Reduction of food intake by intestinal macronutrient infusion is not reversed by NMDA receptor blockade

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Covasa, M., R. C. Ritter, and G. A. Burns. Reduction of food intake by intestinal macronutrient infusion is not reversed by NMDA receptor blockade. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R345–R351, 2000.—Rats increase their intake of food, but not water, after intraperitoneal injection of MK-801, a noncompetitive antagonist of N-methyl-D-aspartate-activated ion channels. We hypothesized that MK-801 might enhance intake by interfering with intestinal chemosensory signals. To test this hypothesis, we examined the effect of the antagonist on 15% sucrose intake after an intraduodenal infusion of maltotriose, oleic acid, or phenylalanine in both real- and sham-feeding paradigms. MK-801 (100 µg/kg) significantly increased sucrose intake regardless of the composition of the infusate during real feeding. Furthermore, MK-801 had no effect on reduction of sucrose intake by intestinal nutrient infusions in sham-feeding rats. These results indicate that MK-801 does not increase meal size and duration by interfering with signals activated by intestinal macronutrients.

n-Methyl-D-Aspartate (NMDA)-activated ion channels are expressed by neurons in the central (15, 23, 24, 38) and peripheral nervous systems, including primary vagal afferents (1) and the intrinsic neurons of the gastrointestinal tract (10, 11, 18). Several recent studies have shown that NMDA receptors, as well as other excitatory amino acid receptor subtypes, may participate in the control of food intake (8, 19, 32–34, 40). Results from our laboratory indicate that rats increase their intake of solid food or 15% sucrose solutions, but not water, after intraperitoneal (8) or caudal hindbrain (36) injection of dizocilpine (MK-801), a noncompetitive NMDA-activated ion channel antagonist. MK-801 does not cause rats to initiate food intake. Instead, the antagonist increases the size of meals that are initiated in response to overnight food deprivation or the presentation of a highly palatable diet.

MK-801 does not increase the intake of a palatable, but nonnutritive, saccharin solution in food-deprived rats. Furthermore, MK-801 only increases intake of 15% sucrose when the antagonist is administered before or in the early phases of a meal, but not once a meal has ended. In other words, as the end of the spontane-

ous meal approaches, the response to MK-801 diminishes (9). These results suggest that blockade of NMDA receptors attenuates a nutrient-related satiety signal that is generated early in the meal.

The fact that MK-801 enhances the intake of sucrose, but not saccharin, suggests that NMDA receptor blockade may interfere with satiety signals that are generated when specific macronutrients enter the intestine. Intestinal infusions of isotonic solutions of long-chain fatty acids, amino acids, and glucose oligosaccharides all have been reported to reduce real feeding (41) and sham feeding (26, 43). We hypothesized that MK-801 might increase meal size by interfering with the intestinal detection of one or more macronutrient signals. To test this hypothesis, we examined the effect of MK-801 on inhibition of real feeding and sham feeding by rats receiving intraduodenal infusions of maltotriose, oleic acid, or phenylalanine, which are representative of the three classes of macronutrients.

MATERIALS AND METHODS

Animals

Adult (320–400 g) male Sprague-Dawley rats (n = 28) were individually caged in a temperature-controlled vivarium on a 12:12-h light-dark schedule (lights on at 0700). Except during experiments and overnight fasts, the rats had ad libitum access to a pelleted laboratory rat chow. Water always was available, except during testing.

Drugs and Intestinal Infusates

MK-801 (Research Biochemical International, Natick, MA) was prepared at a concentration of 100 µg/ml in a vehicle of sterile isotonic saline (pH 5.3). In all experiments, MK-801 (100 µg/kg) or vehicle was administered by intraperitoneal injection. Our previously published dose-response work indicates that a 100-µg/kg dose of MK-801 is optimal for increasing food intake. Dose-dependent reductions in performance occur with higher concentrations of the antagonist due to heightened locomotor activity and ataxia (8). Oleic acid (Sigma Chemical, St. Louis, MO) was dissolved in isotonic saline containing sodium taurocholate (10 mM) to achieve a final oleic acid concentration of 0.08 kcal/ml, pH 7.4. Maltotriose and phenylalanine (Sigma) were prepared in distilled water to yield a final concentration of 0.52 and 0.13 kcal/ml, pH 7.4, respectively. Tonicity of the infusate solutions was adjusted to 300 mosM by the addition of sodium chloride.

Experiment 1: MK-801 and Macronutrient-Induced Reduction of Real Feeding

Rats (n = 18) were fitted with chronic duodenal catheters, consisting of a 22-cm length of silicone rubber tubing
R346 MK-801 AND INTESTINAL NUTRIENTS

[0.025 in. (ID), 0.047 in. (OD), Dow Corning, Midland, MI] as described previously (6). Briefly, rats were deprived of food overnight and anesthetized with a 1-mg/kg mixture of intramuscular xylazine (4.6 mg/ml; Haver-Mobay, Shawnee, KS) and ketamine (76.9 mg/ml; Aveco, Fort Dodge, IA). One end of the catheter was inserted through a needle puncture in the duodenal wall 2 cm distal to the pylorus and advanced 6 cm within the duodenal lumen in an aboral direction. A small silicone nub placed 6 cm from the intraduodenal end of the catheter was passed through the duodenal puncture until it rested against the mucosal surface. The entry point of the catheter was then surrounded by a 5 × 5-mm ring of surgical polypropylene mesh (Marlex), which in turn was sealed to the serosal surface of the duodenum by a drop of cyanoacrylic cement. This procedure served both to close the duodenum and to secure the catheter in place. The free end of each catheter was tunneled subcutaneously to exit through a 5-mm skin incision over the dorsal aspect of the cranial portion of the neck. The ventral celiotomy and skin incisions were closed in a routine fashion. The exposed end of each catheter was occluded with a stainless steel wire (obturator), which was removed only for flushing of the catheter and nutrient infusions.

After a 10-day postsurgical recovery period, the rats were trained to ingest a 15% sucrose solution after an overnight (16 h) fast, as previously described (6). Rat chow or powdered diet was removed at 1700 the day before each experiment and was not returned until the completion of an experiment the following day (0930). On experimental days, rats were injected intraperitoneally with either MK-801 (100 µg/kg) or saline. Fifteen minutes later, the duodenal catheters were flushed with 0.1 ml of distilled water to assure patency. The catheters were connected to 10-ml syringes, containing the infusates, via PE-50 tubing. The syringes were mounted in motorized syringe pumps (Razel) set to deliver the infusate at a rate of 0.48 ml/min. The rats were then placed in clear Plexiglas cylinders where, over a period of 10 min, they received an intraduodenal infusion of maltotriose, oleic acid, or phenylalanine. Within 5 min of completing the infusion (0900), the rats were returned to their home cages and presented calibrated drinking tubes filled with 15% sucrose. Intake was measured to the nearest 0.1 ml every 5 min over a 30-min feeding period.

Rats received the combination of intraperitoneal saline or MK-801 injection and intraduodenal vehicle or nutrient infusion in the following order: saline-vehicle, saline-nutrient, MK-801-vehicle, and MK-801-nutrient. A minimum of 48 h elapsed between each intestinal infusion and the administration of MK-801. Each nutrient infusion was bracketed (48 h before and 48 h after nutrient infusion) by an infusion of the 0.0 kcal/ml vehicle solution.

The rats were divided into three groups. During the first part of the experiment, one group of rats received infusions of oleic acid while the second group was infused with maltotriose. To account for possible intestinal adaptation to multiple exposures to a specific nutrient, in the second part of the experiment the groups were reversed. The group that received maltotriose was assigned to oleic acid infusion and vice versa. The third group of rats only received infusions of phenylalanine. A minimum of three tests was performed under each intestinal infusion condition in each group of rats.

Experiment 2: MK-801 and Macronutrient-Induced Reduction of Sham Feeding

Rats (n = 10) were implanted with chronic gastric fistulas and intestinal catheters as described by Yox and Ritter (43). Briefly, the inner flange of a stainless steel gastric fistula (13 mm length; 6 mm (ID); 8 mm (OD)) was inserted through the ventral wall of the nonglandular portion of the stomach near the greater curvature and secured with a purse string suture. A piece of Marlex mesh was placed against the outer flange of the fistula, and the nonflanged end of the fistula was externalized through a left paramedian abdominal incision. The lumen of the fistula was closed with a stainless steel screw, except during experiments. Also, a silicone rubber catheter was inserted into the lumen of the duodenum as described for experiment 2.

The rats were trained to sham feed a 15% sucrose solution after an overnight (16 h) fast. Experiments were not begun until the daily sucrose intake for individual sham-feeding rats varied <10% of their individual mean intakes for the previous 7 days. On experimental days, the rats received an intraperitoneal injection of MK-801 (100 µg/kg) or saline. The stainless steel screws were removed from the gastric fistulas, and stomach contents were gently lavaged with warm tap water. The rats were immediately placed in Plexiglas sham-feeding boxes and were presented calibrated drinking tubes filled with 15% sucrose 15 min later. Sucrose intake was measured to the nearest 0.1 ml every 5 min over a 90-min feeding period. Fifteen minutes after presentation of the drinking tubes, the rats received a 15-min intraduodenal infusion of maltotriose at a rate of 0.48 ml/min. During all sham-feeding tests, gastric drainage was collected in plastic graduated cylinders placed beneath the cages and the volume was recorded. If the volume of gastric drainage was less than the volume that was sham fed or if gastric drainage was not observed within 15 s of the start of sham feeding, the data from that test were discarded (43). The intestinal infusates were colored with green food dye to detect reflux of intestinal infusate back into the stomach. If the green coloring appeared in the gastric drainage, the results of the test were discarded.

Rats received combinations of intraperitoneal saline or MK-801 injection and intraduodenal vehicle or macronutrient infusion in the following order: saline-vehicle, saline-maltotriose, MK-801-vehicle, saline-vehicle, and MK-801-maltotriose. Thus at least 96 h elapsed between MK-801 injection and intraduodenal vehicle or nutrient infusions.

A separate group of rats (n = 9) received intraduodenal infusions of oleic acid instead of maltotriose. The protocol was otherwise unchanged from that described for the first part of this experiment. After the experiment was completed, all rats were tested for MK-801-induced enhancement of sucrose intake in a real-feeding paradigm (with their gastric fistulas closed) to confirm their responsiveness to MK-801.

Data Analysis

Results are expressed as mean sucrose intake (ml) ± SE. For experiment 1, the real-feeding data were analyzed using a two-way repeated measures analysis of variance followed by an all pair-wise multiple comparison procedure (Student-Newman-Keuls method). The sham-feeding data from experiment 2 were analyzed as cumulative intakes within designated time periods. The time periods were determined by their relationship to the intraduodenal infusion, i.e., before infusion (period 1), during infusion (period 2), and four 15-min periods after infusion (periods 3-6, respectively).

RESULTS

Experiment 1

Effect of MK-801 on maltotriose-induced reduction of sucrose intake. Intraperitoneal injection of MK-801...
(100 µg/kg) significantly [F(1,59) = 71.26; P < 0.0001] increased 30-min intake of 15% sucrose (15.05 ± 1.06 ml) compared with saline injection (10.4 ± 0.6 ml). On the other hand, a 10-min intraduodenal infusion of maltotriose (0.52 kcal/min) significantly [F(1,59) = 19.20; P = 0.0006] suppressed sucrose intake to 6.49 ± 0.6 ml. However, when maltotriose infusion was preceded by MK-801, sucrose intake was increased to 10.8 ± 0.72 ml (Fig. 1A). Therefore, MK-801 increased sucrose intake by approximately the same amounts after either saline or maltotriose infusions.

Effect of MK-801 on oleic acid-induced reduction of sucrose intake. Intrapерitoneal injection of MK-801 (100 µg/kg) significantly [F(1,82) = 41.48; P < 0.0001] increased 30-min intake of 15% sucrose (16.67 ± 0.86 ml) compared with saline injection (11.56 ± 0.48 ml). On the other hand, 10-min intraduodenal infusion of oleic acid (0.08 kcal/min) significantly [F(1,82) = 42.32; P < 0.0001] reduced sucrose intake to 6.91 ± 0.68 ml. However, when oleic acid infusion was preceded by MK-801, sucrose intake was increased to 9.97 ± 0.88 ml. Therefore, MK-801 increased sucrose intake by nearly the same amounts after either saline or oleic acid infusions (Fig. 1B).

Effect of MK-801 on phenylalanine-induced reduction of sucrose intake. Intrapерitoneal injection of MK-801 (100 µg/kg) significantly [F(1,79) = 49.86; P < 0.0001] increased 30-min intake of 15% sucrose (16.23 ± 0.88 ml) compared with saline injection (12.41 ± 0.39 ml). On the other hand, a 10-min intraduodenal infusion of phenylalanine at a concentration of 0.13 kcal/min significantly [F(1,79) = 45.14; P < 0.0001] suppressed sucrose intake to 9.4 ± 0.53 ml. However, when phenylalanine infusion was preceded by MK-801, sucrose intake was increased to 13.8 ± 0.89 ml (Fig. 1C). Therefore, MK-801 increased sucrose intake by approximately the same amounts after either saline or phenylalanine infusions.

Similar results were obtained during the second part of the experiment in which the groups were reversed, i.e., rats that previously received maltotriose were infused with oleic acid and those that had been infused with oleic acid first were infused with maltotriose (Fig. 2).

Experiment 2

Effect of MK-801 on maltotriose-induced reduction of sham feeding. Fifteen-minute intraduodenal infusion of maltotriose (0.52 kcal/ml) significantly [F(1,38) = 21.92; P = 0.0009] reduced 60-min sucrose sham intake (30.2 ± 3.57 ml) compared with vehicle infusion (48.1 ± 4.7 ml). Injection of MK-801 (100 µg/kg) had no significant effect on sham feeding (44.5 ± 7.5 ml) compared with injection of saline (48.1 ± 4.7 ml) during any time period [F(1,38) = 0.444; P = 0.5201]. Preceding maltotriose infusion with MK-801 treatment did not attenuate maltotriose-induced suppression of sham feeding (28.1 ± 4.2 vs. 30.2 ± 3.57 ml; P > 0.05; Fig. 3A).

Effect of MK-801 on oleic acid-induced reduction of sham feeding. Intraduodenal infusion of oleic acid (0.08 kcal/ml) produced a significant [F(1,38) = 76.03; P = 0.0001] reduction of 60-min sham feeding of sucrose (34.4 ± 4.59 ml) compared with vehicle infusion (48.3 ± 4.46 ml). Treatment with MK-801 (100 µg/kg) had no effect on sham sucrose intake (47.2 ± 6.8 ml) compared with injection of saline (48.3 ± 4.4 ml) at any time period [F(1,38) = 0.586; P = 0.466]. Furthermore, administration of MK-801 before oleic acid infusion
failed to diminish oleic acid-induced reduction of sham intake (29.7 ± 4.6 vs. 34.4 ± 4.6 ml; P > 0.05; Fig. 3B). Under real-feeding conditions (with gastric fistula closed), the same rats increased their 30-min intake significantly in response to MK-801 (13.9 ± 0.7 ml) compared with saline control injection (10.9 ± 0.4 ml; P < 0.00183).

**DISCUSSION**

Our real-feeding experiments demonstrated that intraduodenal infusions of maltotriose, oleic acid, and phenylalanine substantially reduced 15% sucrose intake by real-feeding rats compared with intraduodenal infusions of vehicle solutions. Nevertheless, pretreatment of the rats with MK-801 resulted in an increase in food intake, regardless of the content of the intraduodenal infusates. In other words, MK-801 proved ineffective in reversing the nutrient-induced suppression of intake. We tested only one caloric concentration for each nutrient, and these concentrations produced slightly different degrees of suppression. However, despite whether a given nutrient-generated satiety signal was strong (e.g., maltotriose: >50% suppression of intake) or relatively weak (e.g., phenylalanine: 40% suppression of intake), the inability of MK-801 to reverse these nutrients' effects was consistent.

It is possible that NMDA receptors mediate only a portion of the negative feedback signals that reduce food intake in response to intestinal macronutrient infusions and that NMDA-insensitive mechanisms account for the remainder of the satiation-producing effects of these intestinal infusions. For example, one could argue that, in the real-feeding design, intestinal nutrient infusion generates enterogastric reflexes that...
MK-801 AND INTESTINAL NUTRIENTS

alter gastric tone or emptying. MK-801 may alter the behavioral response to direct macronutrient stimulation of the intestine, but not to enterogastrically induced changes in intragastric pressure, etc. This interpretation is somewhat unsatisfying, however, because the caloric content of the macronutrient infusions (0.384–2.496 kcal) was much less than the caloric content of the ingested sucrose itself (>12 kcal). Thus, if MK-801 increased real feeding by attenuating calorie-related macronutrient signals, one would expect that MK-801 would return intake to the levels observed when the antagonist was administered before intestinal vehicle infusion. Furthermore, because enterogastric reflexes appear to rely on similar afferent mechanisms to those that control feeding (30), it seems somewhat unlikely that MK-801 would attenuate macronutrient effects on feeding without causing similar effects on gastric function. This argument is especially germane in relation to maltotriose infusions, because maltotriose is a carbohydrate and likely reduces food intake via a mechanism similar to that of sucrose itself. Of course, we cannot yet rule out the possibility that NMDA receptors are specifically involved in transmission of signals provided by colligative or chemical properties of 15% sucrose that are not mimicked by our intestinal macronutrient infusions.

One might argue that failure of MK-801 to completely reverse the nutrient-induced suppression of intake could be partly due to the “carry over” effects of MK-801 from one test to the next. Long-lasting effects are a particular concern when dealing with drugs that influence NMDA receptors, because a hallmark of these receptors is their involvement in neural plasticity (13). However, in both real- and sham-feeding paradigms, the MK-801-nutrient infusion condition was bracketed by an NaCl-vehicle infusion condition. In each case, sucrose intakes returned to baseline levels 48 h after MK-801 and/or intraduodenal macronutrient infusions. In addition, no drift in baseline intakes occurred over the course of testing for any of the groups, suggesting that order of testing was not a confounding factor.

In contrast to the significant increase in sucrose intake observed during real feeding, MK-801 did not increase the intake of 15% sucrose by sham-feeding rats. Furthermore, reduction of sham intake produced by intraduodenal infusions of oleic acid and maltotriose was not attenuated or reversed by MK-801. Indeed, sucrose intakes by sham-feeding rats were nearly identical after either MK-801 or saline injection. These results suggest that MK-801’s effects on meal size are not directly mediated by blocking specific macronutrient-derived satiety signals from the intestine. This is an interesting finding, given that MK-801 has been shown (4, 7) to attenuate suppression of intake induced by exogenous CCK. Endogenous CCK is thought to participate in the reduction of sham and real feeding by intestinal infusions of oleic acid (6, 35). Nevertheless, MK-801 did not reverse oleic acid-induced reduction of sham feeding in our experiments. Taken together, these results suggest that, although MK-801 attenuates the suppressive effects of exogenous CCK on food intake, this effect must depend on MK-801’s interference with neural substrates other than CCK-activated nutrient-sensitive afferents. Rather, the results of our sham-feeding experiments would be compatible with the interpretation that MK-801 might actually attenuate feedback signals mediated by gastric feedback mechanisms, some of which are enhanced by CCK (29).

Support for this interpretation may be found in the recently reported work of Zheng and co-workers (44). They demonstrated that fourth ventricular injection of MK-801 reduced gastric distension-induced Fos expression in the dorsal, commissural, and gelatinosal subnuclei of the nucleus of the solitary tract (NTS).

Previous studies showed that MK-801-induced increases in intake are abolished by bilateral subdiaphragmatic vagotomy and attenuated in rats treated with capsaicin (9). Subdiaphragmatic vagotomy eliminates both vagal sensory and motor innervation to the abdominal viscera. By contrast, although capsaicin destroys most vagal sensory nerve endings in the small intestine, it spares much of the vagal sensory innervation to the stomach (5, 17). On the basis of these findings, our current data suggest that NMDA receptors may participate in gastric rather than intestinal satiation mechanisms. Indeed, preliminary results from our laboratory (12) indicate that systemic MK-801 accelerates gastric emptying. Thus the hypothesis that MK-801 interferes with one or more mechanisms that mediate gastric emptying and thereby increased food intake seems plausible.

We recently reported that intake of 15% sucrose is increased by 30-nl injections of MK-801 made into or near the medial subnucleus of the NTS (36). This finding supports the hypothesis that MK-801’s enhancement of food intake may be mediated by altered neurotransmission in the dorsal vagal complex. This area receives dense innervation from vagal sensory fibers from the stomach (27, 39). There also is a substantial amount of both anatomic (24) and electrophysiological (2) evidence for glutamatergic neurotransmission in the medial NTS. Furthermore, anatomic data indicate that NMDA-type glutamate receptors are present both on vagal afferent terminals and postsynaptic cell bodies and dendrites in the medial NTS (28). Finally, recent whole cell patch clamp experiments suggest that NMDA receptor activation of NTS neurons may be caused by vagal afferent inputs (31). Thus the dorsal vagal complex, particularly the medial NTS, is an appropriate site for mediation of MK-801’s action on food intake.

To summarize, our results indicate that MK-801 does not increase food intake by blocking feedback signals generated by macronutrients in the small intestine. Furthermore, although our results do not prove that MK-801 increases food intake specifically by altering gastric sensory feedback or motor influences mediated by NMDA receptors in the dorsal vagal complex, they are consistent with this hypothesis.
Viscerosensory signals from the stomach and intestine provide important direct control of food intake. At this time, the information pertaining to the possibility that NMDA receptors participate in receipt and/or integration of viscerosensory satiety signals is scant. However, such participation could occur at any of the several locations in the neuronal chain linking the gastrointestinal tract with the brain. NMDA receptor mRNA is transcribed by intrinsic neurons of the stomach and intestine in the rat (10, 11). Although there is no direct evidence to implicate these receptors in the control of food intake, some enteric neurons are excited by glutamate. Furthermore, Tsai et al. (37) have reported that glutamate alters gastric acid secretion by the rat stomach in vitro and gastric motility is reported to increase after administration of NMDA antagonists, including MK-801 (37). Despite the fact that gastrointestinal NMDA receptors may play a role in the control of food intake, recent results suggest a predominant role for central NMDA receptors in control of food intake. In this respect, there is increasing evidence to suggest that neurotransmitters in the caudal hindbrain participate in the control of food intake. This assertion is supported by experiments in which administration of MK-801 into the fourth (36) or lateral ventricle (22) as well as the caudomedial NTS (36) is followed by robust increases in sucrose intake.

In addition, the possibility that NMDA receptor blockade increases food intake by modulating the activity of other neurotransmitters has not been extensively investigated. However, at least two groups have reported that depletion of dopamine or dopamine receptor blockade abolishes MK-801-induced increases in intake (4, 22). Also, neurons expressing the D2 and D4 subtypes of the dopamine receptor are concentrated in the medial NTS and the dorsal motor nucleus of the vagus system (16, 25). This suggests that the D2 family of dopamine receptors may be an important element of brain stem mechanisms regulating visceral functions, including those of the gastrointestinal tract. Thus it is possible that MK-801 interacts with dopamine receptors on central hindbrain neurons to delay meal termination.

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MK-801 AND INTESTINAL NUTRIENTS


