Capsaicin-treated rats permanently overingest low- but not high-concentration sucrose solutions

LISA KELLY, SILVIA MORALES, BRENSDA K. SMITH, AND HANS-RUDOLF BERTHOUD
Neurobiology of Nutrition Laboratory, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, Louisiana 70808

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Kelly, Lisa, Silvia Morales, Brenda K. Smith, and Hans-Rudolf Berthoud. Capsaicin-treated rats permanently overingest low- but not high-concentration sucrose solutions. Am J Physiol Regulatory Integrative Comp Physiol 279: R1805–R1812, 2000.—The effect of capsaicin-induced chemical ablation of visceral afferents on 1-h liquid sucrose consumption was investigated in food-deprived rats. We first show that although 10% sucrose is permanently overconsumed by capsaicin-treated (CAPs) compared with vehicle-treated (VEHs) control rats, 40% sucrose is only overconsumed during the first but not subsequent 1-h exposures. Furthermore, one group of CAPs lost the overconsumption response at 20% when exposed to progressively increasing sucrose concentrations of 10–40%, and another group recovered the overconsumption response at 10% when exposed to a series of decreasing concentrations. Control rats ingested relatively constant volumes of sucrose over the range of 10, 15, and 20%, resulting in significantly different energy intakes. In contrast, CAPs generally showed a concentration-dependent decrease in volume intake, resulting in relatively constant energy intake. These results suggest that capsaicin-sensitive visceral afferents, likely from gastric distension and other preabsorptive sensors, provide major control over volume ingested. In the absence of these signals, rats initially overconsume, but rapidly learn to use other signals from capsaicin-resistant preabsorptive or postabsorptive sites, to control future intake. This redundant satiety system appears to be sensitive to the osmotic value or caloric content of the unfamiliar food, but only if this is above a threshold of about 15% sucrose.

visceral afferents; vagal afferents; satiety; capsaicin; gastric distension; glucose sensors

VISCERAL AFFERENTS from the gastrointestinal tract are thought to carry important signals to the brain that are used in the process of food satiation and meal termination. Information from gastric mechanosensors (9, 11, 15, 19), small intestinal chemosensors (12, 13, 22), the hormone cholecystokinin (CCK) (27), and vagal afferents (20, 22, 26) have been most strongly implicated in this pathway. If this information is indeed important for meal termination, then interruption of the signaling cascade at any location should result in delayed satiety and larger meals. For example, blockade of the CCK-A receptor with selective antagonists has been demonstrated to increase meal size (5, 18, 20).

We have recently shown that rats with capsaicin-induced ablation of a portion of both dorsal root and vagal primary afferents overingest, relative to controls, various diets in short-term feeding tests (4, 16). However, this relative overconsumption was not seen during the second and subsequent trials a few days later, and intake of familiar chow was not different for capsaicin- and vehicle-treated rats (4, 16). We hypothesized that although signals mediated by capsaicin-sensitive visceral afferents, such as gastric distension and the arrival of nutrients in the small intestine, play an important role in limiting intake of novel foods, they are relatively unimportant in meal termination with familiar foods. Signals generated by alternative sensors located pre- or postabsorptively and carried to the brain by either capsaicin-resistant afferents (1) or the circulation may be used. They generate associations between the taste/flavor and its metabolic consequences during the first exposure to a novel food, which then guide intake in future trials, known as learned appetite and satiety (2, 24). This interpretation is supported by recent findings of Phillips and Powley (20) that after selective surgical vagal afferent rhizotomy, food intake was significantly increased during the first meals on the first day but not on the second day or thereafter. Furthermore, the results of another recent study, reporting that overconsumption of a relatively low-concentration sucrose solution (10%) was not only observed during initial exposure but was permanently displayed (6), suggest that the low metabolic impact of this calorically dilute food may not have stimulated the postulated alternative or redundant sensors. In contrast to this relatively low-energy sucrose solution, we had used diets with medium (complete liquid diet Ensure) to very high (pure vegetable shortening or corn oil) energy density in our earlier studies (4, 16).

The aim of the present series of studies was to determine the conditions and possible mechanisms that allow either persistent or transient overconsumption of sucrose and other sugar solutions by capsaicin-treated rats. The initial purpose was to determine...
whether we could replicate, within our deprivation regime, the sustained overconsumption effect with 10% sucrose that Curtis and Stricker reported (6). In our design, rats were 12-h food deprived prior to testing, in contrast to Curtis and Striker (6), who used chronically food-restricted rats and, furthermore, allowed the animals 48-h continuous exposure to the solution prior to testing.

MATERIALS AND METHODS

Animals

Thirty-five male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were used, weighing 200–240 g at the time of capsaicin or vehicle treatment. The animals were housed individually in hanging wire-mesh cages under standard laboratory conditions (12:12-h lighting schedule, lights on at 0700, 22 ± 3°C), and 5001 Purina lab chow and tap water was available ad libitum except as noted prior to tests. The rats were maintained on water bottles rather than the automatic watering system to ensure familiarity with spouts. All testing was conducted in the light phase between 0830 and 1300.

Capsaicin Treatment and Tests for Its Effectiveness

Rats were treated consecutively with increasing doses of capsaicin. On each of 3 days, rats were injected under inhalation anesthesia (isoflurane) with either vehicle as a control, or a dose of capsaicin (12.5, 30, and 75 mg/kg ip, 98% grade; Sigma Chemical). Capsaicin was freshly dissolved in a mixture of Tween 80 (10%), ethanol (10%), and sterile saline (80%) at the specific concentration. Following the first injection, all rats exhibited respiratory arrest of between 1 and 5 min. Assistance by manually massaging the chest or artificial respiration induced the resumption of spontaneous breathing. Three rats died during the first injection. During subsequent injections with the higher doses artificial respiration was rarely necessary.

Capsaicin-treated rats weighed the same as vehicle-treated rats within 10 days following treatment (265 ± 3 g) and gained weight at a similar rate throughout the study.

Eight days following capsaicin treatment, one drop of 1% NH₄OH was applied to the left eye with a Pasteur pipette, and the number of eye wipes in 30 s and the latency to the first wipe were recorded. All capsaicin-treated rats fulfilled the criterion of less than three wipes and a latency of >5 s to the first wipe. All vehicle control animals wiped vigorously, with a latency of <1 s and >15 wipes/30 s.

At the end of the experimental period, ~23 wk following capsaicin treatment, animals were tested for the known food intake-suppression effects of exogenous CCK. In overnight food-deprived capsaicin- and vehicle-treated rats, CCK (6 μg/kg ip) or saline as a control was administered 5 min before access to chow, and 30-min intake was then measured. Half the animals of each group received CCK first, and the other half received saline first, with tests 3 days apart.

As expected (12), administration of CCK-8 suppressed intake of chow in the overnight food-deprived rats by more than 80% in vehicle-treated control rats [saline, 6.5 ± 0.2 g; CCK, 1.1 ± 0.2 g; t(11) = 6.88, P < 0.001] but failed to induce significant suppression in capsaicin-treated rats [saline, 6.3 ± 0.2 g; CCK, 5.6 ± 0.3 g; t(19) = 1.32, P = 0.12].
sucrose diets, capsaicin-treated rats consumed more than vehicle-treated rats (Figs. 1 and 2); they consumed ~74% more 10% sucrose and 42% more 40% sucrose solution. For the first 5 min, intake of both 10% \( t(81) = 0.12 \) and 40% sucrose \( t(81) = 1.75 \) was not significantly different in capsaicin-treated rats. With 10% sucrose, intake by capsaicin-treated rats was significantly higher throughout the 60-min test [e.g., at 20 min: \( t(81) = 5.14, P < 0.001 \)]. With 40% sucrose, capsaicin-treated rats significantly overconsumed at 15 min \( t(81) = 2.90, P = 0.019 \) and at 20 min \( t(81) = 2.73, P = 0.031 \), whereas the effect did not reach statistical significance at 60 min (Figs. 1 and 2).

During subsequent trials (Fig. 2), overconsumption of 10% sucrose by capsaicin- vs. vehicle-treated rats persisted, so that in trial 4 they ingested 100% more \( t(28) = 8.02, P < 0.001 \). On the contrary, capsaicin-treated rats significantly decreased 40% sucrose intake during the second trial \( t(28) = 4.23; P < 0.001 \) and then slowly recovered during the next trials, so that intake was similar for both treatment groups during trial 4. There was a significant three-way concentration \( \times \) trial \( \times \) treatment interaction with intake expressed both in milliliters \( F(3,28) = 3.54, P < 0.001 \) or in kilocalories \( F(3,28) = 3.92, P < 0.001 \), confirming that the overconsumption by capsaicin-treated rats persisted and became even larger with each trial with 10%, but was lost with 40% sucrose.

When comparing intake of 10% and 40% sucrose during the last two trials, differences in volume vs. calorie tracking were revealed (Fig. 3). Control rats drank only moderately less 40% compared with 10% sucrose and as a consequence ingested significantly more calories \( t(28) = 15.9–17.5, P < 0.01 \). In contrast, the difference between intake of 40% and 10% sucrose was much larger and significant \( t(28) = 13.0–14.5, P < 0.01 \) in capsaicin-treated rats, and as a consequence their calorie intake was not different \( t(28) = 0.47–0.73, \) not significant (NS).

**Experiment 2**

Because the results of experiment 1 demonstrated that capsaicin-treated rats permanently overconsumed 10% but not 40% sucrose solutions, we wanted to determine the concentration threshold that separates the two outcomes and whether prior experience with either one of the sucrose solutions would have an effect on this threshold. Therefore, in this experiment, the animals exposed to 10% sucrose in this last series of trials were subsequently exposed to trials with 15%, 20%, and finally 40% sucrose (ascending series), and the animals with 40% sucrose experience were exposed to 20%, 15%, and finally 10% sucrose (descending series).

In the ascending series (Fig. 4A), capsaicin-treated rats continued to significantly overconsume 15% sucrose in three consecutive trials [average of last 2 trials: \( t(28) = 7.0, P < 0.001 \), but when switched to 20%, this response was gradually lost, so that in the 3rd and 4th trial, intakes were no longer significantly different \( t(28) = 1.70, P = 0.8 \). When finally exposed to 40% sucrose, capsaicin-treated rats did not consume
more than vehicle-treated control rats in any of the four trials.

In the descending series (Fig. 4B), it was not before the last three trials with 10% sucrose that the capsaicin-treated rats started to consume significantly more than the vehicle control rats [average of last 2 trials: $t(28) = 3.51, P = 0.012$]. There was a significant effect of the order of sucrose concentration on intake of capsaicin-treated [$t(28) = 4.19, P < 0.001$] but not vehicle-treated rats [$t(28) = 1.8, P = 0.16$]. This effect was particularly clear with 15% sucrose. At this concentration, there was a highly significant overconsumption by capsaicin-treated rats in the ascending series but no effect in the descending series.

If looking at the last trials only for a given concentration in the ascending series, the vehicle-treated control rats did not change the volume ingested when they went from 10% to 15% sucrose [$t(28) = 0.04, NS$] and from 15% to 20% [$t(28) = 1.26, NS$] (Fig. 5, top). This was reflected by significantly different energy intakes of 10% vs. 15% sucrose [6.0 ± 0.7 vs. 9.0 ± 0.6 kcal, $t(28) = 3.89, P = 0.007$] and 10% vs. 20% sucrose [6.0 ± 0.7 vs. 10.5 ± 1.1 kcal, $t(28) = 3.36, P = 0.027$] (Fig. 5, bottom). It was only in the last two trials with 40% sucrose that control rats significantly reduced the volume ingested. Although control rats ingested about 50% more calories from 40% compared with 15% sucrose, this difference did not reach significance [$t(28) = 2.43, NS$].
In contrast, capsaicin-treated rats demonstrated a concentration-dependent decrease in volume intake \[F(3,28) = 53.4, P < 0.001; \text{Fig. 5, top}\] and as a consequence showed less change in calorie intake, although the difference between 10% and 15% was significant \[F(3,28) = 9.6, P < 0.01; \text{Fig. 5, bottom}\].

Looking only at the last trials for each concentration in the descending series, the difference in volume vs. calorie tracking between control and capsaicin-treated rats was less evident, but the general trend was also present (Fig. 5). Control rats did not significantly change volume ingested from 20% to 10% \[t(28) = 1.58, \text{NS}\] and from 15% to 10% sucrose \[t(28) = 2.0, \text{NS}\] and as a consequence ingested significantly more calories from 20% than from 10% sucrose \[t(28) = 3.56, P = 0.002\]. However, control rats did significantly increase the volume of 20% compared with 40% sucrose \[t(28) = 4.24, P < 0.003\] and as a result ingested a similar number of calories \[t(28) = 0.84, \text{NS}\].

In contrast and similar to the ascending series, capsaicin-treated rats concentration-dependently reduced volume intake \[F(3,28) = 51.6, P < 0.001\]. The exception was the similar volume of 15% and 20% sucrose ingested (Fig. 5, top). As a consequence, none of the calorie intakes from the different sucrose concentrations was significantly different \[F(3,28) = 3.68, \text{NS}; \text{Fig. 5, bottom}\].

**DISCUSSION**

This study was designed to identify and specify changes in ingestive behavior in rats treated systemically with high doses of capsaicin, a toxin that has been shown to chronically ablate a class of visceral afferents. The results show that changes in ingestive behavior by capsaicin-treated rats depend on the familiarity and energy density of a particular test food. The following conclusions can be drawn from the present study.

1) Capsaicin-treated rats ingest significantly more of various concentrations of sucrose solutions if presented for the first time: they overconsume. This overconsumption is the result of increased meal size, with the initial rate of ingestion similar to controls (delayed satiety). Also, overconsumption of low concentration was more pronounced than that of high-concentration sucrose solutions.

2) The ingestive experience from a single 1-h trial was sufficient to abolish or mask overconsumption of high- but not low-concentration sucrose solutions in subsequent trials. Prior experience with high-concentration sucrose is more potent than experience with low sucrose to abolish overconsumption in subsequent trials.

3) Although vehicle-treated control rats, within a certain range of sucrose concentrations, drank relatively constant volumes, capsaicin-treated rats drank a relatively constant number of calories, by adjusting the volume of the different sucrose concentrations.

**Overconsumption During First Exposure to Novel Food**

We have previously shown that capsaicin-treated rats consume almost twice as much of solid (vegetable
shortening) or liquid (corn oil) pure fat diet if it was new to the rat (4, 16). Here we further demonstrate that although rat chow is consumed in similar amounts, liquid sugars over a range of concentrations are overconsumed on first exposure by capsaicin-treated rats.

However, ingestion rate and total amount ingested (meal size) are not only governed by negative feedback signals from the gut but also by the acceptance of the novel diet. Acceptance depends on palatability (hedonic value) and the individual level of caution characteristic for any neophobic behavior. We have tried to keep this neophobic caution to a minimum by allowing animals to taste and get accustomed to small amounts of the novel food before the first test exposure. If this training did not completely eliminate a neophobic hypophagia, then any overconsumption seen in capsaicin-treated rats could theoretically be ascribed to interference with the mechanism underlying the neural control of neophobic behavior.

In addition, capsaicin treatment could affect hedonic acceptability and palatability of the food. The initial (first 5 min) rate of ingestion, which is often used as an indicator of acceptability, was not significantly different in capsaicin-treated rats for both sucrose concentrations. In addition, we did not find significant differences in the number of licks during the first 10 s of exposure to 0.005 M (0.17%) to 0.1 M (3.4%) sucrose (unpublished observations). A near maximal lick rate of 6 licks/s was already observed with 0.1 M sucrose in both vehicle- and capsaicin-treated rats. Furthermore, Silver and coworkers (25) have reported that capsaicin-treated rats emitted the same number of licks to salty (NaCl), sour (HCl), and low concentrations of bitter (1–5 × 10⁻³ M quinine) stimuli. Only the response to the highest concentration of quinine (10⁻³ M) was slightly impaired. Acceptance of sweet stimuli was not tested by these authors (25). On the basis of these observations, it is thus unlikely that overconsumption was the result of capsaicin-induced damage to gustatory pathways.

The overconsumption observed with 10% sucrose was considerably larger than with 40% sucrose solution. Earlier we showed that solid (vegetable shortening) and liquid (corn oil) fat foods caused large overconsumption, whereas a complete liquid diet (Ensure) and fat-free carbohydrate cookies produced only small or no overconsumption. Our original interpretation was that high fat content was necessary for overconsumption to occur (16), but in the light of the present findings it is no longer tenable. It is more likely that the magnitude of the overconsumption response depends on how rapidly a novel food can generate satiety signals at postgastric and/or postabsorptive sites not damaged by capsaicin treatment. These signals would tend to make up for the lacking satiety signals from gastric distension sensors before the first meal has ended. With a 40% sucrose solution, glucose absorption and portal glucose levels are higher than with 10% sucrose, and in general, glucose absorption is much faster than fat absorption. This would explain why overconsumption of low-concentration sugar solutions and high-fat foods is more pronounced than high-concentration sugar solutions.

Another consideration is the osmolality of the novel food. Osmotic concentration seems to be an important satiety signal (14, 30). Abdominal vagotomy abolished the inhibiting effect of mannitol, a nonmetabolizable sugar, on ingestion (8). It is therefore possible that capsaicin-resistant osmosensors may provide the “missing” negative feedback during the first exposure to 40% sucrose in capsaicin-treated rats. The low osmotic value of fat foods would explain why there is no such intake-inhibiting effect during the first exposure to high-fat foods.

Intake During Subsequent Trials

Repeated presentation of 10% sucrose did not diminish the relative overconsumption response in capsaicin-treated rats, confirming the findings by Curtis and Stricker (6). Together with the observations that water intake induced by either hypertonic saline or polyethylene glycol injections as well as 0.4 M NaCl intake induced by chronic deoxycorticosterone acetate treatment was also significantly increased in capsaicin-treated rats, these authors concluded that it is the lack of early signals of gastric distension that causes the delay in the onset of satiety (6). The fact that this continues to occur even after many exposures suggests that there is no other mechanism to compensate for the lack of these gastric distension signals. It seems that the rats are unable to learn from the postabsorptive consequences of the ingested sucrose. This is surprising, because they ingested a considerable number of calories during each trial. In the present experiment capsaicin-treated rats consumed about 10–12 kcal in each 1-h trial, and in the Curtis and Stricker experiment (6) they had unrestricted access to 10% sucrose for 2 days. Although it is generally accepted that associations between the flavor (taste and smell) and the postigestive consequences of a given food allow an animal to learn and direct future intake of this food (2, 3, 24), it is not clear where the signal for the postigestive consequences is formed. Sensors in the small intestine (23) and in the portal-hepatic axis (28) may provide such a signal. For the small intestine it has been shown that the signal used for feeding suppression but not the signal used for reinforcement of flavor preference is attenuated by capsaicin (17) or vagotomy (23). Thus lack of learning by capsaicin-treated rats may be due to insufficient stimulation of capsaicin-resistant sensor mechanisms or to damage to capsaicin-sensitive sensors that contribute to learning of caloric regulation and satiety.

The major new finding of the present study is that in contrast to 10% sucrose, higher sucrose concentrations abolish the overconsumption exhibited by capsaicin-treated rats. Apparently, this higher concentration was able to produce sufficient postgastric signal to counteract and neutralize the intake-enhancing effect of the missing gastric distension signal by a learning
process as outlined above. It is not clear whether the effect was due to a caloric or osmotic effect. Because osmotic concentration may play an important role in satiation (14, 30), additional experiments with metabolically inactive sugars such as mannitol or 2-deoxy-glucose will be necessary to distinguish between caloric and osmotic stimuli.

**Volume-Sensitive vs. Concentration-Sensitive Regulation**

Vehicle-treated control rats did not adjust their volume intake when exposed to repeated trials with successively increasing or decreasing sucrose concentrations over the range of 10–20%, and as a consequence, they ingested significantly different amounts of calories. In a comprehensive study with different groups of rats for each sucrose concentration, Davis et al. (10) have shown that within the range of ~2–28% sucrose, the volume of 30-min intake remains remarkably stable, resulting in significantly different calorie intakes. Performing sham feeding tests in the same rats, these authors (10) also demonstrated that there are two different types of negative feedback controls operative. At all sucrose concentrations there is a direct negative feedback signal based simply on gastrointestinal filling, whereas at higher concentrations (>0.4 M or ~13%) an additional labile, or conditioned, feedback signal is in effect, possibly based on learned associations between taste and visceral sensory signals.

In contrast to control rats, capsaicin-treated rats in both the ascending and descending series showed a more or less concentration-dependent adjustment in volume consumed, resulting in only small differences in calories consumed. It may indicate the recruitment of a normally inactive or masked energy- or osmolar-based sensory mechanism that is not damaged by capsaicin. Because most of the sucrose concentrations in our experiment were hypertonic, this redundant mechanism that decreased volume intake at higher concentrations may be similar to the labile negative feedback signal demonstrated to operate at higher sucrose concentrations by Davis et al. (10).

That the newly recruited sensor is not of vagal origin is supported by the observation that capsaicin treatment almost completely eradicated traceable vagal afferent fibers and terminals in the small intestinal myenteric plexus (1). This is further supported by the observation that the rate of ingestion and meal size of milk or sucrose decreased following abdominal vagotomy and the conclusion that vagotomy enhanced the volume of 30-min intake remains remarkably stable, resulting in significantly different calorie intakes. Performing sham feeding tests in the same rats, these authors (10) also demonstrated that there are two different types of negative feedback controls operative. At all sucrose concentrations there is a direct negative feedback signal based simply on gastrointestinal filling, whereas at higher concentrations (>0.4 M or ~13%) an additional labile, or conditioned, feedback signal is in effect, possibly based on learned associations between taste and visceral sensory signals.

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**Perspectives**

The results suggest that the class of visceral afferents destroyed by capsaicin is important for limiting meal size, particularly when novel foods with low osmotic value or energy content are ingested. Why is this effect smaller when the sucrose concentration is higher, and why is it absent when the food is familiar? The most parsimonious explanation is that negative feedback signals not mediated by capsaicin-sensitive visceral afferents make up for the missing signals mediated by capsaicin-sensitive afferents (redundant systems). The fact that the overingestion response on first exposure to high-energy fat foods (4, 16) is as large as to low sucrose concentrations suggests that the redundant mechanism may be based on osmosensitive signals rather than calories. This mechanism could be identical to the one described by Davis et al. (10) on the basis of different meal sizes with real and sham feeding. The labile or conditionable character of this mechanism would explain why the effect is usually only seen during first exposure to the particular food. However, we cannot exclude other sensory signals mediated by capsaicin-resistant visceral or vagal afferents or directly affecting the brain. Glucose-sensory mechanisms in the small intestine and/or liver could theoretically also provide the redundant control mechanism.

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Present address of L. Kelly: Department of Psychology, University of Ottawa, Ottawa, Canada.

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