NMDA channels control meal size via central vagal afferent terminals

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Gillespie, B. R., G. A. Burns, and R. C. Ritter. NMDA channels control meal size via central vagal afferent terminals. Am J Physiol Regul Integr Comp Physiol 289: R1504–R1511, 2005. First published July 14, 2005; doi:10.1152/ajpregu.00169.2005.—The N-methyl-d-aspartate (NMDA) ion channel blocker MK-801 administered systemically or as a nanoliter injection into the nucleus of the solitary tract (NTS), increases meal size. Furthermore, we have observed that ablation of the NTS abolishes increased meal size following systemic injection of dizocilpine (MK-801) and that MK-801-induced increases in intake are attenuated in rats pretreated with capsaicin to destroy small, unmyelinated, primary afferent neurons. These findings led us to hypothesize that NMDA receptors on central vagal afferent terminals or on higher-order NTS neurons innervated by these vagal afferents might mediate increased food intake. To evaluate this hypothesis, we examined 15% sucrose intake after 50-nl MK-801 injections ipsilateral or contralateral to unilateral nodose ganglion removal (ganglionectomy). On the side contralateral to ganglionectomy, vagal afferent terminals would be intact and functional, whereas ipsilateral to ganglionectomy vagal afferent terminals would be absent. Three additional control preparations were also included: 1) sham ganglionectomy and 2) subnodose vagotomy either contralateral or ipsilateral to NTS cannula placement. We found that rats with subnodose vagotomies increased their sucrose intake after injections of MK-801 compared with saline, regardless of whether injections were made contralateral (12.6 ± 0.2 vs. 9.6 ± 0.3 ml) or ipsilateral (14.2 ± 0.6 vs. 9.7 ± 0.4 ml) to vagotomy. Rats with NTS cannula placements contralateral to nodose ganglionectomy also increased their intake after MK-801 (12.2 ± 0.9 and 9.2 ± 1.1 ml for MK-801 and saline, respectively). However, rats with placements ipsilateral to ganglionectomy did not respond to MK-801 (8.0 ± 0.5 ml) compared with saline (8.3 ± 0.4 ml). We conclude that central vagal afferent terminals are necessary for increased food intake in response to NMDA ion channel blockade. The function of central vagal afferent processes or the activity of higher-order NTS neurons driven by vagal afferents may be modulated by NMDA receptors to control meal size.

Several investigators (3, 6, 7, 5, 14) have reported that rats increase their intake of solid food or 15% sucrose solutions, after systemic injection of dizocilpine (MK-801), a noncompetitive N-methyl-d-aspartate (NMDA)-activated ion channel antagonist. Thirty-nanoliter injections of MK-801 directed to the medial nucleus of the solitary tract (NTS) also yielded robust increases in meal size and duration (27). Lesioning of the dorsal vagal complex (DVC), including the area postrema and NTS, abolishes MK-801-induced increases in food intake after peripheral MK-801 administration (28). These results led us to conclude that the NMDA receptors responsible for mediating MK-801’s effects on food intake are located in the DVC.

Satiety signals from the gastrointestinal tract are conveyed centrally via vagal afferent neurons (18). Vagal sensory neurons and NTS interneurons contain glutamate immunoreactivity in vesicles (4), and glutamate is released in the NTS during vagal stimulation (16). Furthermore, removal of the nodose ganglion reduces glutamate binding in the NTS (12). NMDA receptor mRNA has been detected in vagal afferent neurons (22), and NMDA channel subunit immunoreactivity has been reported on vagal afferent terminals in the hindbrain (1). Finally, application of NMDA to both in vivo and in vitro preparations of NMDA receptor-containing NTS postsynaptic neurons evokes excitatory responses (2). Taken together, this body of work suggests that NMDA channels participating in NTS neurotransmission could contribute to the control of food intake.

In support of this hypothesis, we have found (6) that pretreatment of rats with capsaicin, a neurotoxin that destroys small unmyelinated primary afferents, including a subpopulation of vagal afferents (20, 21, 25), attenuates but does not abolish, increases in food intake by MK-801. These results suggest that capsaicin-sensitive vagal afferent neurons play a role in MK-801-induced increases in food intake. However, the fact that the MK-801-induced increase in food intake is attenuated, but not abolished, in capsaicin-treated rats suggests that capsaicin-insensitive neurons may also participate in MK-801-induced increases in food intake. Burns and Ritter (6) reported that subdiaphragmatic vagotomy abolished increases in food intake by systemically injected MK-801, suggesting that vagal fibers or terminals are required for MK-801-induced increased feeding. However, multiple experimental results have strongly suggested that the hindbrain is the site of action for MK-801’s effect on feeding. Presumably, cutting the vagus below the diaphragm would not eliminate the central vagal afferent terminals, because the nodose ganglia remain intact after this procedure. Furthermore, a preliminary replication of the vagotemy experiment by Ritter and colleagues (29) produced equivocal results. Hence, the suspected site of MK-801’s action along the vagal afferent pathway from the gut to the brain remains unresolved.

In light of these findings, we hypothesized that increased food intake in response to MK-801 is mediated by blockade of NMDA channels located either on primary vagal afferent terminals or on higher-order vagal sensory neurons in the NTS. To test this hypothesis, we conducted a series of anatomically based behavioral experiments to systematically evaluate the participation of peripheral vagal fibers, as well as central vagal afferent terminals, in increases in meal size by MK-801.
MATERIALS AND METHODS

Animals

Male adult (350–400 g) Sprague-Dawley rats (Simonsen) were housed in individual hanging cages in a vivarium under conditions of controlled illumination (12:12-h light-dark cycle), humidity (70%), and temperature (22 ± 2°C). Rats were handled daily and habituated to laboratory conditions before surgery or testing began. They had ad libitum access to pelleted chow (Teklad) and water, except during experiments and overnight fasts. Rats were trained to drink 15% sucrose from drinking tubes for 30-min after an overnight fast, before any surgical manipulations. In addition, the vagotomy and sham vagotomy rats were adapted to receiving an intraperitoneal injection of either MK-801 or saline vehicle 15-min before sucrose presentation. Each rat was tested at least twice with MK-801 at 48-h intervals before any surgical procedure. All animal procedures were approved by the Washington State University Institutional Animal Care and Use Committee and conform to “Guide for the Care and Use of Laboratory Animals” [DHEW Publication No. (NIH) 85–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD 20205].

Drugs

MK-801 (Sigma) was dissolved in a vehicle solution of sterile 0.15 M NaCl and administered either intraperitoneally (80 μg/kg) or into the NTS (1 μg/μl) as a 50-nl injection. Injection volume for central injections were verified using a Cahn C-33 microbalance (1 nl = 1 μg). The 50-nl injection volume was the smallest volume we could consistently inject using our motorized microsyringe/injector apparatus. CCK octapeptide sulfate (CCK-8; American Peptide, Sunnyvale, CA) was dissolved in a vehicle solution of sterile 0.15 M NaCl. CCK-8 was delivered intraperitoneally at the concentration of 2 μg/ml (2 μg/kg) at a dose of 0.1 ml/100 g body wt. All drugs were dissolved into solution immediately before the start of the experiments on each testing day.

In addition, the retrograde tracer Fluoro-Gold (Biotium, Hayward, CA) was used to verify the completeness of the vagal manipulations using methods modified slightly from Powley et al. (17). Two separate (0.5 ml) injections (2 mg/ml) per rat were given 4–5 days before death.

Surgical Procedures

Subdiaphragmatic vagotomy. On completion of their presurgical training, rats underwent either a total surgical resection of both dorsal and ventral subdiaphragmatic vagal trunks (n = 12) or sham vagotomy (n = 10) in a manner consistent with previously described procedures (6, 31). Briefly, rats were induced to a surgical plane of anesthesia with a drug cocktail containing ketamine, acepromazine, and xylazine (0.1 ml/100 g body wt) and maintained at this plane of anesthesia with inhaled isofluorane. Rats were placed in dorsal recumbency, and the dorsal and ventral vagal nerve trunks were carefully isolated via a ventral midline approach to the peritoneal cavity. A 5-mm section of each vagus nerve was removed from the trunk rostral to the hepatic and accessory celiac vagal branches. The cut ends of each trunk were then touched with a cautering pen. The celiotomy incision was closed with a combination of 4-0 gut suture and skin staples. Sham surgeries were conducted in an identical fashion except that, rather than resecting the nerves, the vagal trunks were merely touched with a cotton-tip applicator.

Subnodose cervical vagotomy. Rats underwent unilateral cervical or sham vagotomy in the midcervical region, as previously described (9). Briefly, an area distal to the nodose ganglion in the middle region of the cervical area was approached via a midline incision, and a 10-mm section of cervical vagus was gently isolated using blunt dissection and resected using fine iris scissors. The cut ends of the cervical vagus were cauterized before closing of the skin incision with silk suture. An equal number of right and left cervical vagotomies were done. For sham control surgeries, the same cervical area was isolated, but rather than resecting the nerve trunk, the cervical vagus was gently touched with a sterile cotton applicator.

Unilateral nodose ganglion removal. To investigate the participation of the central terminals of vagal afferent neurons in increases in meal size and duration by MK-801, rats underwent unilateral nodosectomy or sham nodosectomy, as previously described (10). Briefly, removal of the nodose ganglion was achieved from a small incision in the dorsal aspect of the neck extending from the region of the base of the tongue to just cranial to the manubrium. The nodose ganglion was exposed by retracting the overlying muscles. The cranial cervical vagus was first resected at the caudal end of the nodose, thereby allowing lifting of the nodose stump to more easily visualize the vagus rostral to the nodose. In this way, complete removal of the ganglion could be assured. The distal cut end of the vagus was then touched with a cautering pen. In another group of rats, an identical cervical incision was made and the nodose ganglion was touched but not removed.

NTS cannula placements. Sham-operated rats, unilateral-subnodose vagotomized rats, and rats with unilateral nodosectomies were divided into five subgroups for implantation of unilateral injection cannulas aimed for the medial NTS: 1) unilateral nodose ganglionectomy with ipsilateral NTS cannula (n = 15), 2) unilateral nodose ganglionectomy with contralateral NTS cannula (n = 14), 3) unilateral subnodose (cervical) vagotomy with ipsilateral NTS cannula (n = 6), 4) unilateral subnodose (cervical) vagotomy with contralateral NTS cannula (n = 6), and 5) sham surgery (n = 17).

In each case, a 26-gauge guide cannula that accommodated a 33-gauge obturator and injector was directed into the medial NTS as previously described (27). The 33-gauge injectors used with this system extended 1 mm beyond the guide cannula tips. Hindbrain coordinates were as follows: 0.0 mm on the occipital crest, 0.8 mm lateral to the midline, and 7.9 mm ventral to the surface of the skull. Each cannula was then secured to the skull using two stainless steel bone screws and methyl methacrylate bone cement.

Feeding Trials

Subdiaphragmatic vagotomy. Vagotomized and sham-treated rats were allowed to recover from surgery and were maintained on powdered rat chow. Recovery time was defined as the time required for a rat to attain a body weight within 20 g of its presurgery weight or 2 wk, whichever was longer. After reaching their presurgery weight, rats were injected intraperitoneally with MK-801 or saline vehicle following an overnight (16 h) fast. Fifteen minutes after the intraperitoneal injection, rats were presented drinking tubes filled with 15% sucrose and intakes of this solution were measured to the nearest 0.1 ml at 5-min intervals for 30 min. MK-801 tests were alternated with saline tests at 48-h intervals. A total of two MK-801 tests were conducted after the rats had regained their presurgical weights.

Cervical vagotomy and nodosectomy. After recovery and achievement of a baseline sucrose intake, 30-min intake of 15% sucrose was recorded in fasted (16 h) rats after a 50-ml injection of either saline vehicle or MK-801 (1 μg/μl) into the NTS. After a 1-min delay, rats were returned to their individual cages and presented with sipper-tube burettes containing 15% sucrose. Intakes were measured every 5 min over a 30-min feeding period. MK-801 tests were alternated with saline tests at 48-h intervals. By the end of the experiments, each rat received a total of three injections of MK-801.

Verification Procedures

Subdiaphragmatic vagotomy. An intact vagus nerve is essential for CCK-induced suppression of food intake (23). To behaviorally test for the integrity of the subdiaphragmatic vagi, rats were treated with CCK-8 in a manner previously described (19, 31). Briefly, following

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a 16-h fast, vagotomized and sham-vagotomized rats were intraperitoneally injected with CCK. Sucrose intake was measured to the nearest 0.1 ml at 5-min intervals over a 30-min feeding period. The a priori standard for complete vagotomy was any rat that showed a <30% attenuation of intake compared with intraperitoneal saline. Data from animals whose intake of sucrose was suppressed by 30% or more were not included in the study.

After the CCK tests, rats from this experiment were injected intraperitoneally with Fluoro-Gold (two separate injections of 2 mg/ml, 0.5 ml each injection) 4–5 days before death to verify completeness of vagotomy histologically (18). The animals were exsanguinated via a transcardiac cannula, perfused with PBS, and fixed with 4% formalin. Brains were then removed, postfixed in 4% formalin, and cryoprotected in 20% sucrose/PBS. Hindbrains were sliced on a cryostat at 30 μm and alternate sections were floated in PBS for Fluoro-Gold labeling with nickel/diaminobenzidine and thaw mounted on glass slides for fluorescent verification of vagal integrity. The dorsal motor nucleus of the vagus was carefully examined for evidence of Fluoro-Gold labeling.

Cervical vagotomy and nodosectomy. After a baseline and three MK-801 tests were completed, rats were injected intraperitoneally with Fluoro-Gold tracer to verify completeness of the vagotomy and nodosectomy procedures. All rats from both studies were killed 4–5 days after Fluoro-Gold injection. They were transcardially perfused with PBS followed by a 4% formalin solution. Brains were then removed, postfixed, and cryoprotected in 20% sucrose in PBS. Hindbrains were sliced on a cryostat at 30 μm and alternate sections were floated in PBS. Half of the sections were thaw mounted on glass slides and carefully examined for the presence of Fluoro-Gold labeling. The remainder of the sections was thaw-mounted on glass slides and counterstained with Neutral Red for histological verification of cannula placement.

Statistical Analysis

Intakes for all rats are expressed as means ± SE in milliliters. Differences between intakes were evaluated by Student’s t-test or two-way repeated-measures ANOVA, followed by Tukey’s pairwise multiple comparison tests using Sigma statistical software.

RESULTS

Before surgery, all rats significantly (t = 21.78; P < 0.001) increased their 30-min intake of 15% sucrose (Fig. 1) after MK-801 (14.1 ± 0.5 ml ip) compared with intakes after saline vehicle injection (9.7 ± 0.5 ml). This increase in feeding was comparable to increases in intake reported in previous studies (7).

Subdiaphragmatic Vagotomy

As previously reported (6, 13), rats with subdiaphragmatic vagotomies consume more 15% sucrose over 30 min than sham-operated animals (Fig. 2). In other words, in our study vagotomized rats (n = 12) exhibited larger sucrose intakes (15.1 ± 1 ml) over a 30-min feeding period (Fig. 2B) compared with their own sucrose intake before surgery (9.8 ± 0.6 ml; F1,23 = 31.71, P < 0.001). Sucrose intake of sham-operated rats after surgery (n = 10; 9.6 ± 0.7 ml) did not significantly differ from their intake before surgery (8.1 ± 0.6 ml; P = 0.446; Fig. 2A). Presurgical intakes for sham and vagotomized rats did not differ (8.1 ± 0.6 ml for sham rats and 9.8 ± 0.6 ml for vagotomized rats; P > 0.5).

When data from all vagotomized rats (n = 12) are evaluated without regard to postvagotomy baseline intakes, it appears that vagotomized rats (Fig. 3A) did not significantly increase their intake of sucrose in response to MK-801 compared with saline (17.1 ± 1.1 and 15.1 ± 1.1 ml, respectively; P > 0.1). In contrast, sham-vagotomized rats (Fig. 3B) did significantly (F1,19 = 38.10, P < 0.001) increase their sucrose intake in response to MK-801 compared with saline (12.7 ± 0.9 ml vs. 9.6 ± 0.7 ml). However, when vagotomized rats were subdivided into two groups based on their postvagotomy sucrose intake after saline injection, rats (n = 5) that consumed >15
Rats did consume significantly (F(1,110) = 15.1, P = 0.001) their intake of 15% sucrose in response to injection of MK-801 into the NTS, regardless of whether the antagonist was injected contralateral (n = 4; 9.6 ± 0.3 vs. 12.6 ± 0.2 ml) or ipsilateral (n = 5; 9.7 ± 0.4 vs. 14.2 ± 0.6 ml) to vagotomy.

When MK-801 was injected into the NTS contralateral to a surgical nodosectomy (n = 10 positive cannula placements) seven of the ten rats increased their sucrose intake (see Table 1) such that the average intake of this group was significantly increased after MK-801, relative to their intake after saline injection (9.2 ± 1.1 and 12.2 ± 0.9 for saline and MK-801, respectively; F(1,13) = 14.69, P = 0.009; Fig. 6). However, when MK-801 was injected into the NTS ipsilateral to a unilateral nodosectomy (n = 10 positive cannula placements) none of the rats increased their intake (8.3 ± 0.4 and 8.0 ± 0.5 for saline and MK-801, respectively) (P > 0.5; Fig. 6).

All sham-vagotomized rats (n = 14 positive cannula placements) significantly (F(1,27) = 34.21, P = 0.001) increased their sucrose intake after MK-801 injected into the NTS either ipsilateral (n = 6) or contralateral to sham surgery (n = 8). MK-801 injection increased sucrose intake in all of the sham rats, (9.7 ± 0.5 for saline and 14.1 ± 0.5 for MK-801), regardless of whether the injection was ipsilateral or contralateral to the surgery (Fig. 6).

In assessing for any intact vagal afferents after subdiaphragmatic vagotomy, cervical vagotomy, and nodosectomy in the

ml of sucrose (Fig. 4A) did not significantly increase their sucrose intake after MK-801 compared with saline (15.9 ± 1.9 ml and 19.2 ± 0.7 ml, respectively; P > 0.1). On the other hand, vagotomized rats (n = 7) that drank < 15 ml of sucrose after saline injection (Fig. 4B) did significantly (F(1,13) = 51.72, P = < 0.001) increase their intake of sucrose after MK-801 (18.8 ± 1.3 ml) compared with saline (12.6 ± 0.5 ml).

The octapeptide CCK-8 did not significantly attenuate 15% sucrose intake (14.8 ± 1.4 ml) compared with saline injection (15.1 ± 1.1 ml) in any of the subdiaphragmatically vagotomized rats (P > 0.5). On the other hand, sham-vagotomized rats did consume significantly (F(1,19) = 108.99, P = <0.001) less 15% sucrose intake after CCK (3.1 ± 0.6 ml) compared with saline (9.6 ± 0.7 ml).

Cervical Vagotomy and Nodosectomy with NTS Cannula

In this series of experiments, all groups of rats as a whole (preoperatively) significantly (t = 37.46; P < 0.001) increased their intake of 15% sucrose after peripheral injection of MK-801 (14.2 ± 0.4 ml) compared with saline (10.2 ± 0.3 ml). When the preoperative and postoperative sucrose intakes following saline injections were compared in all rats (9.7 ± 0.2 and 9.3 ± 0.3 ml, respectively), no significant differences were seen (P > 0.1) (see Table 1).

Rats (n = 11 positive cannula placements) with unilateral cervical vagotomies (Fig. 5) significantly (F(1,17) = 109.65, P = < 0.001) increased their intake of 15% sucrose in response to injection of MK-801 into the NTS, regardless of whether the antagonist was injected contralateral (n = 4; 9.6 ± 0.3 vs. 12.6 ± 0.2 ml) or ipsilateral (n = 5; 9.7 ± 0.4 vs. 14.2 ± 0.6 ml) to vagotomy.

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experimental animals, sections of the DVC were carefully examined for the presence of retrogradely transported Fluoro-Gold tracer. Fluoro-Gold was not found in any of the sections from lesioned rats, whereas Fluoro-Gold was consistently found in the DVC of sham-treated animals (Fig. 7B), confirming the presence of intact vagus nerves. In addition, histologic sections of the hindbrain from all animals with NTS cannulas were stained with Neutral Red and coverslipped. The location of each cannula tip was determined by examining the corresponding stained sections in conjunction with the rat brain atlas of Paxinos and Watson (15). Location of individual cannula tips in sham-treated, nodose ganglionectomized, and cervically vagotomized rats is depicted in Fig. 8. MK-801 delivered via cannulas placed into the medial nucleus of the NTS yielded feeding responses consistent with previous reports (31) with the exception of the three cannulas in the contralateral nodosectomized group (represented by open stars in Fig. 8). These three rats did not show the typical increase in feeding in response to NTS injection of MK-801 (see DISCUSSION and Fig. 6). The antagonist did not increase intakes of any rat when injected into cannulas whose tips were in locations outside the medial nucleus of the NTS. Data from rats whose cannulas were not positioned in the medial NTS were not included in the statistical analysis.

### DISCUSSION

We hypothesized that increased food intake following MK-801 injection depends on NMDA receptors expressed on vagal afferent terminals or on higher-order vagal sensory neurons in the NTS. Initially, it appeared that the relevant NMDA receptors might be located in the periphery because Burns and Ritter (6) reported that subdiaphragmatic vagotomy attenuated increased food intake after injection of MK-801. Subsequent results, however, indicated that the site of MK-801 action was in the dorsal hindbrain, probably the NTS (27, 28). Our current results, contained in this report, indicate that subdiaphragmatic vagotomy does not abolish increased food intake by MK-801. Instead, these results suggest that central vagal elements, vagal afferent terminals, or postsynaptic neurons driven by these afferents, are essential for increased food intake following MK-801 administration.

Data from the first experiment in our current study suggest that failure to observe MK-801-induced increases in food intake after subdiaphragmatic vagotomy are the consequence of an increase in liquid meal size that results from bilateral vagotomy. Snowden and Epstein (24) reported that vagoto-

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**Table 1. Numbers and intakes for rats receiving vagal manipulation and either contralateral or ipsilateral NTS cannula**

<table>
<thead>
<tr>
<th>Surgical Treatment</th>
<th>Starting Number</th>
<th>Final Number</th>
<th>Average Presurgery Intakes</th>
<th>Average Postsurgery Intakes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral nodose ganglionectomy with ipsilateral NTS cannula</td>
<td>15</td>
<td>10</td>
<td>9.7 ± 0.5</td>
<td>15.2 ± 0.8</td>
</tr>
<tr>
<td>Unilateral nodose ganglionectomy with contralateral NTS cannula</td>
<td>14</td>
<td>10</td>
<td>9.9 ± 0.4</td>
<td>15.5 ± 0.7</td>
</tr>
<tr>
<td>Unilateral cervical vagotomy with ipsilateral NTS cannula</td>
<td>6</td>
<td>5</td>
<td>10.5 ± 0.5</td>
<td>13.7 ± 1.1</td>
</tr>
<tr>
<td>Unilateral cervical vagotomy with contralateral NTS cannula</td>
<td>5</td>
<td>4</td>
<td>9.1 ± 0.5</td>
<td>13.7 ± 0.8</td>
</tr>
<tr>
<td>Sham surgery with unilateral NTS cannula</td>
<td>17</td>
<td>14</td>
<td>9.6 ± 0.6</td>
<td>13.0 ± 0.7</td>
</tr>
</tbody>
</table>

Intakes represent group averages ± SE in milliliters of saline dizocilpine (MK-801) injections. NTS, nucleus of the solitary tract.
mized rats ate smaller, more frequent meals over a 24-h period. However, these investigators also found that if vagotomized rats were deprived of food and were subsequently offered a palatable liquid diet, they ate larger meals than sham-operated rats. We and others (6, 30) have subsequently replicated this increase in meal size after vagotomy. For reasons that are unclear, however, the magnitude of vagotomy-induced increase in food intake varies considerably between vagotomized rats. Examination of hindbrains for retrogradely transported Fluoro-Gold confirmed that all of the vagotomized rats used in the present study had undergone complete, bilateral subdia-
phragmatic vagal denervation. Therefore, we do not have an explanation for the variability of intake in vagotomized rats. Nevertheless, in our hands, some vagotomized rats consumed such large liquid meals that we suspected they were unable to increase their intake in response to MK-801. Indeed, when we examined the intake of vagotomized rats that consumed <15 ml of sucrose solution after saline injection, we found that they significantly increased their meals size in response to MK-801 injection. By contrast, rats whose intake was >15 ml after saline failed to exhibit further increase following MK-801. Thus it appears that prior reports of attenuation of MK-801-induced food intake after vagotomy may represent a ceiling effect due to increased meal size in vagotomized rats.

Continued efficacy of MK-801 for increasing food intake in vagotomized rats is in keeping with the conclusion that MK-801 does not act on peripheral vagal afferent terminals to increase food intake. This evidence against a peripheral site of NMDA action in control of food intake is consistent with our previous findings, which established that increased food intake after MK-801 is mediated by a site in the dorsal hindbrain. For example, we found that injection of MK-801 directly into the NTS increased food intake (27), whereas lesioning of the NTS eliminated increased food intake after intraperitoneal MK-801 injection (28).

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**Fig. 7.** Fluorescence micrographs of coronal sections through the dorsal hindbrain of rats injected ip with Fluoro-Gold, 96 h before death. Vagotomized rats exhibited complete lack of retrograde Fluoro-Gold labeling in the dorsal motor nucleus of the vagus nerve (A), indicating successful vagotomy, whereas sham rats exhibited bilateral labeling of the dorsal motor nucleus (B). Label in the area postrema is an indication of accumulation of blood-borne Fluoro-Gold in both sham and vagotomized groups, indicating a successful injection.

**Fig. 8.** Hindbrain locations of cannulas directed at the NTS in all rats used in statistical analysis. Figure represents brain stem locations −13.24 to −14.08 mm from bregma. Serial sections are listed in groups according to the type of vagal manipulation surgery received and the location of the manipulation (ipsilateral or contralateral) to the cannula. Filled stars, “positive” cannulas (placed in the area of the NTS that has been shown previously to respond to MK-801 injection); filled circles, cannulas placed outside the location of the NTS; open stars, 3 “positively” placed cannulas whose placement and subsequent MK-801 administration did not result in increased feeding.
The fact that MK-801 acts in the caudal hindbrain to increase food intake does not preclude vagal participation in this phenomenon. The cell bodies and central terminals of abdominal vagal afferent neurons are not destroyed by subdiaphragmatic vagotomy. Therefore, it is possible that the central terminals of vagal afferent neurons participate in increased food intake following MK-801. For example, it is possible, even in subdiaphragmatically vagotomized rats, that vagal afferent terminals exhibit a basal release of glutamate that inhibits intake through action on postsynaptic NMDA receptors. It is also possible that descending pathways facilitate glutamate release from vagal afferent terminals that then activate postsynaptic NMDA receptors. Finally, it is possible that NMDA receptors on the vagal afferent terminals themselves modulate the excitation of postsynaptic neurons. Thus, without removal of vagal afferent terminals in the NTS, the potential participation of vagal afferents in increased food intake after NMDA receptor blockade remains uncertain.

An ideal test for the involvement of central vagal afferent terminals in MK-801-induced increase in food intake would involve bilateral removal of the nodose ganglia or bilateral vagal afferent rhizotomy. The problem with this approach is that bilateral nodosectomy or rhizotomies are lethal. However, we reasoned that unilateral removal of the nodose ganglion would be survivable and would result in the degeneration of vagal afferent terminals in the NTS ipsilateral but not contralateral to nodose removal. If central vagal afferent terminals were involved in increased feeding by MK-801, then the effect would be attenuated when MK-801 was administered ipsilateral to nodosectomy but would be retained when MK-801 was administered contralateral to nodosectomy where vagal afferent terminals remained intact. Indeed, we found that injection of MK-801 contralateral to nodose removal evoked an increase in food intake in 7 of 10 rats tested, whereas all 10 rats implanted with cannulas ipsilateral to nodose ganglionectomy showed no increase in food intake in response to MK-801 injection. We interpret these results to suggest that the central terminals of vagal afferent neurons participate in increased food intake by MK-801.

Three of the ten rats injected with MK-801 into the medial NTS contralateral to nodosectomy did not increase their food intake in response to MK-801 (Fig. 8), although these cannulas were histologically positive for placement in the NTS. We cannot offer a definitive explanation for the responses in these rats, because all rats before surgical manipulation responded to MK-801. Examination of retrograde labeling of the DVC with Fluoro-Gold confirmed that nodosectomy completely transected the vagus in these and all other nodosectomized rats. With injection volumes of just 50 nl, it is conceivable that the entire dose of MK-801 may have not cleared the cannula tip and was left inside the cannula itself after withdrawal of the injector.

It might be argued that increased food intake induced by MK-801 injection into the DVC could be attributed to an action of the antagonist on vagal efferent cell bodies and does not depend on intact central vagal afferent terminals. This argument is not defensible, however, because unilateral section of the cervical vagus just below the nodose ganglion, which like nodose removal severs vagal motor axons, did not abolish overconsumption of sucrose. Rats with cervical vagotomies increased their sucrose intake after injection of central MK-801, regardless of whether the antagonist was injected into the contralateral or ipsilateral NTS. Even if disruption of vagal motor fibers in the cervical vagus produced some degeneration of vagal efferent cell bodies, it seems very unlikely that degeneration would be more extensive after nodose removal than it would be after subnodose vagal transection. Finally, it is difficult to conceptualize a mechanism by which vagal efferents could participate in the effects of MK-801 after the fibers that connect them with their peripheral targets have been severed. Therefore, the inefficacy of MK-801 injected ipsilaterally to nodosectomy seems to be attributable to loss of the vagal afferent terminals or to the loss of glutamatergic signals imparted from these terminals to the postsynaptic cell bodies in the NTS.

The mechanism(s) by which central vagal afferent terminals influence food intake after their peripheral axons have been severed, either subdiaphragmatically or cervically, is a matter for speculation. If MK-801 is administered into the NTS where vagal afferents terminate, how is food intake altered at the vagal terminals without peripheral vagal input? One possibility is that release of glutamate from the intact central terminals could be triggered by axoaxonal influences from local or descending central fibers terminating on vagal afferent terminals. MK-801 may increase meal size by antagonizing glutamate released by these axoaxonal influences. Another possibility is that afferent neurons themselves still maintain basal activity even after their peripheral projecting axons are severed (11). Such basal activity at central afferent terminals is suggested to contribute to phantom limb pain. This phenomenon depends on firing of dorsal root neurons that survive peripheral axotomy after amputation (26, 32). Basal activity of nodose neurons would produce glutamate release centrally to act on second-order neuron cell bodies in the NTS. MK-801 might increase meal size by antagonizing basal release of glutamate.

Within each surgical group, an equal number of manipulations (resections or sham treatments) of the right or left vagus nerve or nodose ganglion were done. Tract tracing studies (8, 17) have shown that the left and right vagal trunks supply different abdominal structures. The left dorsal vagal rootlets carry sensory fibers from the common hepatic, accessory celiac, and ventral gastric vagal branches, whereas the right dorsal vagal rootlets receive sensory fibers from the celiac and dorsal gastric vagal branches. In other words, lesioning of right vagal pathways would ablate sensory inputs from the gastric fundus and areas of the proximal duodenum, whereas interruption of the left vagus would eliminate outflow from the cardiac sphincter, lesser curvature of the stomach, most of the pyloric regions of the stomach, and the proximal duodenum. By including examples of left- or right-sided preparations, we were able to examine whether increases in intake induced by MK-801 might be correlated with portions of the stomach and duodenum that are innervated by the left vs. the right vagus nerve. In the end, we were not able to discern any feeding differences that were dependent on a right- or a left-sided cannula placement or manipulation. Of note, the side of the lesion and cannula placement did not account for the absence of an expected response to MK-801. Of the three rats that did not increase sucrose intake in response to MK-801, two had a contralateral cannula in the right NTS and one had a contralateral cannula in the left NTS.
In summary, results of this study indicate that intact subdiaphragmatic vagal fibers are not necessary for increased food intake following MK-801. The data further suggest that what appeared to be an attenuation of the response to MK-801 after complete subdiaphragmatic vagotomy may be due to a vagotomy-induced increase in meal size (ceiling effect). However, our most important conclusion is that central vagal afferent terminals are necessary for increased feeding seen in response to NMDA ion-channel blockade. The necessity of central vagal afferent terminals for increased feeding in response to MK-801 is consistent with a role for NTS NMDA receptors in the control of food intake. The participating NMDA receptors may be located presynaptically on vagal afferent terminals themselves or on higher-order NTS neurons activated by vagal afferent input.

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REFERENCES


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