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Satietin: Route of Injection, Dose Response, Effect on Food and Water Intake and on Running-Wheel Activity in the Rat[1]

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MENDEL, V E , L L BELLINGER, F E WILLIAMS AND R A IREDALE *Satietin Route of injection, dose response, effect on food and water intake and on running-wheel activity in the rat* PHARMACOL BIOCHEM BEHAV **24**(2) 247–251, 1986 —Semipurified satietin significantly ($p<0$ 05) reduced food intake when injected subcutaneously at 10, 15, 20 mg/kg into 48 hr fasted rats with no indication of a dose response When infused intracerebroventricularly (ICV) at 12 5, 25 and 50 μg/rat (10 μl vol) into ad lib fed rats at the end of the light period there was no effect on food intake for the first hour but 24 hr food intake was ($p<0$ 001) reduced at all doses The ICV dose response curve was shallow, with similar suppression at both 12 5 and 25 μg doses, but a ($p<0$ 05) greater suppression with the 50 μg dose An ICV threshold between 6 25 μg and 12 5 μg appears to exist since no suppression occurred after a dose of 6 25 μg Four consecutive daily ICV infusions of satietin (25 μg/rat) in two rats progressively suppressed food intake to low levels, suggesting a cumulative effect Following termination of satietin treatment daily food intake slowly returned towards normal without evidence of rebound feeding In other ad lib fed rats, four ICV infusions of semipurified satietin, on days alternated with no infusion, reduced food intake ($p<0$ 001), water intake ($p<0$ 003) and running wheel activity ($p<0$ 001) on the first day of injection but not on subsequent injection days Suppression of activity approached significance on the second injection day Highly purified satietin infused ICV produced similar responses These findings may indicate a general disruption of behavior by satietin, thus, it may not play a physiological role in feeding behavior because of its apparent non-specificity

Satietin Anorexia Food intake Water intake Activity Dose response

THE discovery of an endogenous anorexigenic substance has recently been reported by Knoll [4] He has reported that its molecular weight is approximately 50,000 daltons, that it is a glycoprotein which contains 14–15% protein, 70–75% carbohydrate and 5–10% water [5] Very interestingly, Knoll has reported finding satietin in the plasma of several species of animals including, human, horse, all species of rodents, guinea pig, rabbit, cattle, cat, dog and goose [5] He and his coworkers have shown that satietin is a powerful anorectic agent when injected subcutaneously, intravenously, orally and intracerebroventricularly (ICV) in rats [5] They have also provided evidence that satietin infused ICV causes a linear dose-response reduction of food consumption in 96 hr fasted rats with no apparent effects on water consumption or activity [5] These observations are potentially of great interest to clinicians who attempt food intake reduction in the obese and to investigators attempting to increase food intake in domestic animals

It is necessary that others verify the results of Knoll and coworkers if this potentially important molecule is to be of value to society The objectives of this paper are to re-evaluate (1) the dose-response characteristics of partially purified satietin on food intake in both fasted and ad lib fed rats, (2) investigate two routes of injection of satietin, and (3) investigate the effect of satietin on activity and water intake in rats

In an effort to increase precision we have chosen to use different methods to measure food intake than Knoll and coworkers who weighed the animals prior to and after feeding We have also used chronic cannulas rather than the acute method of Noble *et al* [9] Chronic cannulas increase accuracy of placement of satietin and reduce stress to the animals Moreover, use of an anesthetic before feeding can be avoided Running wheels have been used to measure activity rather than to use an open-field method [6]

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METHOD

Male, Sprague-Dawley rats from the Animal Science Department small animal colony were utilized in the experiments Animals were caged individually in a temperature controlled (22°C) room The light cycle was L D 12 12, lights out at 12 00 hr The rats were offered laboratory chow (Purina No 5001) and tap water ad lib throughout the experiments unless otherwise noted

Data were analyzed by ANOVA, ANOVA for repeated measures, Duncans multiple range test and Student's *t* test (paired and unpaired)

Preparation and Source of Satietin

Human satietin was prepared from outdated human plasma obtained from the Sacramento Blood Bank The plasma was prepared according to the method of Knoll [4] The lyophilized material was stored in sealed containers at room temperature since the molecule appears to be quite heat insensitive Nagy *et al* [8] have recently reported further improvements in satietin isolation and purification procedures which consist of electrophoresis on Whatman paper followed by passage of the material through a concanavaline A-Sepharose column The human satietin (h-SAT) used in Experiments 1 and 2 was not prepared according to this newer method but some of the h-SAT used in Experiment 3 was so prepared [8] and generously supplied to us by Dr J Knoll Most biological data reported on satietin's effects have been obtained using the older method of preparation [4]

Subcutaneous Injections

In Experiment 1, rats (n=13, 303 0±8 1 g BW) were injected subcutaneously with 1 ml of saline or h-SAT (3, 10, 15, 20 mg/kg) 1 hr prior to refeeding after a 48-hr fast, 24-hr food consumption was then recorded All rats were trained to subcutaneous injections on 7 consecutive days prior to testing Food intake was measured and corrected for spillage in this and all other experiments reported here (spillage collected on a paper towel placed beneath the cage was dried and weighed)

Dose Response Study

In Experiment 2, male Sprague-Dawley rats were used (412 5±5 0 g BW) Following chloral hydrate (Sigma, 40 mg/100 g body weight) anesthesia a stainless steel guide cannula (22 gauge, Plastic Products Co , Roanoke, VA) was placed in the third ventricle using a Kopf stereotaxic instrument (coordinates AP, 0 8 mm behind the bregma, depth, 3 0 mm above ear bar zero, lateral, on the midsaggital suture) The cannula was secured to the skull with three stainless steel screws and dental cement and then occluded with an obturator Cannula placement was verified several days later by a drinking response to intracerebroventricularly (ICV) infused angiotensin II (Sigma, 100 ng/rat, 10 μl vol, infusion over 10 sec) Infusions were made using a Hamilton syringe connected to an injector cannula (Plastic Products), the tubing was filled with sterile saline If the rat did not drink in response to ICV angiotensin II it was removed from the study, rats were then accustomed to the infusion procedure for several days

After this period of adjustment, food was removed at 11 30 hr and the rats were infused (10 μl) with saline or h-SAT at 12 5, 25 0 or 50 0 μg/rat (dissolved in saline, 10 μl over 10 sec) Food was returned 1-hr later and intakes recorded at 1-hr and 24-hr Food intake was measured as in Experiment 1 When food was measured during the dark period a flashlight with a red lens was used to reduce illumination as much as possible Care was also taken to be as quiet as possible when removing or introducing food so as to distrub the animals as little as possible The procedures were repeated 9 days later except the rats receiving saline and 50 μg of h-SAT had their doses reversed as did those animals receiving 12 5 and 25 0 μg of h-SAT This gave a group size of saline, n=13, 12 5 μg, n=13, 25 μg, n=11 and 50 0 μg, n=8 After observing the results of the 12 5 μg dose 6 of the rats previously used in Experiment 2 were injected with 6 25 μg/rat These food intake data were compared with those obtained in the same 6 rats for three days immediately before injecting satietin

Later, two rats were ICV infused (25 μg/rat) on four consecutive days to obtain preliminary data on continuous satietin use

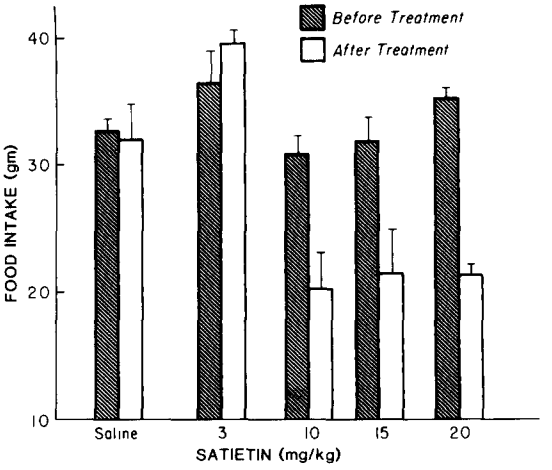

FIG 1 Experiment 1, 24-hour food intake of 48-hour fasted rats following subcutaneous injection of semi-purified human satietin or sterile saline in 1 ml volume

Effect of Satietin on Running Wheel Activity

In Experiment 3, male Sprague-Dawley rats weighing 285 1±10 4 g (n=12) were cannulated as described in Experiment 2 and then following a 7 day recovery period placed in Wahmann LC-34 activity cages Daily activity (revolutions), food consumption and water intake (using Wahmann calibrated bottles with ball stoppers) were measured During each of the following 6 days the rats were handled and given a mock ICV infusion Following this period the rats were started on a regimen of no infusions (non-infused control day, NIC) alternated with ICV infusions of sterile saline (10 μl) at 11 00 hr until 3 saline and 3 NIC days were recorded Next, NIC days were alternated with infusions of h-SAT (25 μg/rat dissolved in saline) until 4 h-SAT infusions were made To compare the effects of semi-purified satietin with those of highly purified material an additional experiment was run six days later using seven of the rats from Experiment 3 They received a NIC day, then a saline day, and finally an infusion day where they received 25 μg/rat of highly purified h-SAT [8]

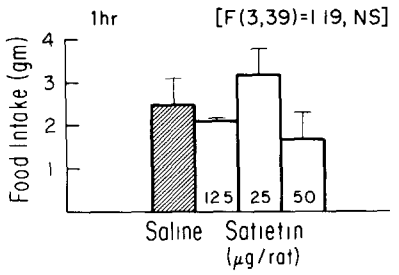

FIG 2 Experiment 2, one hour food intake of ad lib fed rats following infusion of three different doses of satietin or of sterile saline into the third cerebral ventricle (saline, n=13, satietin, 6 25, n=6, 12 5 μg, n=13, 25 μg, n=11, 50 μg, n=8) Satietin was infused ICV one hour before food was presented to the rats

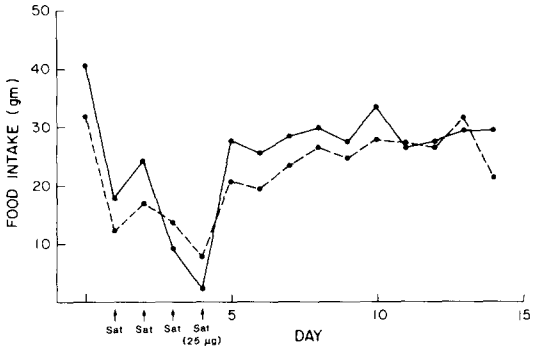

FIG 4 Experiment 2, food intake following ICV infusions of 25 μg of human satietin for four consecutive days in ad lib fed rats

RESULTS

Experiment 1

Semi-purified h-SAT when injected subcutaneously into rats fasted for 48 hr reduced ($p<0$ 05) food intake from 32 7 g/day to 21 0 g/day at doses of 10, 15 and 20 mg/kg but had no effect when injected at 3 mg/kg (Fig 1) There was no indication of a dose response in these data at doses above 10 mg/kg since suppression of food intake was approximately the same at 10, 15 and 20 mg/kg

Experiment 2

Injection of h-SAT into the third ventricle of non-fasted rats at 12 5, 25 and 50 μg/rat (10 μl vol) had no effect on food intake, F(3,39)=1 19, n s , during the first hour of feeding (Fig 2) However, twenty-four-hr food intake was significantly, F(3,39)=16 87, $p<0$ 001, reduced at all dose levels (Fig 3) While the 50 μg dose reduced food consumption more ($p<0$ 05) than the other two h-SAT doses, or saline, there was no difference in the magnitude of suppression seen after 12 5 or 25 μg/kg The dose response curve is therefore very shallow The animals injected with 6 25 μg/kg of h-SAT showed no suppression of feeding ($p>0$ 05) Thus, the food suppression threshold dose of h-SAT appears to be between 6 25 and 12 5 μg/kg

Consecutive daily ICV infusions of h-SAT (25 μg/kg) in two rats produced a continuous, and progressive suppression of food intake, such that by the fourth day of infusion the rats' food ingestion was reduced from over 30 g a day to

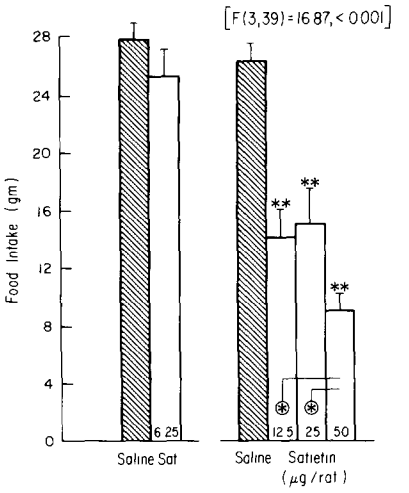

FIG 3 Experiment 2, 24-hour food intake of ad lib fed rats following infusion of three different doses of satietin or of sterile saline into the third cerebral ventricle See Fig 2 for numbers per group

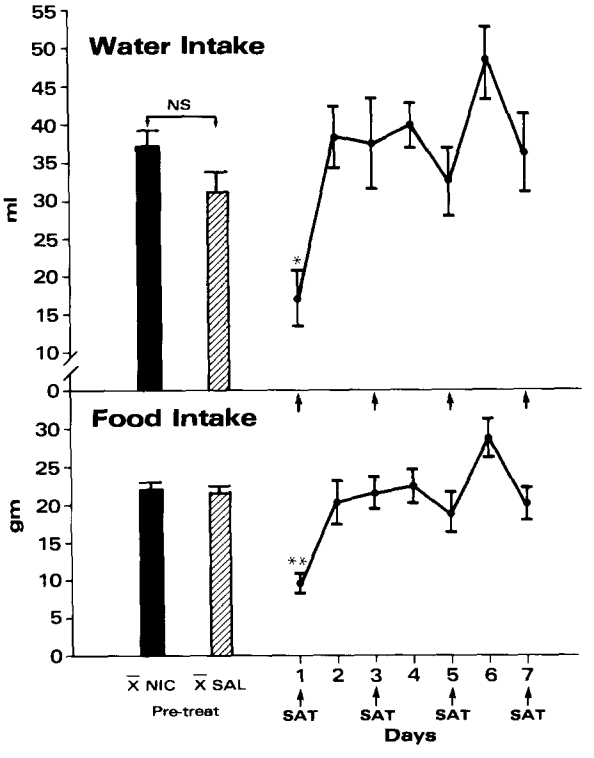

FIG 5 Experiment 3, water and food intake of rats ICV injected with 25 μg human satietin on alternate days Sterile saline injections were alternated with satietin injections

2 3 g/24 hr in one rat and 7 3 g/24 hr in the other (Fig 4) Nine days following the last infusion, food intake was approaching pretreatment levels The animals did not experience a quick rebound in consumption or compensatory overeating These two rats had previously been used in Experiment 2 It should be noted that in this and in Experiment 3 seven rats were used twice (once with semi-purified satietin and later

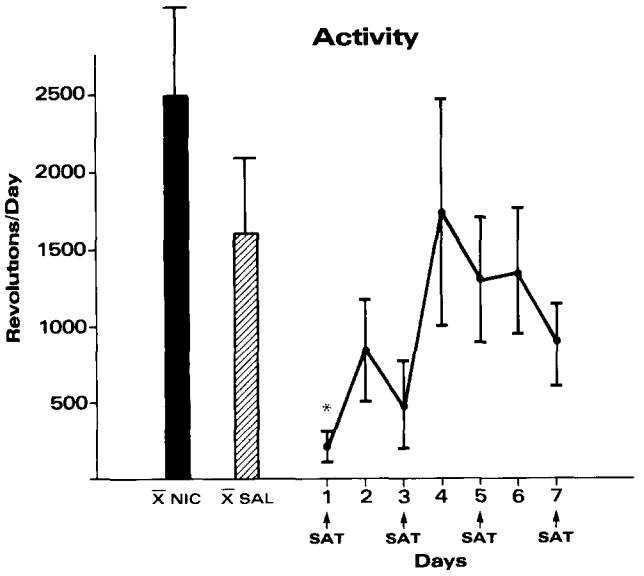

FIG 6 Experiment 3, running-wheel activity (revolutions) of rats ICV injected with 25 μg human satietin on alternate days Sterile saline injections were alternated with satietin injections

with purified satietin) In no other circumstances did rats appear in more than one experiment

Experiment 3

In comparison to saline infusions or non-infused control days h-SAT injected ICV on alternate days for four days produced an effect on food, $F(5,55)=9\ 01$, $p<0\ 001$, and water, $F(5,55)=4\ 00$, $p<0\ 003$, consumption (Fig 5) along with running wheel activity, $F(5,55)=6\ 33$, $p<0\ 001$ (Fig 6) Further analysis revealed that h-SAT significantly suppressed food and water consumption (Fig 5) only on the first day of h-SAT infusion Activity was suppressed significantly ($p<0\ 05$) after the first h-SAT infusion and approached ($p<0\ 06$) significance after the second h-SAT infusion Thereafter, activity remained low but significance was not attained

In comparison to saline infusions the seven additional rats that received a single dose of highly purified h-SAT also showed suppression in the above parameters (food intake, $32\ 2\pm3\ 0$ vs $14\ 0\pm3\ 3$ g, $p<0\ 01$, water intake, $45\ 2\pm5\ 2$ vs $18\ 0\pm6\ 1$ ml, $p<0\ 01$, activity, 1073 ± 442 vs $600\ 1\pm379$ revolutions, n s)

DISCUSSION

Knoll [6] reports that satietin is a highly specific anorectic agent in that it does not affect water consumption, activity in an open-field system, metabolic rate, unconditioned avoidance reactions, development of conditioned reflexes, learning and retention, copulatory behavior, blood sugar, blood insulin or glucagon concentrations or, contraction of the m tibialis anterior to hind paw stimulation in acutely spinalized rats and it is non-aversive Earlier studies [4] report a linear dose response to satietin in 96-hr fasted rats Among the anorectic drugs tested, Knoll and coworkers [6] point out that only calcitonin and satietin significantly reduce food intake in rats fasted for 96-hr [6] All other drugs are only active after shorter fasting periods and are effective for relatively short periods (15 min–2 hr) Both calcitonin and satietin are effective for 24 hr or more However, calcitonin plasma concentrations are far below that of satietin and well below that necessary to reduce feeding [6] Thus, these workers do not believe that satietin and calcitonin are equivalent [6]

Our data indicate that satietin is a powerful anorectic substance in rats subjected to no fast or a 48-hr fast, thus confirming and extending Knoll's contention that h-SAT is a powerful anorexigenic substance when given either peripherally or centrally

While food intake was reduced in the majority of experiments reported herein there are a number of arguments against accepting satietin as a true satiety agent For example, while satietin significantly reduced 24-hr food intake when injected subcutaneously, suppression of feeding behavior was not closely correlated with dose Because subcutaneously injected satietin is deposited as a pool in the tissue from whence it diffuses into the systemic circulation it probably arrives in the circulation in a low concentration The low systemic concentration may not accurately reflect the real dose size, thus, a poor correlation between dose and feeding behavior could result This possibility, however, is unlikely in Experiment 2 where satietin was injected into the third ventricle where tissue volume and dilution would be less variable Here a nonlinear response was again observed A possible explanation of these results (Experiment 2) is that at doses of 12 5 and 25 μg/rat high affinity satietin receptors were activated while at 50 μg/rat both high and low affinity receptor populations were activated It is suggested from our data that the threshold dose for ICV infused satietin is between 6 25 μg/rat and 12 5 μg/rat This correlates well with the previously reported ICV threshold dose of 10 μg/kg of satietin by Knoll *et al* [6] It seems reasonable to expect a linear dose response to ICV infused h-SAT in ad lib fed rats if the response is linear after similar infusions in 96-hr fasted animals Since we did not find that to be so it raises a question as to whether under normal feeding conditions satietin is a true physiological anorectic substance It is possible that our results are different than Knoll's because we used a different strain of rats and that should be examined

Food intake was seriously reduced when 25 μg/rat was injected for four consecutive days Knoll [6] has reported that satietin actively suppresses food intake for up to 30 hr post-injection so a cumulative effect in our study was not unexpected However, from our experiment it was not possible to determine whether food intake was reduced by satietin producing satiety or causing illness These data do, however, suggest that small, consecutive doses of satietin may be very effective in reducing food intake with little or no rebound feeding on termination of infusions On the other hand, when satietin was ICV infused on alternate days, anorectic activity was lost after the first treatment It is unclear why different results were obtained with the two methods of administering satietin

Running wheel activity was also suppressed on the first two days of satietin infusion with a return of running wheel activity to normal after subsequent infusions of satietin There are at least two possible explanations for reduced running wheel activity First, satietin may make the animals sick or experience malaise resulting in reduced activity Second, a true satiety agent is likely to induce somnolence with resulting inactivity It is not possible to determine which is the case from these data However, water intake was also reduced after the first satietin ICV infusion, suggesting that

satietin does not specifically affect feeding This leads to the possibility that satietin may cause a more general disruption of behavior but it should be noted that somnolence could produce similar results With regard to this latter possibility we have recently reported [1] that satietin administered, at doses similar to those used in these experiments, resulted in taste aversion [2] Thus, somnolence may not cause the changes observed in the behavior of the rats

According to the hypothesis of Billington *et al* [3] an aversive substance will reduce feeding to the same extent regardless of the length of fast, whereas, a true satiety agent will become less potent as fast length increases Satietin has been reported to significantly suppress feeding in 96-hr fasted rats during the initial hour of refeeding Our data from Experiment 2 reveals that satietin is not effective in suppressing feeding at the start of the dark phase, when ad lib fed rats normally start to feed [7] Therefore, satietin does not fit the criterion of Billington *et al* [3] regarding a satiety agent This coupled with our findings of a shallow dose response curve, suppression of water intake and activity after satietin infusion raises the question as to whether satietin is a true satiety agent

While it is not possible from the experiments presented here to definitively determine whether satietin plays either a physiological or pathological role in feeding behavior, these data do caution against the premature acceptance of satietin as a true satiety agent Development of suitable satietin assays will help to determine whether it is physiologically related to feeding behavior Development of such assays are currently underway

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