NMDA receptor participation in control of food intake by the stomach

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Covasa, Mihai, Robert C. Ritter, and Gilbert A. Burns. NMDA receptor participation in control of food intake by the stomach. Am J Physiol Regulatory Integrative Comp Physiol 278: R1362–R1368, 2000.—We previously reported that MK-801 (dizocilpine), an antagonist of N-methyl-D-aspartate (NMDA)-type glutamate receptors, increased meal size and duration in rats. MK-801 did not increase sham feeding or attenuate reduction of sham feeding by intraintestinal nutrient infusions. These results suggested that the MK-801-induced increase in meal size did not depend on antagonism of postgastric satiety signals. Consequently, we hypothesized that the NMDA antagonist might increase food intake by directly antagonizing gastric mechanosensory signals or by accelerating gastric emptying, thereby reducing gastric mechanoreceptive feedback. To test this hypothesis, we recorded intake of 15% sucrose in rats implanted with pyloric cuffs that could be closed to prevent gastric emptying. Sucrose intake was increased when the pyloric cuffs were open, allowing the stomach to empty. However, intake was not increased when the pyloric cuffs were inflated, causing gastric retention of all ingested sucrose. Direct measurements of gastric emptying revealed that MK-801 accelerated the emptying of 5-ml loads of 0.9% NaCl and 15% sucrose. Furthermore, MK-801 also accelerated the rate of emptying of freely ingested sucrose regardless of the volume ingested. Taken together with our previous findings, these results indicate that blockade of NMDA receptors with MK-801 does not increase food intake by antagonizing gastric mechanosensory signals. Rather, it accelerates gastric emptying, and thereby may indirectly reduce gastric mechanoreceptive cues, resulting in prolongation of eating. Modulation of gastric emptying rate by NMDA receptors could play an important role in the control of neural mechanisms.

gastric distension; pyloric cuff; satiety

WE PREVIOUSLY REPORTED that rats increase their intake of solid food or 15% sucrose, but not water, in response to an intraperitoneal or caudal hindbrain injection of dizocilpine (MK-801), a noncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist (8, 34). We hypothesized that MK-801 increased meal size by attenuating satiation signals arising from the gastrointestinal tract. Recently, however, we (11) reported that MK-801 does not attenuate reduction of food intake induced by intestinal infusion of macronutrients, indicating that NMDA receptor activation is not essential for intestinal satiation. Interestingly, our prior work also revealed that increased intake after MK-801 is abolished by bilateral subdiaphragmatic vagotomy but not by systemic capsaicin treatment (9). Subdiaphragmatic vagotomy eliminates both vagal sensory and motor innervation to the abdominal viscera. However, systemic capsaicin does not damage vagal motor fibers (5). Furthermore, although capsaicin destroys most vagal sensory nerve endings in the small intestine (4, 13), it spares much of the vagal sensory innervation to the stomach (4, 25). Collectively, these results suggest that MK-801 might increase food intake by interfering with gastric rather than intestinal satiation mechanisms. Specifically, the NMDA antagonist may increase food intake by directly antagonizing gastric mechanoreceptive inputs. Alternatively, MK-801 could indirectly reduce gastric mechanosensitive feedback by accelerating gastric emptying. To test these possibilities, we performed a series of three experiments. First, we examined the effect of MK-801 on intake of 15% sucrose in rats with indwelling pyloric cuffs that could be inflated to reversibly prevent gastric emptying. We reasoned that if MK-801 directly reduced gastric mechanosensation, the NMDA antagonist would increase intake both when the pylorus was open and when it was closed. On the other hand, should the MK-801-induced enhancement of feeding be due to acceleration of gastric emptying, the antagonist would not increase intake when the cuff was inflated to prevent gastric emptying. MK-801 would increase intake only when the cuff did not occlude the pylorus. In a second experiment, we measured the effect of MK-801 on gastric emptying of infused loads of 15% sucrose or 0.9% saline to determine whether MK-801 actually altered gastric emptying. Finally, in a third experiment, we measured gastric emptying in rats that ingested a small fixed load or a large ad libitum load of 15% sucrose to determine whether altered gastric function occurs under ambient conditions in which MK-801 increases food intake.

METHODS AND RESULTS

Subjects

Adult (350–400 g) male Sprague-Dawley rats were individually housed in a temperature-controlled vivarium with ad libitum access to a standard pelleted rat chow and water, except during experiments. In addition, rat chow was not available during overnight fasts. The rats were maintained on a 12:12-h light-dark
schedule (lights on at 0700) and were habituated to the laboratory conditions for at least 5 to 7 days before surgery.

Surgical Procedures

Pyloric cuff. Pyloric cuffs were constructed and surgically implanted according to previously described methods (12, 22, 36). Briefly, the cuffs consisted of Silastic sheeting (0.005-in. thick) cut into 0.6-mm strips. At one end of each strip, a 20-cm length of Silastic tubing (ID 0.03 in., OD 0.056 in.) was affixed with silicone adhesive (Dow-Corning). Half of the length of each strip was glued flat, whereas the margins of the remaining half were joined to form a watertight pouch. This enabled only one-half of the resulting cuff to inflate and deflate (22). Rats were deeply anesthetized with Metofane, and their stomachs were exteriorized via a midline celiotomy. A cuff was placed around the pyloric portion of the stomach, and the two free ends of the cuff were joined with sutures to form a loose ring. Care was taken to avoid entrapping nerves or blood vessels in the ring. The inflatable half of the cuff was positioned along the ventral aspect of the pylorus. The cuff was then inflated with 0.5–0.7 ml of water to verify its integrity and proper positioning. Finally, the Silastic tubing was inserted through an incision in the muscles of the abdominal wall and tunneled subcutaneously to emerge at the dorsal aspect of the neck. The rats were allowed to recover from surgery for 7 to 10 days before testing began.

After completion of experiment 1, the effectiveness of each cuff was assessed. The first test entailed measuring plasma glucose concentration after gavage of a glucose solution (28). A series of seven tail blood samples were collected over a 3-h period from rats that were deprived of food for 17 h. The first two samples were collected at 15-min intervals. Before the second sample, the pyloric cuff was inflated as it had been during the experimental tests. After the second sample, 10 ml of a 10.5% glucose solution was delivered by gavage. With the cuff still inflated, three more blood samples were taken 20, 60, and 120 min after the gavage. The cuff was then deflated, and two more blood samples were taken 15 and 30 min later. The samples were centrifuged, and plasma glucose concentration was measured using a Beckman glucose analyzer. If blood glucose values for a given rat were the same or higher when the cuff was inflated than when it was deflated, we assumed the glucose solution had escaped through the inflated cuff into the duodenum, and data obtained from that animal were discarded.

The second test performed to evaluate the effectiveness of cuff occlusion involved a postmortem examination (21). Each rat was overdosed with pentobarbital sodium, and its stomach, pyloric cuff, and proximal duodenum were exteriorized via midline celiotomy. The cuff was inflated with 0.5 ml of water, and the rat was gavaged with 15 ml of a dye solution. A hemostat was applied to the cardiac portion of the stomach. After 5 min, a 2-cm segment of the duodenum, immediately distal to the cuff, was resected, opened, and blotted (mucosa side down) on a piece of filter paper. If dye was visible on the filter paper, data for that animal were discarded.

Gastric fistula. Rats participating in experiment 2 were implanted with gastric fistulas according to a previously described procedure (37). Under inhalation anesthesia (Metofane), a stainless steel gastric cannula was inserted through the ventral wall of the nonglandular portion of the stomach near the greater curvature. A piece of Marlex mesh encircled the inner flange of the cannula. The cannula was secured with a purse string suture. The nonflanged end of the cannula was externalized through a left paramedian abdominal incision. The cannula was occluded with a stainless steel screw except during experimental tests.

Experiment 1

Rationale. Previous results from our laboratory demonstrate that MK-801 does not attenuate satiation signals generated by nutrient stimulation of the small intestine (11). However, it is possible that MK-801 increases meal size by attenuating gastric mechanoreceptive signals either by direct sensory blockade or by altering gastric motor function. To examine these possibilities, we measured the intake of 15% sucrose after MK-801 treatment in rats implanted with pyloric cuffs. Deflated pyloric cuffs allow the stomach to empty normally. Therefore, if MK-801 increases food intake by accelerating gastric emptying, then increases in meal size should only occur when the cuff is deflated. When inflated, the cuff occludes the pylorus, effectively retaining all ingesta within the stomach during the experimental period. Thus closure of the cuff makes it possible to examine the effect of MK-801 on intake when all postingestive cues are limited to those arising from the stomach.

Experimental design. Rats (n = 15) weighing from 320 to 350 g at the time of surgery were trained to drink a 15% sucrose solution and then subjected to pyloric cuff surgery, as described above. The rats were allowed a minimum of 10 days to recover from surgery and were refamiliarized with 15% sucrose before experimental testing began. Beginning on postoperative day 2, however, the rats were habituated to cuff manipulations by inflating the cuff with increasing volumes of water. When testing began, the rats were tested every other day, including weekends. On nontesting days, the rats were handled and pyloric cuffs were inflated and deflated so that the rats would be less likely to associate sensations resulting from cuff manipulations with the presentation of food.

On test days, food-deprived (17 h) rats received an intraperitoneal injection of either saline or MK-801 (100 µg/kg) with their cuffs either open (deflated) or closed (inflated). The order of testing was as follows: saline/cuff open, MK-801/cuff open, saline/cuff closed, MK-801/cuff closed. Graduated drinking tubes filled with 15% sucrose were presented 15 min after the injection. Intakes were recorded every 5 min over a 30-min feeding period. A minimum of three tests was conducted under each condition. The resulting data...
were analyzed using two-way ANOVA with repeated measures. Post hoc analyses were performed with paired t-tests adjusted for multiple comparisons.

Results. Four rats failed the cuff verification tests, and one rat developed a pyloric stricture. Therefore, the data from 9 of the original 15 animals were statistically analyzed.

Injection of saline had no significant effect on 30-min sucrose intake in rats with the cuff-open (13.2 ± 0.7 ml) compared with the cuff-closed (11.6 ± 0.7 ml) condition (P > 0.5). Treatment with MK-801 increased intake significantly in the cuff-open condition (18.2 ± 1.4 ml) (P < 0.01) but not in the cuff-closed condition (12.4 ± 1.0 ml) (Fig. 1).

Interpretation. If NMDA receptors participate in monitoring gastric distention, one would expect MK-801 to increase intake when the pyloric cuffs closed. Instead, the antagonist enhanced the intake of sucrose in the cuff-open condition, but not the cuff-closed condition, suggesting that mechanoreceptive signals are unaffected by NMDA receptor blockade.

The fact that MK-801 increased the intake only when the pyloric cuff remained open is consistent with the possibility that MK-801 indirectly attenuates gastric mechanoreception by accelerating gastric emptying. Thus, under the influence of NMDA receptor blockade, ingesta would empty rapidly enough from the stomach such that gastric distension receptors would be minimally activated. Consequently, we conducted two additional experiments to assess whether MK-801 accelerates gastric emptying.

Experiment 2

Rationale. Results from experiment 1 demonstrated that MK-801 does not increase intake when the stomach is prevented from emptying by occluding the pylorus. Therefore, it is conceivable that MK-801 might delay meal termination by altering the rate of emptying. If this hypothesis is correct, then MK-801 should increase gastric emptying. To assess this possibility, we measured gastric emptying of both 0.9% NaCl and 15% sucrose after injection of MK-801 or isotonic saline.

Experimental design. Rats (n = 12) equipped with gastric fistulas were deprived of food for 17 h. At 0900 on test days, they were removed from their cages, the stainless steel screw was removed from their gastric cannulas, and their stomachs were gently lavaged with warm tap water (37°C). After a drainage tube had been attached to their open cannula, the rats were placed in Plexiglas sham-feeding boxes. Their stomachs were flushed twice with warm 0.9% saline, via the drainage tubes, using a 10-ml syringe. A third saline wash contained phenol red (60 mg/l), designed to saturate the gastric mucosa, thereby reducing its absorption during subsequent emptying measurements. At the completion of the three washes, the stomachs were allowed to drain over the ensuing 60 min. The rats then received an intraperitoneal injection of MK-801 (100 µg/kg) or saline (pH 5.3). Fifteen minutes later, 5 ml of warm 0.9% NaCl or 15% sucrose, containing phenol red as a marker, were instilled into the stomach via the drainage tube, after which the tube was clamped. After a 10-min period, the stomach contents were collected, and the rats' stomachs were washed several times with warm saline, which also was collected. Finally, the remaining gastric contents were allowed to drain freely into a graduated cylinder containing the collected gastric contents and washes for an additional 60 min. The contents of the graduated cylinders were measured and centrifuged. The phenol red concentration in the instilled and recovered fluids was measured by spectrophotometry, which was then used to calculate the rate of gastric emptying (19). Gastric-emptying determinations were made on alternate days until emptying of each solution was tested at least three times in response to both saline and MK-801 injections.

Results. Results are expressed as milliliters (means ± SE) emptied per 10-min emptying period. As demonstrated in Fig. 2, intraperitoneal injections of MK-801 accelerated 0.9% NaCl emptying (4.72 ± 0.4 ml) compared with injections of saline (3.54 ± 0.3 ml), [F(1,34) = 7.09, P = 0.01]. MK-801 also significantly increased the rate of 15% sucrose (4.76 ± 0.5 ml) emptying compared with saline (2.93 ± 0.23 ml) [F(1,36) = 14.6, P = 0.001].

Interpretation. The results of experiment 2 suggest that the mechanism by which MK-801 increases food intake in the cuff-open condition is acceleration of gastric emptying. MK-801 accelerated the emptying of both 15% sucrose and isotonic saline solutions when they were administered via gastric cannulas as fixed 5-ml loads. However, during deprivation-induced sucrose ingestion, intakes are two to four times the size of our fixed loads. Therefore, in experiment 3, we assessed the potential for MK-801 to accelerate emptying of sucrose, which was ingested either in limited or unrestricted amounts.

![Graph](http://example.com/graph.png)

**Fig. 1.** Mean 30-min intake of 15% sucrose after an intraperitoneal injection of 0.9% saline or dizocilpine (MK-801) (100 µg/kg) with deflated (open) or inflated (closed) pyloric cuffs. There was no significant difference in intake of sucrose between cuff-open (●) and cuff-closed (○) conditions after intraperitoneal saline. Treatment with MK-801 produced a significant increase in intake of 15% sucrose in cuff-open condition (▼) but not in cuff-closed condition (▼). Error bars indicate means ± SE.
Experiment 3

Rationale. On the basis of the results from experiment 2, increases in intake after treatment with MK-801 may be attributed to accelerated gastric emptying of 5-ml loads. However, in real-feeding situations, sucrose intakes are considerably larger than 5 ml. Evidence suggests that the stomach is very sensitive to changes in volume (21), and it is possible that MK-801 might not alter gastric emptying when these larger volumes of sucrose are ingested. To test this possibility, we examined gastric emptying of 15% sucrose in rats allowed to consume sucrose ad libitum and in rats whose intakes were limited.

Experimental design. A total of 30 rats were assigned to a 2 × 2 factorial design experiment (2 treatment conditions: 0.9% NaCl vs. MK-801, and 2 intake conditions: limited vs. ad libitum). After a stable baseline of sucrose intakes had been established, food-deprived rats (17 h) were given an intraperitoneal injection of either saline or MK-801 (100 µg/kg). Graduated drinking tubes filled with a solution of 15% sucrose were presented 15 min later. Half of the rats were allowed to drink a fixed amount of sucrose (6 ml), after which the drinking tubes were immediately removed, whereas the remaining rats were allowed ad libitum access to the drinking tubes over a 15-min period. All rats were euthanized with CO2 15 min after sucrose presentation. To measure the gastric contents, the stomachs were exposed via a midline celiotomy, ligated at the pylorus and cardia, resected, and weighed. The resected stomachs were incised and rinsed of any residual test meal. Excess liquid was blotted, and the empty stomachs were weighed. The weight of the test meal was calculated by subtracting the initial weight from the final weight of the stomach. Data were analyzed using a two-way ANOVA followed by a Student's t-test.

Results. Two-way ANOVA revealed a significant treatment effect [F(1,27) = 10.23, P = 0.0039] independent of the volume of sucrose ingested [F(1,27) = 0.94, P = 0.34]. Rats offered a fixed volume of sucrose and treated with MK-801 emptied significantly more sucrose during the 15-min feeding test (34.5 ± 2.6% emptied) compared with rats given saline control injection (25.1 ± 3.7% emptied). Likewise, rats that had ad libitum access to sucrose during testing emptied significantly more sucrose after MK-801 injection (31.9 ± 1.9% emptied) compared with rats that received saline injection (21.8 ± 3.2% emptied) (Fig. 3). There was no significant treatment × volume interaction [F(1,27) = 0.01, P = 0.89].

Interpretation. MK-801 accelerated gastric emptying of 15% sucrose compared with saline injection. Rats that drank large amounts of sucrose ad libitum exhibited comparable increases in emptying to those permitted to drink only 6 ml. Thus MK-801 increases gastric emptying under conditions during which it also increases food intake. Inasmuch as both electrophysiological (18) as well as behavioral evidence (20, 21) indicate that gastric mechanoreceptors respond to small changes in gastric distension, our results are consistent with the hypothesis that MK-801-induced acceleration of gastric emptying is responsible for increased meal size after NMDA ion channel blockade.

DISCUSSION

The results of this study suggest that MK-801-induced increases in food intake are due to an increase in the rate of gastric emptying, rather than direct interference with gastric mechanoreceptive cues. MK-801 increased 15% sucrose intake only when the inges-tate was permitted to empty from the stomach. On the other hand, the antagonist failed to increase the intake when the ingested sucrose solution was prevented from leaving the stomach by an occluding pyloric cuff. Finally, direct measurements of gastric emptying revealed that emptying of either ingested or intragastrically infused 15% sucrose was significantly accelerated by prior treatment with the NMDA receptor antagonist.
Enhancement of food intake after treatment with NMDA and non-NMDA receptor agonists or antagonists has been reported independently by several groups (8, 30, 31, 32, 35), but the mechanism(s) by which NMDA receptor blockade produces this effect is not known. Our previous work (6) has shown that the efficacy of MK-801 to increase food intake is a within-meal effect. MK-801 no longer increases intake after a meal has been spontaneously terminated. Consequently, effects of NMDA receptor blockade most likely are due to interference with feedback signals that occur before most postabsorptive effects of ingested food. MK-801 does not increase sham intake of 15% sucrose (11). Taken together, these observations could suggest that MK-801 attenuates signals related to the nutrient or osmotic properties of the ingesta in the gastrointestinal tract. However, our recently reported intraduodenal infusion data clearly demonstrate that MK-801 does not interfere with macronutrient-generated intestinal feedback signals (11). Therefore, if increased meal size observed after NMDA receptor blockade is the result of altered visceral feedback, the alterations are likely to be to gastric feedback.

The results of our pyloric cuff experiments demonstrate that MK-801 does not increase intake when ingesta are confined to the stomach. These findings indicate that MK-801 does not enhance food intake by increasing the compliance of the stomach or by directly antagonizing gastric mechanoreceptive signals. Instead, as suggested by our gastric-emptying experiments, MK-801 may accelerate the rate of emptying of ingesta from the stomach, thereby indirectly diminishing mechanoreceptive feedback signals.

Our previous work indicates that MK-801 increases the size of meals in response to overnight food deprivation or the presentation of a highly palatable diet (8). The rate of emptying of a highly palatable meal under the influence of MK-801 may be rapid enough that filling of the stomach sufficient to terminate feeding is slowed, thereby delaying meal termination. Moreover, in view of the fact that MK-801 does not interfere with macronutrient-generated intestinal feedback signals, it is plausible that the main effect of the antagonist is to facilitate gastric emptying throughout the course of the meal by an action on motor inputs to the stomach. Presumably, meal termination then is accomplished as a result of unimpaired intestinal and/or postabsorptive satiation signals.

The mechanism by which MK-801 accelerates gastric emptying is not known. However, the rate of liquid emptying is controlled by adjustments in gastric tone, intragastric pressure, pyloric resistance, and duodenal pressure (33). Gastric vagal efferents are shown to be potent modulators of each of these gastromotor variables (2). We have shown that the action of MK-801 is dependent on an intact vagus, as subdiaphragmatic vagotomy abolishes its effect on feeding (9). In addition, other specific sources of gastromotor control are documented, including contributions from efferent projections of the splanchnic and hepatic branches of the vagus, intrinsic neurons, and circulating hormones (4, 14). MK-801 might also interfere with one or more of these levels of gastric control. For example, Burns et al. (10) demonstrated that some gastric intrinsic neurons express NMDA receptor mRNA. Therefore, it is possible that some gastric effects of MK-801 are mediated via a direct gastric neuronal action of the drug.

Work by Burns et al. (7) and Bednar et al. (3) indicated that reduction of food intake by CCK is attenuated by prior treatment with MK-801. CCK is a peptide released from intestinal endocrine cells in response to specific nutrients in the intestine. Furthermore, CCK is implicated in the termination of food intake by intestinal nutrients (24). However, mechanoreceptors in the corpus of the stomach are also shown to be sensitive to CCK (23). Furthermore, Schwartz and colleagues (27) provide compelling evidence that CCK may control food intake, in part, via enhancement of gastric mechanoreceptive signals. Therefore, attenuation of CCK-induced reduction of food intake by MK-801 remains compatible with a gastric mode of action for MK-801-induced increase in food intake.

Taken collectively with our previous observations, our current results indicate that NMDA receptor blockade does not interfere with intestinal nutrient detection or gastric mechanoreception. Rather, MK-801 increases the rate of the stomach emptying. The net effect of this accelerated emptying may be an indirect reduction in or delay of gastric mechanoreceptive satiation cues.

Perspectives

Several investigators (15) have reported that removal of ingesta from the stomach evokes ingestion sufficient to replace the volume removed. These reports suggest that gastric fill must reach some critical level for mechanoreceptive signals to inhibit further ingestion. Consequently, it is plausible that modulation of gastric-emptying rate during an ongoing meal may provide an important mechanism for the physiological control of meal size. In this regard, it is interesting that Kaplan and co-workers (15) demonstrated that gastric emptying of a glucose solution is as much as four times more rapid during a meal while ingestion is ongoing than it is after ingestion has terminated. Furthermore, they also reported that gastric emptying during ongoing gastric fill with glucose is under vagal control (16), and therefore could be altered by modulating gastric motor function.

The data presented in our current report provide compelling support for the hypothesis that NMDA receptors participate in control of meal size by modulating the rate of gastric emptying during the meal, thereby delaying the onset or diminishing the intensity of mechanosensitive feedback signals from the stomach. As discussed previously, NMDA receptors are expressed by intrinsic gastric neurons (10), as well as by neuronal elements in the central nervous system (1). Systemically administered MK-801 could enhance gastric emptying through actions at central and/or peripheral sites. Nonetheless, several observations of our own, as well as those from other laboratories, strongly
support the hypothesis that acceleration of gastric emptying and increased meal size are mediated by NMDA actions in the dorsal vagal complex. First, Shinozaki et al. (29) demonstrated that systemic MK-801 increases gastric motility in the rat and that anticholinergic agents or vagotomy both attenuate the MK-801-induced increase in motility. Similarly, our own prior work (9) demonstrates that MK-801-induced increases in meal size are abolished in vagotomized rats. More recently, Treece et al. (34) reported that direct injection of MK-801 into the medial nucleus of the solitary tract increases meal size. Taken together, these results suggest that MK-801’s effects on gastric motor function and meal size are mediated by similar, if not identical, vagal mechanisms.

The source(s) of excitatory amino acid afferents to the dorsal vagal complex and the precise sites where such afferents might increase gastric emptying and meal size are unknown. However, the presence of glutamate in primary vagal sensory neurons (29) and the existence of NMDA receptors both on axon terminals of vagal sensory neurons and on postsynaptic targets of these terminals (1) suggest that modulation of vagal motor output could be mediated by adjusting responsiveness of primary and higher order visceral sensory neurons within the dorsal vagal complex. Such NMDA receptor-mediated modulation of gastric motor function could be of great importance for control of meal size and, hence, control of daily food intake.

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