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The Non-Competitive NMDA Antagonist MK-801 Increases Food Intake in Rats

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BURNS, G. A., AND R. C. RITTER. *The non-competitive NMDA antagonist MK-801 increases food intake in rats.* PHARMACOL BIOCHEM BEHAV **56**(1) 145–149, 1997.—A role for excitatory amino acids in the control of feeding behavior has not been extensively investigated. Nevertheless, there is direct and circumstantial evidence to indicate that some circuits involved with feeding behavior include glutamatergic elements. To test the hypothesis that endogenous glutamate participates in the control of food intake, we performed experiments to determine whether MK-801, a non-competitive *N*-methyl-D-aspartate (NMDA) ion channel antagonist, is capable of altering intake of liquid and solid foods in hungry or satiated rats. Following a 16 h fast, intake of 15% sucrose was significantly enhanced by systemic treatment with MK-801. Water intake was not altered by the NMDA antagonist. Rats did not ingest more rat chow after MK-801, unless they had been fasted. When a more palatable food (cookies) was offered, MK-801 did increase intake. Thus MK-801 enhanced food intake only when feeding was initiated by food-deprivation or increased palatability. In conclusion, our results support the hypothesis that endogenous glutamate plays a role in the control of food intake. Blockade of NMDA receptor function by MK-801 may diminish or delay satiety signals, rather than initiate feeding behavior per se.

Feeding Intake Satiety Glutamate *N*-methyl-D-aspartate MK-801 Receptor Antagonist

EXCITATORY amino acids (EAA), such as glutamate, or their binding sites have been described in brain areas that are implicated in the control of food intake. For example, neurons or terminals in the nucleus tractus solitarii (NTS) release glutamate (1, 9). Areas of the hypothalamus, associated with the control of food intake, express mRNA for ionotropic glutamate receptors (21). In addition, mRNA coding for the R1 subunit of the *N*-methyl-D-aspartate (NMDA) receptor has been localized to intrinsic neurons of the stomach and small intestine (5). Thus, the central and peripheral distribution of EAA or EAA receptors is compatible with their participation in the control of food intake.

To test the hypothesis that endogenous glutamate participates in the control of food intake, we examined intake of liquid and solid foods following systemic administration of MK-801, a non-competitive NMDA receptor antagonist. We observed that, when feeding was driven by prior food deprivation or increased palatability, MK-801 increased intake. However, in non-hungry rats with ad lib access to rat chow, MK-801 did not increase food intake.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (300–450 g) were caged individually in a temperature-controlled vivarium on a 12/12 light/dark schedule (lights on at 0700). Except as noted, they had ad lib access to pelleted laboratory rodent chow and water. All experiments were begun at 0900 h and conducted at intervals of at least 48 h, unless otherwise specified.

Drugs and Treatments

Dizocilpine (MK-801, Research Biochemical International, Natick, MA) was prepared for injection in a vehicle of sterile isotonic saline (pH 5.3). In each of the following experiments, an intraperitoneal injection of MK-801, or its vehicle, was administered 15 min prior to the presentation of sucrose, water, rat chow, or cookies.

Experimental Protocols

For Experiment 1, rats (n = 12) were adapted to ingest a 15% sucrose solution, following an overnight (16 h) fast, as

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previously described (3). Briefly, rat chow was withheld beginning at 1700 h on the day prior to a test. At 0900 the next day, 15% sucrose was presented in calibrated tubes and intake was measured to the nearest 0.1 ml every 5 min for a total of 30 min. The sucrose was removed and rat chow was returned immediately after each 30 min trial. On experimental days, an intraperitoneal injection of MK-801 or vehicle was given 15 min prior to the presentation of sucrose. The rats were tested with MK-801 in a descending series of doses (300, 100, and 50 μg/kg). Intake after each dose of MK-801 was compared to the average intake, following vehicle injections given 48 h prior to and 48 h after each MK-801 dose.

For Experiment 2a, rats (n = 12) were deprived of drinking water, but not food, for 16 h. Fifteen minutes after an injection of MK-801 (100 μg/kg) or vehicle, water was returned and its intake was recorded at 5 min. intervals for 30 min. In Experiment 2b, a separate group of 6 rats was water deprived for 16 h. When water was returned, intake was measured hourly for 6 h and finally at 24 h after vehicle or MK-801 injection. In the above experiments (2a and 2b), each treatment was administered two times. The order of treatment was: vehicle on the first experimental day and MK-801 on the second experimental day. Then, the same 6 rats were retested with MK-801 on the first day and vehicle on the second experimental day.

For Experiment 3, rats (n = 6) were fed laboratory rodent chow ad lib on the cage floor. The rats were deprived of rat chow but not water overnight (16 h). At 0845 h, the rats received injections of vehicle or MK-801 (100 μg/kg). Rat chow was returned on the floor of the cages at 0900 h and food and water intake were measured hourly for 6 h and also at 24 h after injection. The order of treatment was: all 6 animals received vehicle on the first experimental day and MK-801 on the second experimental day, 48 h later. One week later, this experiment was repeated with the rats receiving MK-801 on the first experimental day and vehicle on the second experimental day, 48 h later.

In Experiment 4, we examined the effect of MK-801 on intake of laboratory rat chow by non-deprived rats (n = 6). The animals were floor fed and on experimental days, the amount of rat chow and water consumed, following intraperitoneal injection of MK-801 (100 μg/kg) or vehicle, was recorded hourly for the ensuing 6 h, beginning at 0900 h. A 24 h measurement was also taken at 0900 h the following day.

In preparation for Experiment 5, rats (n = 12, composed of the 6 rats used in Experiment 4 and 6 naive rats) were adapted, over a period of 3 weeks, to eating Nabisco vanilla wafers (cookies) from the floor of their cage, with intake measured 2 h after wafer presentation. Experimental testing began when each animal's intake of wafers did not vary by more than ± 0.5 g on the last three days of their adaptation period. Wafer intake experiments were scheduled at least 72 h apart to assure that animals were satiated for rat chow on the morning of the experiment. Thus, food intake was driven solely by the presentation of preferred vanilla wafers to presumably non-hungry rats. On experimental days, wafers were presented at 0900, 15 min after injection of vehicle or MK-801 (100 μg/kg). Intake of vanilla wafers was recorded 2 h later.

Statistical Analysis

All results are expressed as means ± SE. For experiments where a single intake measurement was made at 2 h (Experiments 4 and 5), comparisons between MK-801 (100 μg/kg) and saline were made using Student's t tests. All other comparisons consisted of appropriate repeated measures analysis of variance, followed by multiple comparisons with Bonferroni t-tests.

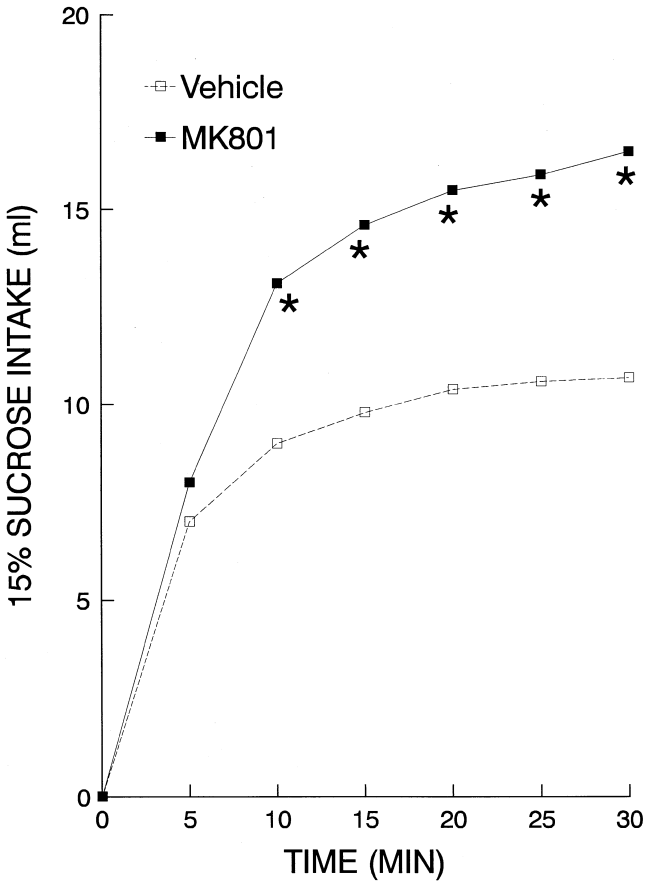

FIG. 1. Thirty minute intake of 15% sucrose solution by rats (n = 12) deprived of food for 16 h and then treated with MK-801 (100 μg/kg) or vehicle. Rats consumed significantly more [$F(3, 264) = 223.38$, $p < .001$] 15% sucrose after receiving an intraperitoneal dose of MK-801 than after vehicle injection. MK-801 significantly increased intake beginning at 10 min and this effect continued throughout the remainder of the experiment (30 min).

RESULTS

Repeated measures ANOVA detected significant differences in 15% sucrose intake following treatment with 0, 50, 100 or 300 μg/kg MK-801 [$F(3, 264) = 223.38$, $p < .001$]. Post hoc multiple comparisons revealed 30 minute sucrose intake was significantly greater following injection of 50 (12.9 ± 0.8 ml) or 100 fg/kg (16.6 ± 0.7 ml) MK-801 compared to injection of saline (10.7 ± 0.5 ml) ($p < 0.05$). Thus, increased sucrose intake after MK-801 appeared to be dose-dependent. However, following the 300 μg/kg dose, rats were uncoordinated and ataxic, which interfered with their ability to approach and lick the spout of the burette. Hence, a 300 μg/kg dose of MK-801 caused a significant reduction of sucrose intake (4.6 ± 2.1 ml). Figure 1 illustrates the 30 min intake of 15% sucrose for the optimal 100 fg/kg MK-801 dose. Note that this intake was significantly increased, relative to that which followed an injection of saline vehicle, as early as 10 min. post-MK-801.

When animals were deprived of water, but not food, for 16 h, MK-801 injection did not increase water intake relative

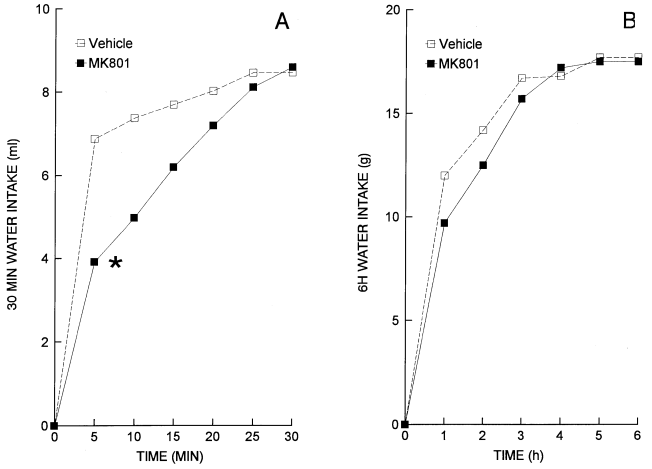

FIG. 2. Drinking following 16 h of water deprivation in rats treated with MK-801 or vehicle. A. After rats ($n = 6$) were deprived of water, but not food, for 16 h, MK-801 (100 mg/kg) did not increase 30 min water drinking relative to intake following isotonic saline (vehicle) injection. A statistically significant [$F(1, 25) = 6.63$, $p < .05$] decrease in water intake occurred 5 min after the MK-801 injection, but at no time did the rats drink more water. B. In a separate experiment, MK-801 (100 mg/kg) did not significantly increase [$F(1, 30) = 0.434$, $p = 0.539$] 6 h water intake by 16 h water-deprived rats ($n = 6$).

to intake following isotonic saline (vehicle) injection (See Fig. 2a,b for details).

MK-801 significantly increased [$F(1, 30) = 54.58$, $p < .001$] the intake of pelleted rat chow following a 16 h fast (Fig. 3). Following MK-801 (100 μg/kg), rats fasted for 16 h ate 9.2, 11.6, 13.2, and 13.7 ± 0.5 g at 1, 2, 3 and 4 h. After vehicle injection, the same rats ate 6.0, 8.0, 9.4, and 11.1 ± 0.5 g at 1, 2, 3 and 4 h, respectively.

MK-801 treatment did not significantly increase [$F(1, 30) = 0.0587$, $p = .818$] the intake of rat chow in non-deprived animals (Fig. 4a). However, increased food intake could be induced in non-deprived animals by providing a highly palatable ration. After MK-801, non-deprived rats ingested more ($p < .02$) vanilla wafers over the 2 h test period (10.9 ± 0.8 g) than they did following vehicle treatment (7.8 ± 0.7 g) (Fig. 4b).

DISCUSSION

Intraperitoneal injection of MK-801, a selective, noncompetitive antagonist for NMDA-activated ion channels, enhanced food intake in satiated rats, presented with a highly preferred food, or those eating after 16 h of food deprivation. Food-deprived rats, treated with MK-801, consumed significantly more of a 15% sucrose solution than they did after a control injection. MK-801 also increased intake of pelleted rodent chow, following a 16 h fast. However, MK-801 did not increase water intake, after an identical period of water deprivation. These results indicate that MK-801 selectively increases food intake and suggest that EAAs may participate in the control of food intake via an action at NMDA receptors.

Rats did not ingest more rat chow in the presence of MK-801, unless they had been fasted. In other words, MK-801 did not elicit intake of rodent chow. Rather, the NMDA antagonist enhanced or prolonged intake that was initiated in response to deprivation. Nevertheless, prior food deprivation was not a strict prerequisite for MK-801-induced increase in food intake. When a highly palatable solid food (cookies) was substituted

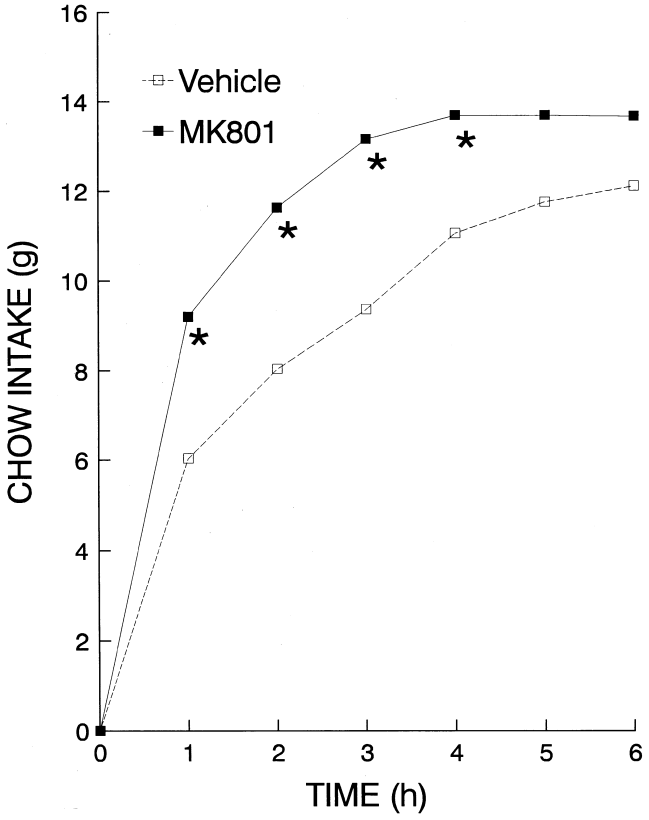

FIG. 3. Intake of rat chow in animals deprived of food for 16 h prior to receiving an injection of MK-801 (100 mg/kg) or vehicle. MK-801 significantly increased [$F(1, 30) = 54.58$, $p < .001$] intake of pelleted rat chow in 16 h food deprived rats. Intake following MK-801 was significantly greater beginning at 1 h post injection. At 2 h post-injection, MK-801 significantly ($p < .003$) increased food intake.

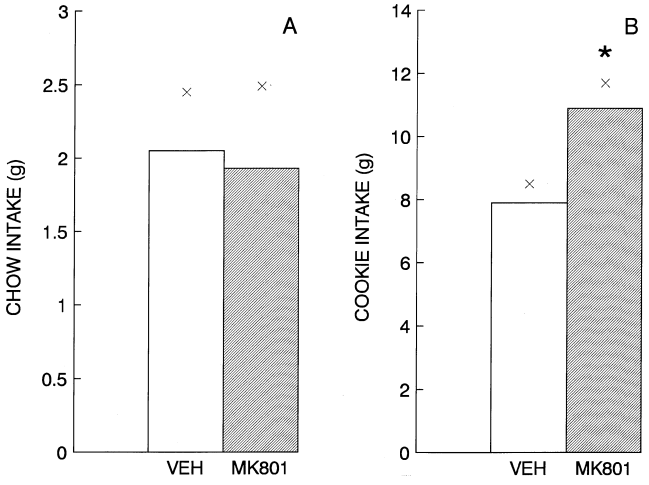

FIG. 4. Two hour intake of rat chow or cookie (mean ± SEM) by non-food deprived rats treated with MK-801 (100 mg/kg) or vehicle. A. In the absence of prior food deprivation, MK-801 treatment was not associated with any significant [$F(1, 30) = 0.0587$, $p = .818$] change in intake of rat chow ($n = 6$). B. MK-801 did increase food intake ($n = 6$).

for rodent chow, satiated rats did increase their intake in the presence of MK-801. These observations indicate that NMDA receptor antagonism enhances ongoing ingestion or delays its termination.

It is possible that the action of the antagonist is to induce a non-specific perseverative effect on an ongoing behavior. Perseveration, a condition in which responses or activities are inappropriately continued or repeated, has been described for suckling in genetically obese mouse pups (22) and adults with damage to the basal ganglia (8, 12). Induction of perseveration by MK-801 seems unlikely, however, because the antagonist did not increase water drinking, a behavior with a similar topology to 15% sucrose consumption.

An alternative interpretation is that NMDA receptor blockade interferes with the processing of signals that participate in termination of eating -- satiety signals. This interpretation is consistent with previous data (2), indicating that MK-801 delayed rejection of liquid food infused directly into the mouth via a cheek fistula in reserpinized rats. However, under their rather complex experimental conditions, prolongation of consumption might be a reflection of altered oral reflexes associated with higher dosages of MK-801 than we used in our experiments. Our experimental setting, which requires neurologically competent appetitive behavior, obviates this possibility. It is possible, however, that NMDA receptor antagonism prolongs intake by enhancing the positive orosensory qualities of food in some way, once feeding has been initiated. The results of these experiments do not allow one to confidently rule out either of the latter two interpretations.

The neuroanatomical site(s) of action through which MK-801 increases feeding is unknown. The potential exists for NMDA receptor involvement in the control of food intake at several levels of the central and peripheral nervous system. Intake of both food and water can be elicited in non-deprived rats by the local administration of NMDA receptor antagonists into the median raphe (23, 24). These results differ from our own in that, in our study, MK-801 did not increase water intake when food was not present. In addition, MK-801 did not increase water intake following water deprivation. Therefore, while the median raphe is a site that merits further analysis with regard to NMDA receptor participation in control of food intake, it would be premature to conclude that the effects observed after peripheral MK-801 administration are referable to an action in the raphe.

The investigations of Stanley and coworkers (16) have shown that a number of EAA agonists, including kainic acid, D, L-alpha-amino-3-hydroxy-5-methyl-isoxazole proprionic acid (AMPA), and NMDA, when injected into the lateral hypothalamus of satiated rats, elicit transient, intense eating. Apparently, the lateral hypothalamus contains neurons that can be activated by any of several EAA receptor subtypes. Recently, Stanley and Butterfield (15) reported that food intake, elicited by an intra-hypothalamic injection of NMDA, could be blocked by co-injecting 7-chlorokynurenic acid, an antagonist of its glycine receptor site. Furthermore, administration of this NMDA-glycine receptor antagonist by itself reduced food intake in 24 h fasted rats. These data suggest that EAA release in the lateral hypothalamus might contribute to the initiation of food intake. Our results indicate that systemic exposure to MK-801, an NMDA ion channel antagonist, actually increased food intake. However, in our hands, MK-801 did not elicit food intake by satiated animals. Rather, MK-801 appeared to selectively delay the termination of eating. These results suggest a non-lateral hypothalamic site of MK-801 action and suggest a role of EAA in a process distinct from initiation of feeding.

Food intake also has been reported to increase with the systemic (10) or intracerebroventricular (17) administration of glutamate. These increases are opposite to what one might predict from our results, using an NMDA receptor antagonist. However, it should be recalled that receptors for EAA are widely distributed in the central nervous system. Therefore, we should not be surprised by elevated food intake, induced by EAA applied in the vicinity of neurons involved with increasing food intake. Likewise, elicitation of food intake by systemically injected EAA may produce pharmacological activation of feeding neurons in circumventricular structures, such as the area postrema (11). It is our contention that our observation of increased food intake, in the presence of MK-801, reflects a blockade of endogenous EAA released by neurons that participate in the process of satiation.

Alteration of gut-associated, primary afferent inputs to the central nervous system might be the means by which MK-801 increases feeding behavior. Some reports suggest that, in the rat, vagal afferent neurons may not utilize glutamate as a neurotransmitter (18, 19). However, results from other investigators suggest glutamatergic transmission by primary vagal afferents in the cat (1). In addition, there is evidence that primary vagal afferent neurons may communicate sensory information to glutamatergic interneurons in the NTS (4, 7, 25). Since the NTS receives vagal inputs that appear to participate in the termination of food intake (6, 14), a role for NTS NMDA receptors in the control of food intake should be considered.

It is possible that NMDA receptors located outside the central nervous system are responsible for the increased feeding we observed following MK-801 injection. NMDA receptor mRNA has been localized to intrinsic neurons of the stomach and intestines of the rat (5). Although there is no direct evidence for participation of these receptors in control of food intake, Tsai et al. (20) have reported that glutamate alters gastric acid secretion by the rat stomach in vitro. Furthermore, gastric motility is also reported to increase, following systemic administration of NMDA antagonists, including MK-801 (13). Therefore, it is plausible that gastrointestinal NMDA receptors participate in the control of food intake, either directly or indirectly through modulation of gastrointestinal motility or secretion.

In summary, we find that MK-801, an NMDA ion channel antagonist, selectively increases food intake following food deprivation or introduction of a highly palatable food. Our results support the hypothesis that endogenous glutamate plays a role in the control of food intake. Blockade of NMDA receptor function by MK-801 may diminish or delay satiety signals, rather than initiate feeding behavior per se.

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