Cholinergic neurotransmission participates in increased food intake induced by NMDA receptor blockade

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Covasa, Mihai, Robert C. Ritter, and Gilbert A. Burns. Cholinergic neurotransmission participates in increased food intake induced by NMDA receptor blockade. Am J Physiol Regul Integr Comp Physiol 285: R641–R648, 2003.—MK-801, a noncompetitive N-methyl-d-aspartate (NMDA) receptor antagonist, enhances gastric emptying while increasing food intake. Although we previously reported results implicating the vagus in MK-801’s effect on feeding, it is not clear whether vagal motor fibers participate in the feeding response. Control of gastric emptying is exerted, in part, by cholinergic vagal motor neurons. Therefore, we examined the ability of MK-801 to increase meal size in the presence or absence of the muscarinic receptor antagonist atropine methyl nitrate. Both central and systemic administration of MK-801 significantly increased intake of 15% sucrose. Intraperitoneal injection of atropine abolished MK-801-induced increase in sucrose intake, whereas administration into the fourth ventricle had no effect. To determine whether augmentation of cholinergic tone produces an enhancement of food intake in the absence of MK-801, we tested the ability of cisapride, a gastric prokinetic agent that promotes acetylcholine release through an action on presynaptic serotonin (5-HT4) receptors, to increase sucrose consumption. Cisapride (500 μg/kg ip) induced a small but significant increase in 15% sucrose intake (15.5 ± 0.5 ml) compared with NaCl (13.0 ± 0.6 ml). Furthermore, when MK-801 (100 μg/kg ip) was given in combination with cisapride, intake was significantly higher (19.8 ± 0.9 ml) than following either agent given alone. Pretreatment with atropine abolished the cisapride-induced increase in intake (12.1 ± 0.9 ml) as well as the increased intake induced by combining MK-801 and cisapride. These results suggest that blockade of NMDA-gated ion channels in the hindbrain increases food intake, in part, via a peripheral muscarinic cholinergic mechanism.

atrope; cisapride; gastric emptying; muscarinic blockade.

OVER THE PAST DECADE, EVIDENCE supporting brain glutamate participation in control of food intake has steadily accumulated (2, 6, 10, 11, 21, 26, 40, 43–45). Several laboratories (21), including our own (5–7, 10), have demonstrated that systemic administration of the noncompetitive N-methyl-d-aspartate (NMDA)-activated ion channel antagonist MK-801 increases food intake in rats by delaying the process of satiation. Also, we (43) have shown that microinjections of the NMDA receptor antagonist into the fourth ventricle or the dorsal vagal complex (DVC) of the hindbrain increase meal size. Finally, electrolytic lesions of the DVC abolish MK-801-induced enhancement of food intake (44). These results, and others, were among the first indications that NMDA receptors in the DVC participate in control of food intake.

Identity of the neural elements on which MK-801 acts within the DVC to control food intake is uncertain. There are ample immunohistochemical data to suggest that NMDA receptors are located on vagal motor neurons and vagal sensory terminals in the NTS (34). In addition, in situ hybridization studies (37) reveal that vagal afferent cell bodies in the nodose ganglia express mRNA for NMDA receptor subunits. Finally, our own previous work (7) and that of others (3) suggests that vagal sensory and/or motor neurons mediate increased food intake after MK-801 administration.

Although there is strong support for the notion that vagal neurons and their central connections are necessary for MK-801-induced increase in food intake, the relative importance of vagal motor vs. vagal sensory neurons in MK-801’s effect has not been assessed. Many vagal motor effects are mediated by acetylcholine released at muscarinic synapses (27, 28, 35). In fact, Shinozaki et al. (39) demonstrated that systemic MK-801 increases gastric motility in the rat and that anticholinergic agents or vagotomy attenuates MK-801-induced increase in motility, suggesting that MK-801-induced changes in gastric emptying are mediated by cholinergic vagal fibers.

We hypothesized that cholinergic mechanisms might contribute to increased meal size, after the administration of MK-801. To test this hypothesis, we examined the ability of centrally or systemically administered MK-801 to increase meal size in the presence or absence of the muscarinic receptor antagonist atropine methyl bromide. Because muscarinic receptors are expressed in the DVC as well as on the peripheral targets.

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of vagal motor neurons, we also compared the effect of central vs. peripheral administration of atropine on MK-801-induced increases in meal size. In addition, we tested the ability of cisapride, an agent that increases acetylcholine release by acting on presynaptic serotonin (5-HT₄) receptors, to enhance MK-801-induced increase in food intake.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Simonsen) were housed in individual suspended cages under conditions of controlled illumination (12:12-h light-dark cycle; lights on at 0700), humidity, and temperature (22 ± 2°C). Rats were handled daily and habituated to laboratory conditions before surgery or before testing began. They had ad libitum access to pelletized chow (Teklad) and water except during experiments and overnight fasts.

Drugs

MK-801 (dizolcilpine; RBI, Natick, MA), bombesin (BBS; Sigma), atropine methyl nitrate (Sigma), and cisapride were dissolved in a vehicle of sterile 0.15 M NaCl. All drugs were dissolved in 0.9% NaCl immediately before use.

Cerebral Cannulae

Intracerebroventricular cannula implantations were performed as described previously (43). Briefly, rats were anesthetized with a mixture of acepromazine, ketamine, and xylazine. Guide cannulae (23 gauge) that accommodated 30-gauge obturators and injectors were stereotaxically aimed at the fourth ventricle. The stereotaxic coordinates used to guide cannula placements were derived from the atlas of Paxinos and Watson (29) as previously described (43). The cannulae were anchored with methacrylate and stainless steel screws affixed to the skull. The rats were allowed to recover for at least 10 days after surgery. By this time, all rats exceeded their presurgical body weights and were eating and drinking normally.

Verification of cannula patency and placement. Functional as well as histological verifications of cannula placement and patency were conducted. To functionally verify that the cannulae provided access to the fourth ventricle, rats were tested for reduction of food intake by microinjection of BBS. Fourth ventricular injections of BBS have been shown to reduce food intake (22) and consequently the response to BBS has been used to verify placements of fourth ventricular cannulae. All rats were pretreated with BBS before the MK-801 experiments and they were retested for their response to BBS after completion of their respective experiments. Each rat received a 50-ng injection (2.0 μl) of BBS after an overnight fast (17 h). Five minutes later, rats were presented with 15% sucrose solution, and intake was recorded every 5 min for the ensuing 30-min feeding period. Data from animals that failed to decrease sucrose intake by 40% compared with vehicle control injection in both the pre- and postexperiment tests were discarded from the analysis. This procedure ensured that cannulae used to assess the behavioral response to fourth ventricle injection of MK-801 or atropine actually accessed the fourth ventricular space during the experiment. When verification experiments were completed, the rats were anesthetized and received a 3.0-μl injection of India ink into their cannulae. The brains were removed, postfixed in 4% formalin for 4 h, and examined grossly for reflux of the ink injectate around the cannulae tracts. None of the rats that responded to BBS exhibited reflux. The fixed brains were cryoprotected in 20% sucrose for 24 h and the hindbrain was cut into 30-μm sections with a cryostat. Sections were mounted on glass slides, stained with cresyl violet, and examined microscopically to assess the location of the cannula tract.

Experimental Design

All experiments were routinely started between 0830 and 0930 after an overnight (17 h) fast. For intraventricular studies, the obturators were removed from the guide cannulae, and an injector attached to a dispenser via a Hamilton syringe was inserted into the cannulae. Each injection was made over a period of 1 min and the injector was allowed to remain seated in the guide cannulae for an additional 1 min. Subsequently, the injector was removed, the obturator was replaced, and the rats were returned to their home cages. Calibrated drinking tubes filled with a 15% (wt/vol) sucrose solution were then immediately presented, and intake was recorded. In all experiments, each drug test was bracketed by a saline test. All injections were separated by at least 48 h during which no experimental manipulations occurred. Each series of the treatment combinations was performed at least twice.

Study 1. Effects of peripheral cholinergic blockade on increases in food intake after fourth ventricular administration of MK-801. This study was performed to assess the role of peripheral cholinergic mechanisms in mediating increases in food intake induced by MK-801 when administered into the fourth ventricle. Thus rats were pretreated with atropine injected intraperitoneally. A separate group of rats (n = 6) implanted with fourth ventricle cannulae were injected with one of the following drug combinations: NaCl/NaCl, NaCl/MK-801, atropine/NaCl, or atropine/MK-801. Rats were pretreated intraperitoneally with atropine (500 μg/kg, 1 ml/kg) 15 min before microinjection of MK-801 (1.0 μg/kg, 1 μl volume) into the fourth ventricle. Thereafter, 15% sucrose was presented, and intake was recorded for the ensuing 30 min.

Study 2. Effects of central atropine administration on increased food intake after fourth ventricular administration of MK-801. As previously noted, muscarinic receptors occur in the DMV as well as at peripheral sites (20, 24). Although atropine methyl bromide does not cross the blood-brain barrier (1, 30), the possibility remains that this agent could reach neural elements in the DMV, given that the DMV is more permeable to blood-borne substances than most other brain areas (19, 30). Consequently, an action of atropine at central vagal sites cannot be totally ruled out solely on the basis of the results of peripheral atropine methyl bromide administration. The aim of this experiment was to determine whether increases of food intake after intraventricular administration of MK-801 are mediated by hindbrain muscarinic mechanisms. Adult Sprague-Dawley rats, implanted with 23-gauge guide cannulae, aimed at the fourth ventricle were habituated to consume a meal of 15% sucrose for 24 h and the hindbrain was cut into 30-μm sections with a cryostat. Sections were mounted on glass slides, stained with cresyl violet, and examined microscopically to assess the location of the cannula tract.
tion, previously reported results demonstrate that atropine doses at or below this concentration are sufficient to block other centrally mediated cholinergic responses (15, 17). We chose the 1.0 μg/kg dose of MK-801 because, in previously published dose-response studies (43), we demonstrated that this dose is optimal for increasing food intake when injected into the fourth ventricle.

Calibrated drinking tubes filled with 15% sucrose were presented immediately after the second injection and intakes were recorded every 5 min over a 30-min feeding period. Injections were conducted every 48 h until rats were tested at least twice under each drug combination.

**Study 3. Effects of peripheral cholinergic blockade on systemic MK-801-induced increases of food intake.** Our previously published results (43) suggest that the effects of peripherally injected MK-801 are mediated by its action at hindbrain synapses. Nevertheless, it remains possible that peripheral NMDA receptors might contribute to control of food intake. Conceivably, such peripheral NMDA receptors could act by modulating noncholinergic neurotransmission at peripheral sites. To control for this seemingly remote possibility, we investigated the role of peripheral muscarinic cholinergic neurotransmission in increased food intake after systemically administered MK-801. Overnight food-deprived rats (420–460 g, n = 6) trained to 15% sucrose drinking were injected with one of the following drug combinations: NaCl/NaCl, NaCl/MK-801, atropine/NaCl, atropine/MK-801. Atropine (500 μg/kg ip) was injected 15 min before MK-801 (100 μg/kg ip). Fifteen minutes later, the rats were presented with 15% sucrose and intakes were recorded every 5 min over a 60-min feeding period.

**Study 4. Effects of cisapride on MK-801-induced increases of food intake.** If peripheral cholinergic receptors participate in increased food intake after MK-801, then perhaps increasing acetylcholine release by another mechanism could increase food intake and further enhance the effect of MK-801 on meal size. To test this possibility, we examined food intake by rats treated with cisapride, an agent that acts presynaptically to increase acetylcholine release from gastrointestinal cholinergic terminals (42). Overnight fasted adult Sprague-Dawley male rats (310–340 g, n = 12) trained to consume a 15% sucrose solution received one of the following doses of cisapride: 0, 100, 300, 500, 750, or 1,000 μg/kg ip. Ten minutes later, rats were presented with burettes filled with 15% sucrose and intakes were recorded every 5 min for the following 30-min period. A minimum of two tests was conducted for each dose of cisapride. In the second part of the experiment, the same rats were injected with one of the following drug combinations: NaCl/NaCl, NaCl/MK-801, cisapride/NaCl, or cisapride/MK-801. Cisapride (500 μg/kg) was given 5 min before MK-801 (100 μg/kg). Sucrose was presented 15 min after MK-801 administration, and intake was recorded for the ensuing 30 min.

**Study 5. Effects of atropine on increases in food intake induced by systemically administered cisapride.** The presence of NMDA peripheral receptors has been documented (8, 23, 31, 33). NMDA-induced stimulation of these receptors results in increase in intestinal excitation, which appears to be mediated by myenteric cholinergic neurons (25). Similarly, cisapride has been shown to exert its effect via facilitation of enteric cholinergic neurotransmission. The administration of increased food intake (study 4). Because muscarinic cholinergic transmission is necessary for increased food intake after MK-801 administration, the objective of this study was to examine whether the feeding effects of cisapride also depend on muscarinic cholinergic transmission. A separate group of rats (380–440 g, n = 12) received one of the following drug combinations: NaCl/NaCl, atropine/NaCl, NaCl/cisapride, atropine/cisapride. Atropine (500 μg/kg ip) was given 5 min before cisapride (500 μg/kg ip) administration. Fifteen minutes later, rats were presented with 15% sucrose and intakes were recorded every 5 min over a 60-min feeding period.

**Data and Statistical Analysis**

Only rats that reduced their food intake in response to BBS on both functional tests and whose cannulae allowed ink to enter the fourth ventricle at the time of euthanasia were included in the numbers for the cannulation experiments and were analyzed statistically. Intakes for all rats are expressed as means ± SE in milliliters. Data for all experiments were statistically analyzed with two-way repeated-measures ANOVA, with treatment and time as main factors, using Sigma statistical software. Differences between individual treatments were evaluated by the Student-Newman-Keuls test. P < 0.05 was considered significant.

**RESULTS**

**Study 1. Effects of Peripheral Cholinergic Blockade on Increases in Food Intake by Fourth Ventricular Administration of MK-801**

When examining the effects of systemic administration of atropine on fourth ventricular MK-801-induced increases in food intake, two-way ANOVA revealed significant effects of treatment \( F(3,431) = 37.03, P < 0.001 \), time \( F(5,431) = 13.69, P < 0.001 \) and their interaction \( F(15,431) = 1.8, P = 0.029 \) on food intake. As shown in Fig. 1 and consistent with previous studies, intraventricular administration of MK-801 (1.0 μg/kg) produced a significant increase in 30-min sucrose intake (16.8 ± 1.4 ml) compared with the administration of saline (11.5 ± 0.4 ml, \( P < 0.001 \)). Intrapericapsidal MK-801 and Cholinergic Neurotransmission

![Fig. 1. Effects of peripheral administration of atropine on increases in food intake by fourth ventricular administration of MK-801. Intraventricular administration of MK-801 (1.0 μg) produced a significant increase in sucrose intake compared with the administration of saline. Intraperitoneal administration of atropine (500 μg/kg) alone produced a significant reduction in 30-min sucrose intake compared with saline control test. Pretreatment with atropine produced a partial reversal of MK-801-induced increase of sucrose intake. The data are expressed as means ± SE.](http://ajpregu.physiology.org/DownloadedFrom/)
Data are expressed as means ± SE.

Fig. 2. Effects of fourth ventricular administration of atropine (2 μg) on MK-801-induced increase in food intake. Rats consumed significantly more sucrose after receiving fourth ventricular administration of MK-801 (1.0 μg) compared with the saline-treated group. Atropine alone did not produce any significant changes in sucrose intake compared with saline. Prior treatment of rats with atropine had no effect on MK-801-induced increase in sucrose intake. The data are expressed as means ± SE.

There was a significant overall effect on food intake by treatment [F(3,431) = 82.1, P < 0.001], time [F(5,431) = 22.4, P < 0.001] and their interaction [F(15,431) = 1.8, P = 0.028]. Figure 3 shows the results of this analysis. Specifically, systemic administration of MK-801 (100 μg/kg) produced a significant increase in 30-min sucrose intake (20.8 ± 1.5 ml) compared with intake after saline administration (12.6 ± 0.9 ml, P < 0.001). Intraperitoneal injection of atropine (500 μg/kg) alone caused a small but significant reduction in sucrose intake (10.4 ± 0.9 ml) compared with intake after saline administration (P < 0.01). Prior treatment with atropine completely abolished MK-801-induced increases in sucrose intake (11.8 ± 1.3 ml, P < 0.001).

Study 3. Effects of Peripheral Cholinergic Blockade on Systemic MK-801-Induced Increases of Food Intake

There was an overall significant difference in sucrose intake between treatment conditions [F(3,575) = 69.9, P < 0.001]. Also, there was a significant treatment by time interaction [F(15,575) = 2.1, P = 0.007]. Figure 4 shows that intraperitoneal administration of MK-801 (100 μg/kg) produced a significant increase in 30-min sucrose intake (14.8 ± 0.7 ml) compared with intake after saline administration (11.6 ± 0.6 ml, P < 0.001). The administration of cisapride alone (500 μg/kg ip) caused a significant increase in 30-min sucrose intake (13.4 ± 0.5 ml) compared with intake after saline (P < 0.001). Prior administration of cisapride produced a

Study 4. Effects of Systemic Cisapride on MK-801-Induced Increases of Food Intake

There was an overall significant difference in sucrose intake after saline injection when rats received prior treatment with atropine. There was no significant difference between atropine/NaCl and atropine/MK-801 condition (9.3 ± 0.8 vs. 12.4 ± 0.8 ml, P = 0.01). Enhancement of sucrose intake after MK-801 treatment was reduced to the level of intake after saline injection when rats received prior treatment with atropine. There was no significant difference between atropine/MK-801 and NaCl/MK-801 condition (12.4 ± 1.1 vs. 11.5 ± 0.4 ml, P = 1.0).

Study 2. Effects of Central Cholinergic Blockade on Increases in Food Intake by Fourth Ventricular Administration of MK-801

Two-way repeated-measures ANOVA revealed an overall significant difference in sucrose intake for treatment [F(3,95) = 6.49, P = 0.013] and for time [F(5,95) = 24.3, P = 0.001]. The administration of MK-801 (1.0 μg/kg) into the fourth ventricle caused a significant increase in 15% sucrose intake during the 30-min test (17.2 ± 2.2 ml) compared with saline injection (12.0 ± 0.58 ml, P = 0.03). The administration of atropine (2.0 μg) alone did not produce any significant changes in sucrose intake compared with saline (13.7 ± 1.2 ml, P = 1.0). Prior treatment of rats with the 2.0 μg dose of atropine had no effect on MK-801-induced increase in sucrose intake (17.3 ± 1.6 ml, P = 1.00) (Fig. 2).
NMDA ion channel blocker. This ventricular injection of MK-801, a noncompetitive increase in meal size is attenuated in rats treated with capsaicin to destroy small unmyelinated vagal sensory fibers (3, 7). MK-801 appears to increase food intake by an action in the caudal hindbrain. This was demonstrated by the fact that nanoliter injections of the drug into the DVC increase food intake at doses that are one hundredth the threshold dose for increased feeding after peripheral administration (43). Furthermore, lesions of the DVC abolish increased food intake after systemic MK-801 injections (44).

Our previously reported results (11) suggest that changes in gastrointestinal function accompany MK-801-induced increases in meal size. For example, we have found that systemic MK-801 injection increases gastric emptying of liquid meals and nutritive preloads. The increase in gastric emptying after MK-801 is relatively small compared with the increase in food intake that accompanies administration of the drug. Nonetheless, it is possible that changes in vagovagal gastric reflexes are causally related to increased feeding after MK-801. The fact that atropine attenuated increased feeding induced by MK-801 supports this hypothesis. Control of gastric emptying is mediated, in part, by vagally released acetylcholine, acting at gastric muscarinic receptors. Antimuscarinic agents, such as atropine methyl bromide, reduce antral contractility and substantially reduce gastric emptying rate (13, 28, 41). Glutamatergic inputs to vagal cholinergic substrates may be important not only for gastric emptying but also in enhancing gastric motility. Shinozaki et al. (39) have demonstrated that systemic MK-801 increases gastric motility in the rat and that anticholinergic agents or vagotomy attenuate MK-801-induced increases in motility. Thus inhibition of gastric motility might be a plausible explanation for our observation that atropine eliminated MK-801-induced increase in sucrose intake.

Although a cholinergically mediated alteration of gastrointestinal activity may be necessary for MK-801-induced increases in food intake, it is unlikely that

**Study 5. Effects of Atropine on Cisapride-Induced Increases of Food Intake**

ANOVA detected significant differences between treatments \( F(3, 575) = 40.8, P < 0.001 \). Figure 5 shows that intraperitoneal administration of cisapride (500 μg/kg) produced a significant increase in sucrose intake (13.3 ± 0.6 ml) compared with intake after saline injection (11.5 ± 0.3 ml, \( P < 0.001 \)). The administration of atropine alone (500 μg/kg) caused a significant reduction in sucrose intake (9.9 ± 0.7 ml, \( P < 0.001 \)). Prior treatment with atropine completely eliminated cisapride-induced increase in food intake (8.8 ± 0.8 ml, \( P < 0.001 \)).

**DISCUSSION**

Our most important result is that systemic but not fourth ventricular injection of atropine reverses increased food intake induced by peripheral or fourth ventricular injection of MK-801, a noncompetitive NMDA ion channel blocker. This finding indicates that activation of muscarinic cholinergic receptors in the periphery is necessary for the increase in food intake induced by hindbrain NMDA receptor blockade. Several research groups, in addition to our own, have reported that systemic administration of the NMDA ion channel blocker, MK-801, increases meal size in rats (5, 6, 10, 21, 32). This effect appears to involve vagal sensory neurons, because MK-801-induced increase in meal size is attenuated in rats treated with capsaicin to destroy small unmyelinated vagal sensory fibers (3, 7). MK-801 appears to increase food intake by an action in the caudal hindbrain. This was demonstrated by the fact that nanoliter injections of the drug into the DVC increase food intake at doses that are one hundredth the threshold dose for increased feeding after peripheral administration (43). Furthermore, lesions of the DVC abolish increased food intake after systemic MK-801 injections (44).
increased gastric emptying itself is causally related to increased food intake for two reasons. First, there is not a one-to-one relationship between the increase in food intake and increased gastric emptying of liquid preload after the administration of MK-801. In other words, for every milliliter increase in load emptied, rats increase their intake by 3–4 ml. Therefore, it is clear that increases in gastric emptying after MK-801 cannot be explained on the basis of replacing the emptied load milliliter for milliliter. Second, MK-801 increases intake of solid as well as liquid foods. Yet, we have found that MK-801 does not increase gastric emptying of solid food (unpublished observations). Therefore, the relationship between gastric emptying and increased food intake by MK-801 either is complex or indirect. The fact that systemic atropine completely eliminated MK-801-induced increase in food intake suggests that cholinergic receptors other than those directly involved in gastric emptying mediate NMDA participation in control of food intake. One possibility is that MK-801 increases gastric tone, which is attenuated by atropine. Increased tone may lead to both increased emptying of liquids and decreased satiation. This intriguing hypothesis has not yet been put to a direct experimental test.

Consistent with our previously reported work, central as well as peripheral administration of MK-801 significantly increased food intake in food-deprived rats. In fact, there is compelling experimental evidence indicating that the site of MK-801’s action on food intake is in the DVC of the hindbrain (26, 43, 45). Neurons in this area of the brain express muscarinic receptors (12, 18, 20, 38). Furthermore, the DVC appears to be much more permeable to blood-borne substances than other brain areas (19, 30). Although atropine methyl bromide exhibits minimal penetration of the blood-brain barrier (1, 30), it might easily enter the DVC and block muscarinic synapses there. However, fourth ventricular administration of atropine methyl bromide did not attenuate MK-801-induced increase in food intake, indicating that NMDA receptor blockade does not depend on hindbrain muscarinic receptor activation. We cannot rule out the possibility that atropine methyl bromide acts elsewhere in the brain to inhibit the effect of MK-801. However, this interpretation seems unlikely, because other candidate areas for muscarinic/NMDA receptor interaction in the control of food intake do not exhibit the deficiency of blood-brain barrier present in the DVC.

As noted above, the administration of MK-801 (100 μg/kg) significantly increased food intake, consistent with previously reported results from this laboratory. However, the magnitude of this increase varied between studies. For example, MK-801 produced a larger increase in food intake in study group 3 compared with study group 4. We have previously observed variability in the magnitude of the response to MK-801 among groups of animals. We cannot account for the source of this variability in response, and would only note this sort of variation. In any event, it is notable that atropine attenuates MK-801-induced increase in meal size in all our groups, regardless of variation in magnitude of response to MK-801. We believe that an appreciation of the source of variation in MK-801 responsiveness might help to illuminate the role of NMDA receptors in control of meal size. On the other hand, the fact that we do not understand the source of this variation does not diminish the strength of the data supporting our conclusion that muscarinic cholinergic transmission is necessary for increased food intake after MK-801 administration.

We found that food intake was increased after the administration of cisapride when it was given alone. In addition, we found that the coadministration of cisapride and MK-801 increased food intake more than the administration of either substance by itself. Cisapride enhances release of acetylcholine from intrinsic myenteric neurons, by acting as an agonist at 5-HT4 receptors on their presynaptic terminals (4). This action of cisapride presumably enhances cholinergic neurotransmission stimulated by other inputs, such as preganglionic vagal inputs. Laboratory studies using animal models and humans indicate that cisapride enhances propulsive activity in the stomach, small intestine, and colon (14, 16, 27, 35). The motility-enhancing effects of cisapride extend to the entire gastrointestinal tract. Cisapride enhances antral contractile amplitude, improves antroduodenal coordination and accelerates gastric emptying of liquid, semi-solid, and solid test meals (36). Cisapride exhibited a bell-shaped dose-response curve for enhancing sucrose intake (data not shown). This dose relationship is similar to those obtained for cisapride’s effects on the motility index of antrum, ileum, and colon muscle strips in guinea pigs, suggesting that cisapride may be autoinhibitory and that receptors for cisapride might consist of high- and low-affinity sites (9). Not only did cisapride alone induce an increase in sucrose intake, the effect of cisapride was added to that of MK-801, further increasing food intake. The fact that atropine methyl bromide abolished increased feeding after cisapride alone, as well as after the coadministration of cisapride and MK-801, indicates that both drugs depend on muscarinic cholinergic transmission for their effects on feeding. However, the fact that the effects of the two agents are additive suggests that the mechanisms by which they may alter cholinergic transmission are different. As mentioned earlier, we have demonstrated that MK-801 acts in the DVC to increase food intake. That effect probably is mediated by enhancement of vagovagal control of gastrointestinal motility or tone. We did not determine whether centrally administered cisapride could alter food intake. However, we suspect the cisapride’s effect on feeding is mediated by its well-documented action at peripheral enteric terminals. Therefore, we would suggest that MK-801 increases the activity of cholinergic enteric neurons by blocking NMDA receptors that influence vagal preganglionic input to the myenteric plexus. Cisapride, on the other hand, would further enhance enteric cholinergic transmission by increasing acetylcholine release from the enteric neuron terminals.
In conclusion, peripheral administration of atropine methyl bromide attenuates increased food intake induced by central or peripheral administration of MK-801, a noncompetitive NMDA ion channel blocker. This suggests that MK-801’s effect on feeding is mediated by a vagally driven modulation of peripheral cholinergic muscarinic transmission. The fact that cisapride, an agent known to increase acetylcholine release by acting on the terminals of enteric neurons, also induced an atropine-sensitive increase in food intake suggests that gastrointestinal cholinergic transmission may play a role in control of food intake. Although we have demonstrated small increases in gastric emptying after MK-801, it is unlikely that alteration in gastric emptying itself accounts for cholinergically mediated effects of MK-801, because changes in emptying are too small to account for the robust increases in food intake that follow central or peripheral administration of the NMDA channel blocker. Rather, it is likely that cholinergically induced changes in gastrointestinal tone affect satiation signals generated in vagal afferent neurons. This hypothetical mechanism is consistent with our previous results indicating that MK-801-induced increases in food intake are attenuated by capsaicin treatment, which damages vagal sensory neurons but not vagal motor neurons.

DISCLOSURES

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