth [2] stating that if a substance is a true satiety agent, its effectiveness in suppressing feeding should diminish as the test animal is deprived of food for longer time periods (i.e., its hunger increased). Agents that are aversive and, thus behaviorally disruptive, would not lose their ability to suppress feeding [2]. Using this model it has been suggested that cholecystokinin was a satiety agent and lithium chloride an aversive substance [2].

Our data indicate that satietin is significantly less effective in supressing feeding during the first hour of refeeding when the length of fast is increased from 12 to 46 hours. On the other hand, 24-hr food consumption by both satietin treated groups was statistically similar but less than their controls. It should be noted that when 24-hr intake was computed on the basis of body weight the 46-hr deprived rats ate significantly more than the 12-hr fasted, satietin-treated group. Thus, while satietin was effective in suppressing feeding in both fasted groups it appeared to be less so in the 46-hr deprived group. Weight gains on the first day of refeeding supports this conclusion with the 46-hr fasted, satietin-treated group gaining significantly more weight than their 12-hr fasted counterparts. According to the hypothesis of Billington *et al.* [2] these data indicate that satietin, may in fact, be a satiety agent. In support of such an interpretation satietin has been shown (with some reservations as to methods employed) not to affect metabolic rate, body temperature, blood pressure, sexual performance and some, but not all, behavioral tests [8, 26, 27].

The validity of the Billington *et al.* [2] model, however, needs to be considered. When comparing long-term to short-term fasted animals one would naturally expect the long-term deprived subjects to have the greatest hunger. This was reflected in our data during the first hour of refeeding with the saline infused 46-hr fasted group consuming significantly more food than their 12-hr fasted counterparts. If the agent to be tested in the above model was not overwhelmingly aversive, but nevertheless aversive, then one would naturally expect the group fasted the longest to eat more food. In other words, at some point the strength of the aversive agent will most likely be nullified by the animals' hunger drive.

In addition we have some grave reservations on the validity of the Billington *et al.* hypothesis because of the conditioned taste aversion caused by satietin and secondly by our finding that water intake was also suppressed after satietin infusion, which questions the specificity of satietin. Thirdly, the possibility that satietin may act nonspecifically is also heightened by our recent findings [18] that running wheel activity is greatly reduced after satietin infusion. Satietin in that experiment also reduced 24-hr water consumption in ad lib fed rats.

While chemical analyses indicate that purified satietin is a homogenous substance [12,21] there is the possibility that impurities, which produce aversion, are in the preparation. Only the availability and testing of synthesized satietin will ultimately resolve this question.

Finally, even if satietin is eventually shown not to be a normal physiological satiety agent it is a naturally occurring compound that is found in high concentration in plasma. Because satietin does have a profound influence on feeding it is important to determine whether it could have a role in any of the pathological anorexias (e.g., cancer).

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