Intracerebroventricular administration of MK-801 increases food intake through mechanisms independent of gastric emptying

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Intracerebroventricular administration of MK-801 increases food intake through mechanisms independent of gastric emptying. Systemic or hindbrain administration of MK-801, a noncompetitive N-methyl-D-aspartate receptor antagonist, increases meal size. To examine whether MK-801 enhances intake by increasing gastric emptying, we administered MK-801 (2.0 μg/3.0 μl) into the fourth ventricle [intracerebroventricular (ICV)] and measured feeding and gastric emptying of 5-ml NaCl or 15% sucrose loads. In a parallel experiment, we examined food intake and gastric emptying following intraperitoneal (IP) injection of MK-801 (100 μg/kg). MK-801, either IP or ICV, increased 30-min sucrose intake compared with control (12.3 ± 0.7 vs. 9.8 ± 0.5 and 16.6 ± 2.0 vs. 10.7 ± 0.7 ml, for IP and ICV administration, respectively). Also, IP MK-801 increased 5-min gastric emptying of NaCl (4.13 ± 0.1 ml emptied) and sucrose (3.11 ± 0.1 ml emptied) compared with control (3.75 ± 0.2 and 2.28 ± 0.1 ml emptied for NaCl and sucrose loads, respectively). In contrast, ICV MK-801 did not alter NaCl emptying (3.82 ± 0.1 ml emptied) compared with control (3.82 ± 0.3 ml emptied) and actually reduced gastric emptying of sucrose (2.1 ± 0.2 and 2.94 ± 0.1 ml emptied, for MK and vehicle, respectively). These data confirm previous results that systemic as well as hindbrain injection of MK-801 increases food intake. However, because ICV MK-801 failed to increase gastric emptying, these results indicate that MK-801 increases food intake through mechanisms independent of altered gastric emptying.

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N-METHYL-D-ASPARTATE (NMDA)-activated ion channels are present in neurons located in the central (4, 16, 17, 24, 26, 40) as well as peripheral nervous systems, including primary vagal afferent (21) and the intrinsic neurons of the gastrointestinal tract (9, 23, 40). Dizocilpine or MK-801, a noncompetitive NMDA-receptor antagonist, has been shown to enhance food intake when administered centrally (12, 25, 35, 45) as well as peripherally (1, 5–7, 10–14). The mechanism by which MK-801 enhances food intake is not yet clear. Our previous published results demonstrated that systemically administered MK-801 does not increase food intake by attenuating satiation signals generated by nutrient stimulation of the small intestine (11). It is possible, however, that blockade of NMDA receptors increases food intake by increasing the rate of gastric emptying, thereby diminishing the intensity of gastric mechanoreceptive feedback signals from the stomach. However, the effects of MK-801 on food intake are large compared with its modest effects on gastric emptying (10). Therefore, it seems unlikely that the increase in gastric emptying is causally related to the increase in food intake.

Systemically administered MK-801 has been shown to increase feeding by acting in the dorsal hindbrain. Several observations support this conclusion. First, direct MK-801 injection into the nucleus tractus solitarii (NTS) increases meal size (35). Second, lesions of the dorsal vagal complex abolish increased food intake in response to systemic MK-801 administration (36). It is not known whether central administration of MK-801 increases gastric emptying. If MK-801-induced increases in food intake are caused by increases in gastric emptying, then hindbrain administration of MK-801 should increase gastric emptying as well as food intake. To directly test the hypothesis of whether the increase in food intake following administration of MK-801 is due to an increase in gastric emptying, we performed two parallel studies comparing feeding and gastric-emptying responses following fourth ventricular administration of MK-801 with feeding and gastric-emptying responses following systemic administration of MK-801. In the first study, we examined 30-min intake of 15% sucrose and gastric emptying of 5-ml 0.9% saline or 15% sucrose infusion loads following administration of MK-801 to determine whether MK-801 actually accelerates gastric emptying when injected into the fourth ventricle. In the second study, 30-min intake of 15% sucrose and gastric emptying of 5-ml 0.9% saline or 15% sucrose infusion loads were measured following intraperitoneal (IP) administration of MK-801.

MATERIALS AND METHODS

Animals and Drugs

Adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) were individually housed in hanging wire-bottom cages in a temperature-controlled vivarium with access ad libitum to standard pelleted rodent chow (Purina 5001) and water, except during experiments or overnight food deprivation, as indicated below. The rats were maintained on a 12:12-h light-dark cycle (lights off at 1800) and were habituated to laboratory conditions for 1 wk before surgery or initiation of experiments.

The drug used in these experiments was MK-801 (dizocilpine, RBI, Natick, MA), a noncompetitive NMDA-receptor antagonist. MK-801 was dissolved in sterile 0.9% saline and administered via intracerebroventricular (ICV) injection in a volume of 3.0 μl per...
Surgical Procedure: ICV Cannula Implantation

Overnight food-deprived rats weighing 280–330 g were anesthetized with a mixture of acepromazine, ketamine, and xylazine. Rats were placed in the stereotaxic apparatus and implanted with a 23-gauge chronic guide cannula directed toward the fourth ventricle. The stereotaxic coordinates used for the placement of the guide cannula were 2.0 mm rostral from occipital crest, 0.0 mm lateral from the midline, and 6.5 mm below the dura. Access to the ventricle was achieved by using a 30-gauge beveled stainless steel injector that extended 1.5 mm below the tip of the guide cannula. The cannulas were secured to the skull by using stainless steel screws, and the assembly was fixed in methacrylate cement. Rats were allowed to recover to preoperative weights before the initiation of behavioral tests.

Adaptation and Test Procedure

Feeding and gastric-emptying responses following ICV administration of MK-801. After recovery from surgery, rats \( n = 12 \) were placed on a food deprivation schedule with regular laboratory chow ad libitum, except for 16 h of overnight food deprivation every other day, including weekends. Rats were trained to drink 15% sucrose from a burette until 30-min sucrose consumption reached a stable baseline (3–4 trials). At the same time, rats were habituated to inserting a 30-gauge injector attached to a PE-10 tube into the guide cannula. For experimental days, rat chow was removed at 1800 the day before each experiment and was returned the following day after the completion of the experiment. Beginning at 1000 on the day of the experiment, rats were removed from their home cages, the obturators were removed from the guide cannulae, and MK-801 (2 µg in 3 µl sterile saline) or saline alone was infused over 30 s into the fourth ventricle by using a 10-µl Hamilton syringe and dispenser attached to the PE-10 tubing and injector. After the injection was completed, the injector was allowed to remain seated in the guide cannula for an additional 60 s. Subsequently, the injectors were removed, obturators were replaced, and rats were returned to their home cages. Calibrated drinking tubes containing 15% sucrose were immediately presented, and intake was measured to the nearest 0.1 ml every 5 min for 30 min, after which pelleted rat chow and water were returned. Each animal was tested at least twice with the drug and vehicle. All injections were separated by a period of at least 48 h, during which no experimental manipulations occurred. The response to MK-801 was compared with the response after saline administered 48 h before and 48 h after MK-801 injection.

For the gastric-emptying studies, the same rats used in the feeding tests were adapted to experimental conditions for 1 wk before the start of testing. Following an overnight fast, water was removed 1 h before ICV drug administration. At 1000, rats received an injection of either MK-801 or saline. Immediately after injection, 5 ml of 0.9% NaCl or 15% sucrose solution (wt/vol) containing 0.006% phenol red were instilled into the rat’s stomach via an orally inserted 8-Fr polyethylene intragastric tube. Rats were immediately returned to their home cages after gastric load. Following a 5-min emptying period, the tube was reinserted into the stomach, and the remaining gastric contents were withdrawn. The stomach was rinsed repeatedly with 0.9% NaCl until withdrawals were void of any visible phenol indicator. Collected volume was measured, and the gastric contents were centrifuged at 3,200 rpm for 10 min to remove any residual matter. Gastric emptying was measured by dye-dilution spectrophotometry, and absorption was read at 550 nm. The volume remaining in the stomach was determined by comparing the concentration of the phenol red in the original 5-ml load solution to the concentration of phenol red in the gastric rinse solution withdrawn at the end of the gastric-emptying test. Volume remaining was calculated by the following formula:

\[ V = \left( C_0/C_1 \right) V' \]

where \( V \) is the volume of the 5-ml intragastric phenol red load remaining at the end of the gastric-emptying test; \( C_1 \) is the concentration of the phenol red in the original gastric load solution, measured in absorbance; \( C_0 \) is the concentration of the phenol red in the gastric contents withdrawn at the end of the gastric-emptying test; and \( V' \) is the volume (in ml) used to rinse the stomach contents, infused at the end of the gastric-emptying test. Each drug treatment was separated by a minimum of 48 h and was bracketed by a control condition. Rats were tested at least twice under each condition, with all rats receiving the same drug treatment on the same day. These experimental techniques have been detailed previously (20, 30).

Verification of cannula patency and placement. The placement and patency were verified both functionally as well as histologically. For functional verification, at the completion of gastric-emptying experiments, rats were tested for their increase of 30-min 15% sucrose intake in response to fourth ventricular injection of MK-801. Data from animals that failed to increase sucrose intake in both the pre- and post-MK-801 tests were discarded from the analysis. This procedure ensured that injection of MK-801 accessed the fourth ventricular space. In addition, the location of the guide cannulae was determined in histological sections. At the completion of the experiments, rats were anesthetized and transcardially perfused with 0.1 M phosphate buffer followed by 4% formalin. Brains were removed, postfixed in 4% formalin for 4 h, transferred in 20% sucrose solution for overnight cryoprotection, and sectioned at 40-µm sections with a cryostat. The sections were stained with cresyl violet and examined under the microscope to determine the location of the cannula tract.

Feeding and gastric-emptying responses following systemic administration of MK-801. A separate group of rats \( n = 8 \) with a body weight of 320–320 g were used to examine feeding and gastric emptying in response to IP administration of MK-801. For the feeding tests, overnight food-deprived rats trained to consume 15% sucrose solution received either MK-801 (100 µg/kg IP) or saline injection. Fifteen minutes later, rats were presented with burettes filled with sucrose, and intakes were recorded every 5 min for the following 30-min period. Injections were given every 48 h until rats were tested at least twice under each treatment condition. For the gastric-emptying tests, the same rats were adapted to experimental conditions for 1 wk before the start of testing. Following an overnight fast, water was removed 1 h before IP drug administration. At 0900, rats received an IP injection containing either MK-801 (100 µg/kg IP) or physiological NaCl. Fifteen minutes following drug administration, 5 ml of 0.9% NaCl or 15% sucrose solution (wt/vol) containing 0.006% phenol red were instilled into the rat’s stomach via an orally inserted 8-Fr polyethylene intragastric tube. Rats were immediately returned to their home cage after gastric load. Following a 5-min emptying period, the tube was reinserted into the stomach, the remaining gastric contents were withdrawn, and stomachs were cleared with saline. Gastric emptying was measured by using the same method as described above.

Statistical Analyses

Data for each respective study were analyzed separately and are expressed as means ± SE. Cumulative sucrose intakes were analyzed with two-way repeated-measure ANOVA, with drug treatment and time as the main variables. Data for liquid gastric emptying are expressed as percentage of the liquid emptied and were analyzed by one-way repeated-measures ANOVA. Comparisons between the results among treatment means (adjusted) were analyzed by Tukey’s test, with \( P < 0.05 \) considered statistically significant. All analyses were made by using PC-SAS (version 8.02, SAS Institute, Carey, NC) mixed procedure.
RESULTS

Effects of ICV Administration of MK-801 on Sucrose Intake and Gastric Emptying

Results of two-way repeated-measures ANOVA showed a significant effect of fourth ventricular injection of MK-801 on sucrose intake. As demonstrated in Fig. 1, ICV administration of MK-801 (2 μg/3 μl) significantly increased 30-min 15% sucrose intake compared with saline injection (16.6 ± 2.0 vs. 10.7 ± 0.7 ml) [F(1,22) = 11.981; P = 0.002]. The effect of MK-801 on sucrose consumption was evident beginning at 5 min post-sucrose presentation and continued to be significant throughout the 30-min feeding period. Figure 2 shows the effects of MK-801 on gastric emptying of saline and sucrose loads. There was a significant difference between gastric emptying of saline load compared with sucrose load. Following control saline injection, instilled sucrose emptied significantly slower (emptying: 58.9 ± 2.5%) from the stomach compared with saline load (emptying: 76.3 ± 2.9%) [F(1,22) = 11.981; P = 0.002]. Pretreatment of rats with MK-801 had no effect on gastric emptying compared with saline treatment (emptying: 76.3 ± 2.9 vs. 76.4 ± 5.6%). However, injection of MK-801 produced an enhancement of sucrose-induced suppression of gastric emptying (emptying: 42.0 ± 4.2%) compared with control (emptying: 58.9 ± 2.5%) [F(1,32) = 26.197; P < 0.001].

Effects of IP Administration of MK-801 on Sucrose Intake and Gastric Emptying

IP administration of MK-801 increased both sucrose intake and gastric emptying in overnight food-deprived rats. As demonstrated in Fig. 3, ANOVA showed that MK-801 (100 μg/kg) significantly increased 30-min sucrose intake (12.3 ± 0.7 ml) compared with saline injection [9.8 ± 0.5 ml; F(1,47) = 7.846; P = 0.007]. Also, compared with saline injection, administration of MK-801 significantly increased gastric emptying of 0.9% saline load [82.6 ± 2.2 and 74.9 ± 3.0%; F(1,31) = 4.432, P = 0.05, for MK-801 and saline injection, respectively], as well as 15% sucrose load [62.2 ± 1.7 and 45.7 ± 2.9%; F(1,31) = 66.795, P < 0.001, for MK-801 and saline injection, respectively] (Fig. 4).

DISCUSSION

The present results demonstrate that administration of MK-801 into the fourth ventricle increases sucrose intake without increasing gastric emptying. Increased food intake following fourth ventricle or hindbrain administration of MK-801 is consistent with previous reports from our laboratory (12, 35) as well as others (45), which indicate that the hindbrain, especially the medial NTS, is the site where both centrally and peripherally injected MK-801 acts to increase food intake. NMDA-type glutamate receptors are present both on vagal afferent terminals and postsynaptic cell bodies and dendrites in

![Fig. 1. Effect of intracerebroventricular (ICV) administration of MK-801 on food intake. Administration of MK-801 (3 μg/3 μl) into the fourth ventricle significantly increased cumulative 30-min 15% sucrose intake compared with control. Values are means ± SE. *P < 0.01 from saline.](http://alpresse.physiology.org/)

![Fig. 2. Effect of ICV administration of MK-801 on gastric emptying. Fourth ventricular administration of MK-801 (2 μg/3.0 μl) had no significant effect on gastric emptying of 0.9% saline compared with emptying rates following saline administration. ICV administration of MK-801 (2 μg/3.0 μl) significantly enhanced sucrose-induced suppression of gastric emptying compared with control. Values are means ± SE. *P < 0.001 from saline.](http://alpresse.physiology.org/)

![Fig. 3. Effect of systemic [intraperitoneal (IP)] administration of MK-801 on food intake. IP injection of MK-801 (100 μg/kg) significantly increased cumulative 30-min 15% sucrose intake compared with intake after saline injection in food-deprived rats. Values are means ± SE. *P < 0.05 from saline.](http://alpresse.physiology.org/)
the medial NTS (29). Fourth ventricular microinjection (35, 45) as well as nanoinjection of MK-801 into the NTS produce large increases in the intake of solid or liquid foods (35). Finally, lesions of the dorsal vagal complex, which extensively damage the NTS, abolish increased feeding following systemic MK-801 injection (36). These results strongly support the dorsal hindbrain as the site of MK-801 action for increasing food intake.

Our results, reported here, confirm our previous findings that systemic administration of MK-801 accelerates gastric emptying (10). However, in the present experiments, we also found that, unlike systemic administration, fourth ventricular injection of MK-801 did not accelerate gastric emptying of either saline or sucrose gastric loads. Thus, whereas MK-801 increases food intake via an action in the hindbrain, increased gastric emptying must be mediated by MK-801 action at a nonhindbrain site. Therefore, our data indicate that increases in food consumption following ICV administration of MK-801 cannot depend on increased gastric emptying. It could be argued that gastric emptying might be affected differently by gastric intubation vs. oral ingestion of sucrose, because, in our experiments, gastric emptying was not measured in the same experiment as was sucrose intake. Kaplan et al. (15) have shown marked differences in emptying profiles for intragastrically delivered corn oil vs. intraooral infusions. However, for glucose, the same amounts emptied under the matched intragastric and intraooral infusion conditions for each concentration tested. Although the possibility of differences in the emptying profiles of sucrose vs. glucose under this paradigm seems unlikely, this has not been tested.

The mechanisms by which MK-801 acts in the hindbrain to increase food intake are not understood. The fact that increased food intake and increased gastric emptying following MK-801 are not causally related in no way rules out the possibility that MK-801 increases food intake by interfering with gastrointestinal sensory signals. Indeed, we previously published results indicate that MK-801 only increases the intake of calorically active substances (5). It does not increase intake of nonnutritive substances, such as saccharin solutions. Calorically active components of a meal contribute to satiation when they enter the small intestine. Satiation evoked by intestinal infusion of most nutrients is attenuated by vagal deafferentation (38, 39, 43) or capsaicin treatment (34, 41, 42), indicating that the contribution of intestinal nutrient stimuli to satiation is mediated by small unmyelinated vagal afferents. Taking these observations together, one might hypothesize that MK-801-induced increase in food intake is due to antagonism of glutamate released by vagal sensory neurons, responding to the entry of nutrients into the intestine. However, we previously demonstrated that MK-801 does not attenuate maltotriose-, oleate-, or phenylalanine-induced reduction of real or sham feeding (11).

Entry of some nutrients into the small intestine is accompanied by secretion of cholecystokinin (CCK) (3, 22). Previous reports from our laboratory (13) and by others (1) demonstrated that MK-801 attenuates CCK-induced reduction of real feeding. However, we recently demonstrated that MK-801 does not attenuate reduction of sham feeding by CCK (13). Therefore, it appears that MK-801 does not directly antagonize the effects of either CCK or intestinal nutrients. The efficacy of MK-801 in attenuating CCK-induced reduction of real feeding, but not sham feeding, suggests that the NMDA antagonist might attenuate satiation signals that are secondary to some action of CCK. One such possibility is that MK-801 might increase meal size by attenuating gastric mechanosensory signals that are enhanced by CCK actions on the full stomach. In fact, Sengupta et al. (31) have recently shown that systemic administration of glutamate receptor antagonists, including MK-801, attenuates firing of gastric vagal afferent fibers stimulated by gastric distension. Similarly, Zhang and Fogel (44), using single-cell recording techniques, have shown that glutamate antagonists, including NMDA-type receptor antagonists, attenuated firing of neurons in the dorsal motor nucleus of the vagus and the NTS in response to gastric or intestinal distention.

The possible involvement of NMDA receptors in medullary processing of gastric distention signals has been previously examined by Zheng et al. (45), using the c-Fos method. However, the interpretation of their studies was complicated by the fact that MK-801 in itself stimulated Fos expression in the NTS. There was no net suppression of gastric distention-induced Fos expression by MK-801, but only a relative suppression in selective sites, such as dorsomedial, commissural, and gelatinousus subnuclei of the NTS. These findings would support a role for NMDA-receptor-mediated glutamatergic transmission in gastric signal processing, assuming that the blocker and distention activate different populations of neurons. Finally, NTS neurons expressing NMDA-receptor subunit immunoreactivity are activated by gastric distention (2). Collectively, the foregoing observations comprise tantalizing circumstantial evidence that interference with gastric and/or intestinal mechanoreceptive signaling might be the mechanism by which MK-801 increases intake. However, further investigation of this hypothesis is necessary to establish causality.

Whereas it is clear that MK-801 acts in the dorsal hindbrain to increase food intake, the site at which systemically administered MK-801 acts to increase gastric emptying is not known. MK-801 is able to cross the blood-brain barrier (37). Therefore, it is possible that increased gastric emptying is mediated by which MK-801 acts in the dorsal hindbrain to increase food intake. Administration of MK-801 (100 μg/kg IP) produced a significant increase in 5-min gastric emptying of 0.9% saline and 15% sucrose compared with control. **P < 0.001 and *P < 0.05 from saline.

![Fig. 4. Effect of systemic (IP) administration of MK-801 on food intake. Administration of MK-801 (100 μg/kg IP) produced a significant increase in 5-min gastric emptying of 0.9% saline and 15% sucrose compared with control. **P < 0.001 and *P < 0.05 from saline.](http://ajpregu.physiology.org/Downloadable/1465)
by MK-801 action at a brain site other than the dorsal hindbrain. Furthermore, the fourth ventricular data, although compelling, do not rule out the possibility that some mechanisms contributing to enhanced feeding after systemically administered MK-801 may be independent of those observed after ICV administration but may utilize overlapping neural substrates. However, to the best of our knowledge, there is no data available to support this hypothesis. Currently, more compelling data exist to support a peripheral, rather than a central, site of MK-801’s action to increase gastric emptying. For example, immunohistochemical studies confirm the existence of glutamatergic neurons in the gut (2, 9, 19, 23). Glutamatergic immunoreactivity has been reported in myenteric and submucosal plexuses of the rat gut and glutamate, and its transporter is found in both intrinsic and extrinsic innervation (23) in the cell bodies of the enteric neurons and ganglionic neuronephil (28). Expression of mRNA for NMDA-receptor subunit is present in a population of intrinsic neurons in the rat intestine (8, 9), and NMDA receptors are present on the peripheral endings of both vagal and spinal gastrointestinal primary afferents of the intestinal wall (18). Pharmacological studies have ascribed a role for enteric glutamate receptors in the neuronal control of gastrointestinal functions such as motility and secretions (33). For example, concentration-dependent contractions of gastric fundus are produced by glutamate and NMDA (33), and MK-801 administration produces an increase in gastric motility (32). Finally, electrophysiological studies provide another line of evidence that enteric glutamate receptors modulate the activity of the gut (27). All of these data suggest that the peripheral visceral innervation should be the focus of attention to determine the role of NMDA receptors in the control of gastric emptying.

In summary, our results demonstrate that blockade of hindbrain NMDA receptors produces a significant increase in food intake, but does not increase gastric emptying. These results indicate that MK-801 increases food intake by a mechanism independent of increased gastric emptying.

GRANTS

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REFERENCES


