Short communication

Effects of perivagal administration of capsaicin on food intake in animals after noxious gastric surgery

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Abstract

Previous studies have shown that the perivagal administration of capsaicin induces greater food intake vs. controls at 24 h after the surgery but a similar intake to that of controls at 48 h. The present study aimed to determine whether the nutritive effect observed after perivagal capsaicin administration is due to the interruption of noxious vagal fibers in rats. For this purpose, postsurgical food intake was analyzed in control and capsaicin-treated animals with (Experiment 2) and without (Experiment 1) noxious lesions in the gastric wall. The results of both experiments showed that the food intake of capsaicin-treated animals was greater vs. control animals at 24 h but not at 48 h after the surgery (p<0.025), as previously demonstrated. However, the food intake of the capsaicin-treated lesion animals in Experiment 2, although still greater than that of the control group, was significantly less than the intake of the capsaicin-treated animals in Experiment 1 (p<0.01). Therefore, it appears unlikely that the demonstrated effect is produced by lesion to noxious vagal fibers destroyed by the capsaicin. On the contrary, it is more likely that the vagal afferent pathways are those related to short-term nutrition.

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Short-term satiety or satiation is the nutritive process responsible for ending food intake. Currently, most authors agree that the mechanisms that regulate this process are neural, especially vagal. The involvement of the vagal nerve in satiation has been studied using different experimental approaches including analysis of the intake pattern of vagotomized subjects (Snowdon, 1970; Mordes et al., 1979; González and Deutsch, 1981; Smith et al., 1981; Kral, 1983; Sclafani and Kramer, 1983; Davis et al., 1994; Furness et al., 2001; Date et al., 2002). These studies, most of which were performed in rodents, generally examine the intake after assurance that the subjects have completely recovered from the surgical intervention. However, some authors recently proposed investigation of this behavior immediately after the surgery, in order to avoid learning factors (or factors of another type) that could mask the effect induced by the absence of neural information (Chavez et al., 1997; Phillips and Powley, 1998).

In accordance with this proposal, we examined the involvement of vagal afferents in postsurgical intake by using the perivagal administration of capsaicin (Zafra et al., 2003), a more specific technique than the traditional surgical vagotomy. In fact, this neurotoxin selectively destroys weakly myelinated A-delta or unmyelinated C afferent fibers (Jancso et al., 1987; Holzer, 1991; Ritter and Dinh, 1992; Blackshaw et al., 2000), fibers particularly abundant in the vagal nerve (Mei et al., 1980; Mei, 1983; Prechtl and Powley, 1990; Sengupta and Gebhart, 1994; Berthoud et al., 1997). In agreement with studies of vagotomized animals (Phillips and Powley, 1998), investigations of capsaicin-treated subjects have shown that the perivagal administration of capsaicin induces a greater food intake vs. controls at 6, 12 and 24 h after the surgical intervention, with a similar intake to that of controls at 48

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and 72 h (Zafra et al., 2003). Given that capsaicin seems to be a neurotoxin that acts selectively on vagal afferents, which are crucial to satiety, it has been proposed that interruption of the sensory component of the nerve affects one of the mechanisms involved in the short-term regulation of intake (Zafra et al., 2003). However, an alternative explanation is possible. Capsaicin-sensitive visceral afferent neurons process both nonharmful and harmful, or potentially harmful, stimuli (Holzer, 1991; Berthoud and Neuhuber, 2000). This may account for the increased intake observed in the subjects treated with this neurotoxin. In fact, the pain or other unpleasant sensations caused by the surgery may have a greater inhibitory effect on food intake in control animals, whose vagal afferents are intact, than in treated animals.

The present study aimed to explore this possibility, determining whether the nutritive effect observed after perivagal capsaicin administration is due to the interruption of noxious fibers. For this purpose, postsurgical food intake was analyzed in control and capsaicin-treated animals with (Experiment 2) and without (Experiment 1) lesions in the gastric wall. It was hypothesized that, if the effect of capsaicin on postsurgical intake is due to the interruption of harmful fibers, a gastric lesion (Experiment 2) would have a greater effect on the intake of the control animals, whereas the intake of the capsaicin-treated animals would be similar to that in Experiment 1. Part of the present study has been reported in abstract form (Zafra et al., 2004).

All experiments were performed on adult male Wistar rats from a breeding colony at the University of Granada. Experiment 1 used 14 male Wistar rats (312–349 g each) which were randomly assigned to the experimental (capsaicin treatment) group (n=7) or control (sham procedure) group (n=7). Experiment 2 used 15 male Wistar rats (250–317 g each) randomly assigned to a lesion (capsaicin treatment) group (n=8) and control (sham procedure) group (n=7). On arrival at the laboratory, the animals were individually housed in 30×15×30 cm methacrylate cages, with unlimited access to food and water. The room was maintained on a 12:12-h light/dark cycle at 21–23 °C. All experimental procedures took place during light periods and were conducted in accordance with the Animal Care and Use Guidelines established by Spanish Royal Law 223/1988.

Capsaicin was administered perivagally using a modified version of the procedure published by Raybould and Taché (1989). After the i.p. administration of 0.15 mg atropine (Sigma, St. Louis, MO) the animal was anesthetized with sodium pentothal (46.3 mg/kg, i.p., sodium thiopental, Abbot Laboratories). A 3-cm incision was then made along the midline of the abdominal wall, and the esophagus was carefully exposed. Paraffin film was placed beneath the esophagus to prevent the capsaicin (Fluka, 98%) spreading to underlying tissues. The esophagus was then surrounded for 30 min by cotton impregnated with capsaicin solution (1 mg capsaicin dissolved in 1 ml of vehicle: 10% Tween 80 in olive oil), which was rewetted every 5 min. The total amount of capsaicin applied was 1 ml (1 mg per rat). After the 30-min operation, the area was washed out with saline solution and dried with sterile material. The incision was then closed with several stitches, and a topical antiseptic (Betadine, Viatris manufacturing, Burdeos, France) was applied to the wound. Finally, 0.1 ml penicillin (100000 IU, Penilevel, Lab. Ern, Barcelona, Spain) was i.m. injected to avoid possible infections. In the control group, after the anesthesia of the animals and administration of the atropine, the abdomen was opened using the same surgical procedure as above and irrigated with saline solution for 30 min. The abdomen was then closed as described above.

Additionally, in Experiment 2, during the 30-min that the animals in both groups remained with the stomach exposed, two full thickness incisions of approximately 2 mm were made in the least irrigated portion of the corpus of the stomach (in the anterior surface near the greatest curvature) and were then immediately sutured.

The amount of food consumed during the 2 days before the surgical intervention was recorded as the baseline value. Immediately after surgery, the animals were returned to their cages where solid food and water was available ad libitum. The food intake was again measured at 24 and 48 h after the end of surgery and was compared with the presurgical measurement.

At the end of the experiments, the vagotomy test proposed by Martin et al. (1978) was applied to the animals to determine whether the vagal nerve had been accidentally damaged during the surgery. This test consists of extracting and weighing the stomach of the animal after 12 h without food (overnight in these experiments). The criterion for vagotomy and exclusion from the study was a proportion between the stomach weight and the animal weight prior to food deprivation of >0.020.

One animal from the capsaicin-treated group of Experiment 2 died after the surgery. Additionally, two capsaicin-treated animals (one from Experiment 1 and another from Experiment 2) were inadvertently vagotomized, and their data were excluded from the statistical analysis.

All statistical analyses were performed by using Statistics, version 5.1 (from Statsoft, Tulsa, USA), with p<0.05 considered statistically significant. The significance of mean differences among groups was determined with analysis of variance (ANOVA Groups×Day).

There were no statistically significant differences in stomach weight (vagotomy-test) between the capsaicin-treated [F(1,10)=0.02; p=0.88; one-way ANOVA] and control [F(1,12)=0.21; p=0.65; one-way ANOVA] animals in either experiment. These results suggest that is unlikely that the incisions made in the stomach in Experiment 2 could have damaged important vagal fibers in the stomach wall (which could influence the data on the food intake). There was no significant difference between the groups in
food intake during the 2 days prior to surgery \((p>0.05; \text{one-way ANOVA})\) in either experiment. Statistical analysis of the postsurgery results was carried out by converting the results for the subjects into percentages with respect to the average intake shown in the 2 days prior to surgery. In both experiments, ANOVA analysis of the data showed significant differences between the capsaicin-treated group and the control group at 24 h \([\text{Experiment 1: } F(1,11)=7.854; \ p<0.017; \text{Experiment 2: } F(1,11)=11.279; \ p<0.006]\) but not at 48 h \([\text{Experiment 1: } F(1,11)=2.0625; \ p<0.178; \text{Experiment 2: } F(1,11)=0.4888; \ p<0.49]\) after the surgery (see Fig. 1A, B). The mean intake (in grams) was 16.31 vs. 12.11 (24 h postsurgery) and 19.46 vs. 17.11 (48 h postsurgery) in Experiment 1, and 9.9 vs. 5.6 (24 h postsurgery) and 15.7 vs. 13.2 (48 h postsurgery) in Experiment 2.

There were significant differences in postsurgical food intake between the capsaicin-treated groups of each experiment \([F(1,10)=45,705; \ p<0.001]\). When the daily intake was analyzed, the differences were significant at 24 h \([F(1,10)=22,425; \ p<0.001]\) and 48 h \([F(1,10)=15,809; \ p<0.003]\) after the surgery (Fig. 1C). As could be expected, the difference between the two control groups (of Experiments 1 and 2) was also significant \([F(1,12)=19,906; \ p<0.001]\). However, the control groups (Fig. 1D) showed significant differences at 24 h after surgery \([F(1,12)=45,389; \ p<0.001]\) but not at 48 h, although there was a tendency towards a significant difference \([F(1,12)=4,22; \ p<0.06]\). Joint analysis of the data from the two experiments showed that both the group variable (capsaicin-treated vs. control) \([F(1,22)=18,27; \ p<0.001]\) and the lesion variable (gastric lesion vs. no gastric lesion) \([F(1,22)=62,36; \ p<0.001]\) were significant. In contrast, the interaction of the two variables \([F(1,22)=0.02; \ p<0.88]\) was not significant, indicating an identical decrease in intake by the control group vs. the experimental group in both experiments.

In both experiments, there was no difference in body weight between the groups, either before or after the surgery (all \(p\) values >0.5).

The present results showed that the food intake of capsaicin-treated animals was greater than that of the control animals at 24 h but not at 48 h after the surgery, as previously demonstrated (Zafra et al., 2003). Therefore, these experiments confirm the short-term food intake effect of perivagal capsaicin. Because the capsaicin was administered perivagal and it is a neurotoxin that selectively affects the primary afferents (Holzer, 1991; Ritter and Dinh, 1992), it is reasonable to propose that the changes in the food intake were due to damage to vagal afferent fibers produced by the drug. This interpretation is supported by the similarity between our findings and the published results of

![Fig. 1](image)

**Fig. 1.** Mean amount of food consumed by the rats in the capsaicin-treated \((N=6)\) and control \((N=7)\) groups of Experiment 1 (A), capsaicin-treated \((N=6)\) and control \((N=7)\) groups of Experiment 2 (B), capsaicin groups of Experiments 1 and 2 (C), and control groups of Experiments 1 and 2 (D), during 24 and 48 h after the surgery. Data are expressed as percentages of the mean intake recorded during the two days prior to surgery for each subject (E1: Experiment 1; E2: Experiment 2. *\(p<0.05\); **\(p<0.01\); ***\(p<0.001\)).
Vagal afferents have been linked to numerous nutritive processes, especially in relation to the satiation mechanism (González and Deutsch, 1981; Smith et al., 1981; Blackshaw and Grundy, 1990; Prechtl and Powley, 1990; Davis et al., 1994; Phillips and Powley, 1998; Schwartz et al., 1999; Schwartz, 2000; Ritter, 2004). Therefore, the greater intake observed during the 24 h after capsaicin treatment can be interpreted as a consequence of the interruption of vagal neural signals involved in short-term food intake control. On the other hand, recent studies implicated the vagal nerve in the detection of nociceptive signals (Berthoud and Neuhuber, 2000). Thus, it cannot be ruled out that the effect observed in the capsaicin-treated animals may result from the disruption of visceral noxious afferents, especially given that capsaicin-sensitive visceral afferents are also sensitive to harmful or potentially harmful stimuli (Hölzer, 1991). Therefore, interruption of the processing of the surgical pain or other unpleasant sensation may, in some way, produce the increased intake observed in the neurotoxin-treated animals. Nevertheless, this alternative explanation appears unlikely in the light of the results obtained in the second experiment. The food intake of the capsaicin-treated animals in Experiment 2, although still greater than that of the control group, was significantly less than the intake of the capsaicin-treated animals in Experiment 1 at 24 h after the surgery. If the nutritive effects observed in Experiment 1 were due to the lesioning of capsaicin-sensitive noxious vagal afferents, there should not be a difference in postsurgical intake between the intact animals (Experiment 1) and those with gastric lesions (Experiment 2). In other words, if the noxious fibers were damaged in both groups, the presence or not of a gastric wall lesion should have no effect, and the intake of the two capsaicin-treated groups should be the same. As this was not the case, the visceral noxious afferents were probably intact in both groups, and the lesion to the gastric wall of the animals was responsible for the consequent inhibitory effect on the intake. Furthermore, joint statistical analysis of the data from both experiments showed that the reduction in intake produced in the Experiment 2 animals with respect to the Experiment 1 animals was identical in both groups (capsaicin-treated and control). This result suggests that the unpleasant information derived from the noxious surgery itself was processed in a like manner in both groups through mechanisms independent of the capsaicin-damaged vagal fibers. These mechanisms could involve vagal afferents unaffected by capsaicin (Berthoud et al., 1997) or, more probably, splanchnic fibers traditionally related to the processing of harmful visceral stimuli (Cervero, 1994). As expected, there were significant differences in the intake at 24 h postsurgery between the control groups of the experiments. In the controls, transmission of both types of information (satiation- and noxious-related stimuli) was intact, so that the effects on food intake reduction were additive.

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