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MK801 increases feeding and decreases drinking in nondeprived, freely feeding rats

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Abstract

The noncompetitive NMDA receptor antagonist MK801 has been reported to increase food intake in rats during scheduled test meals of palatable foods or after food deprivation, but not in nondeprived rats given rodent chow. To determine if MK801 has an effect on spontaneous meals, MK801 (100 μg/kg) was administered 15 min prior to dark onset to nondeprived rats maintained on powdered rodent chow, and spontaneous food and water access was measured. MK801 increased the length of the first meal and the amount of time spent feeding within the meal. Conversely, MK801 decreased the length and size of the first drinking bout. MK801 did not alter the latency to the first meal or drinking bout, nor the intervals between successive meals or bouts. The effects of MK801 on feeding and drinking bouts were partially confirmed by measuring total chow and water intake over the first 2 h of the dark period. Thus, acute MK801 can significantly alter spontaneous chow feeding and drinking in nondeprived rats when administered prior to dark onset.

Keywords: NMDA antagonist; Food intake; Ingestion; Intermeal interval; Drinking

1. Introduction

Several laboratories have demonstrated that glutamate receptor antagonists, including the noncompetitive *N*-methyl-D-aspartate receptor antagonist MK801 (dizocilpine), can increase food intake in rats (Bednar et al., 1994; Burns et al., 1998; Burns and Ritter, 1997; Burns and Ritter, 1998; Burns et al., 1994; Wirtshafter and Trifunovic, 1988). For example, pretreatment with MK801 (100 μg/kg, ip) caused a 50–60% increase in intake of rodent chow or 15% sucrose in 16-h deprived rats, and a 40% increase of palatable cookie intake in nondeprived rats (Burns and Ritter, 1997). MK801 did not increase intake of rodent chow, however, in nondeprived rats (Burns and Ritter, 1997). This suggests that MK801 requires the presence of deprivation- or palatability-induced appetitive signals to increase intake, and does not have intrinsic orexigenic properties that cause it to initiate feeding.

To determine if NMDA receptors contribute to the control of feeding during spontaneous meals, we examined the effects of MK801 on freely feeding rats. Rather than measuring intake by rats during scheduled test meals, the ad libitum access of rats to rodent chow and water was monitored. Deprivation-, schedule-, or palatability-induced appetitive signals were minimized because the rats were not food-deprived prior to MK801 administration, and because they were tested only once on their regular maintenance diet. Endogenous appetitive signals were maximized, however, because MK801 was administered just before the onset of the dark period, when rats are most likely to initiate a meal spontaneously.

2. Methods

2.1. Experiment I. Effects of MK801 on feeding and drinking patterns

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Florida State University. Sixteen adult male Sprague–Dawley rats

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(Charles River) weighing 300–400 g were individually housed in hanging wire cages with ad lib access to tap water and powdered rodent chow under a 12-h light, 12-h dark cycle. The cages, previously described (Spector and Smith, 1984), were equipped to monitor spontaneous feeding and drinking. The rat's access to a food jar containing powdered rodent chow was detected by the interruption of the beam from an infrared-light-emitting diode falling on a phototransistor positioned across the front of the food-jar holder. Infrared photobeams and detectors were also positioned across the spout of each water bottle such that the rat's tongue caused a beam break with each lick of the spout. The position of the spout required that the rat insert its snout into a slot and fully extend its tongue to lick. Thus, compared to drinking from a fully extended drinking spout, drinking in this apparatus while recording licks requires precise motor movements on the part of the rat. The duration (in seconds) of beam breaks across the food jar or number of beam breaks (licks) across the water-bottle spout were cumulated in 6-s bins by computers for up to 23 h.

Rats were housed in the apparatus for 4 weeks prior to drug treatment. Thirty minutes prior to lights out, food jars and water bottles were removed. Beginning 20 min before dark onset, rats were removed from their cages, weighed, and injected with either 100 μg/ml/kg MK801 (Research Biochemicals, Natick, MA) in 0.15 M NaCl ($n=8$, odd-numbered rats) or 1 ml/kg 0.15 M NaCl ($n=8$, even-numbered rats), then returned to their home cages. The last rat was injected 8 min after the first rat, so that the rats were injected between 20 and 12 min before dark onset. Five minutes before dark onset, food and water were returned to the cages. Food jars and water bottles were weighed 30 min after lights on to determine overnight food and water intake.

2.2. Feeding and drinking bout analysis

Meals were defined as beginning with the first 6-s time interval in which the food-jar infrared beam was broken for 3 s or more. When 300 consecutive seconds elapsed without beam breaks, the start of the 300 s was defined as end of the meal. *Meal length* was defined as the number of seconds from beginning to end of the meal. *Feeding time* was defined as the total number of seconds the food-jar beam was broken within a meal. The *intermeal interval* was the number of minutes from the end of one meal to the start of the next meal.

Drinking bouts were similarly defined, but with a criterion of three licks per 6-s time bin defined as the start of a drinking bout. The *size* of each drinking bout was defined as the total number of licks during the bout.

Two-way ANOVAs with drug treatment and bout number as factors were applied to the interbout intervals, length, and size (feeding time or number of licks) of the first four meals or first four drinking bouts. *t* tests were used to compare the effects of MK801 or NaCl injection on individual meal or bout parameters.

2.3. Experiment II. Effects of MK801 on food and water intake

Experiment I measured the patterns of food and water intake, but did not measure total intake (i.e., grams consumed). A second experiment determined the actual amount of chow and water consumed in the first 2 h of the dark period after MK801 or vehicle injections. A second group of 12 adult male Sprague–Dawley rats (Charles River) weighing 300–400 g were individually housed in the same hanging wire cages as in Experiment I, with ad lib access to tap water and powdered rodent chow under a 12-h light, 12-h dark cycle.

Rats in Experiment I were required to fully extend their tongues while positioning their heads in the lickometer block while drinking. In order to reduce any effects of MK801 on the motor ability of rats to lick water through the lickometer blocks, in this experiment the spouts of the water bottles were extended so that they protruded into the rat's cage. In this configuration, the number of licks could not be recorded, but the rats were no longer required to extend their tongues fully through the narrow slot of the lickometer block.

Rats were housed in the apparatus for 4 weeks prior to drug treatment. Thirty minutes prior to lights out, food jars and water bottles were removed, refilled, and weighed. Beginning 20 min before dark-onset, rats were removed from their cages, weighed, and injected with either 100 μg/ml/kg MK801 (odd-numbered rats) in 0.15 M NaCl or 1 ml/kg 0.15 M NaCl (even-numbered rats), then returned to their home cages. Five minutes before dark onset, food and water were returned to the cages. Food jars and water bottles were weighed 60 and 120 min after lights-off to determine food and water intake during the first and second hour of the dark period. Nine days later, rats were again injected but in a crossover design with either 100 μg/ml/kg MK801 (even-numbered rats) or 1 ml/kg 0.15 NaCl (odd-numbered rats), for a total of 12 rats injected with either MK801 or vehicle.

3. Results

3.1. Experiment I. Effects of MK801 on feeding and drinking patterns

Across the entire 12-h dark period, there was no significant difference between MK801-treated and saline-treated rats in number of meals (10.4 ± 0.9 vs. 9.9 ± 0.7), number of drinking bouts (19.5 ± 2.8 vs. 17.3 ± 2.3), overall food intake (21.2 ± 0.9 g vs. 19.9 ± 0.9 g), or overall water intake (39.9 ± 3.8 g vs. 37.0 ± 2.4 g). Significant differences were found between the groups, however, within the first few meals or bouts of drinking.

Two-way ANOVAs with drug treatment and bout number as factors were applied to the preceding intervals (latency or intermeal interval), length, and feeding time of

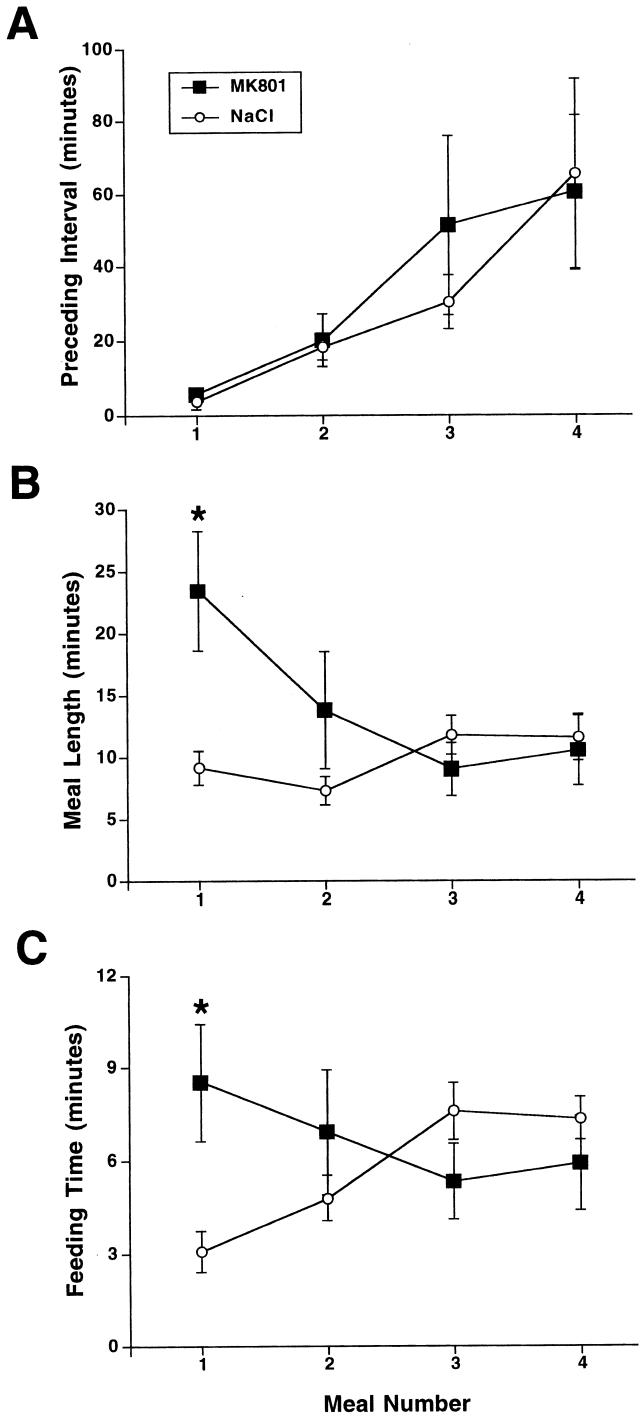

Fig. 1. Effects of MK801 (black squares) or vehicle (white circles) on latency and intermeal interval (A), meal length (B) and feeding time within each meal (C). MK801 had no effect on latency to the first meal and intermeal interval, but significantly increased the length and feeding time of the first meal. * $P < .05$ vs. saline.

the first four meals of each rat (see Fig. 1). Eleven of the sixteen rats (four MK801- and seven NaCl-treated rats) initiated their first meal within 5 min prior to dark onset. However, there was no significant effect of drug on latency or preceding intermeal intervals, although there was a significant effect of meal number [$F(3,56) = 5.6$, $P < .005$], such that the intermeal interval increased across the first four meals.

For meal length, there was a significant interaction of drug and meal number [$F(3,56) = 3.6$, $P < .05$]. The first

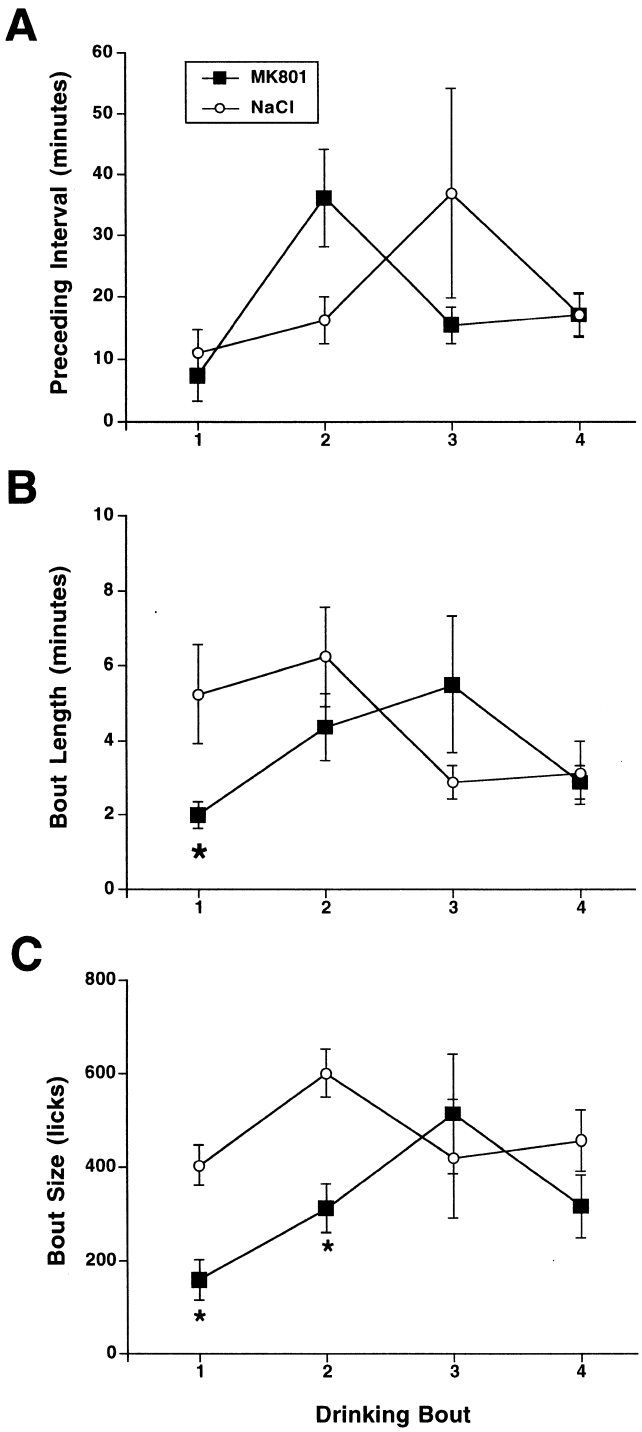

Fig. 2. Effects of MK801 (black squares) or vehicle (white circles) on latency and interbout interval (A), drinking bout length (B), and licking within each bout of drinking (C). MK801 had no effect on latency to the first drinking bout and interbout interval, but significantly decreased the length and size of the first drinking bout. * $P < .05$ vs. saline.

meal was significantly longer in MK801-treated rats compared to saline controls [$t(14)=2.9$, $P<.05$]. For feeding time, there was no significant effect of drug or meal size, but there was a significant interaction [$F(3,56)=3.7$, $P<.05$]. Feeding time during the first meal was significantly longer in MK801-treated rats compared to saline controls [$t(14)=2.7$, $P<.05$].

Two-way ANOVAs with drug treatment and bout number as factors were applied to the intervals preceding bouts, length, and size (number of licks) of the first four drinking bouts of each rat (see Fig. 2). For preceding intervals, no significant effects of drug or bout number were detected, but there was a trend towards a significant interaction [$F(3,56)=2.7$, $P=.056$].

For drinking bout length, there was a significant interaction of drug and bout number [$F(3,56)=2.9$, $P<.05$]. The first drinking bout was significantly shorter in length in MK801-treated rats compared to saline controls [$t(14)=-2.4$, $P<.05$]. For bout size, there was a significant effect of drug [$F(3,56)=6.4$, $P<.05$], but no effect of bout number and no interaction. There were fewer licks in the first and second bouts of drinking in MK801-treated rats compared to saline controls [$t(14)=-2.2$, $P<.05$ and $t(14)=-3.4$, $P<.005$].

3.2. Experiment II. Effects of MK801 on food and water intake

Measuring the amount of chow or water consumed by rats during the first 2 h of the dark period partially confirmed the effects of MK801 observed in the feeding and drinking patterns (Fig. 3). Two-way ANOVA found a significant effect of time [$F(1,44)=39.2$, $P<.0001$] but not drug on cumulative food intake, although comparison of cumulative 2-h intake by *t* test showed a significant increase of intake after MK801 compared to NaCl injection [$t(22)=1.7$, $P=.05$]. Significant effects of drug and time (but no interaction) were found for drinking [$F(1,34)=4.3$, $P<.05$]; MK801 significantly reduced the cumulative amount of water consumed in the first and second hour of the dark period.

4. Discussion

MK801 (100 μg/kg) increased spontaneous meal length and feeding time of nondeprived rats when administered prior to dark onset. MK801 also decreased the size of drinking bouts and 2-h water intake. It has been reported by Burns and Ritter (Burns and Ritter, 1997) that while MK801 increases food intake during scheduled test meals if rats are 16-h food-deprived or given a highly palatable meal, it has no effect on intake of rodent chow or 15% sucrose in nondeprived rats. Our results suggest that there is an interaction between circadian phase, deprivation, and test schedule that modulates the effects of MK801, such that MK801 is more potent at increasing meal length in freely feeding rats at the start of their nocturnal feeding period.

In Experiment I, the actual mass of food and water consumed during each bout was not measured. In Experiment II, however, we attempted to confirm the effects of MK801 on food and water intake by directly measuring the amount of food and water consumed after the first and second hour of the dark period. It has been previously demonstrated in the same apparatus that the length of food access is correlated very closely with the amount of food ingested for most rats (Thaw et al., 1998). Likewise, the volume of individual licks at a drinking spout is very consistent, and thus the total number of licks is a reliable measure of water intake. The decrease in water intake was confirmed: by repositioning the spouts of the water bottles so that they were more accessible within the cage, we reduced the possibility that the decreased drinking was due to an MK801-induced deficit in motor function. An increase in food intake after MK801 was not detectable by two-way ANOVA when 2-h cumulative food intake was

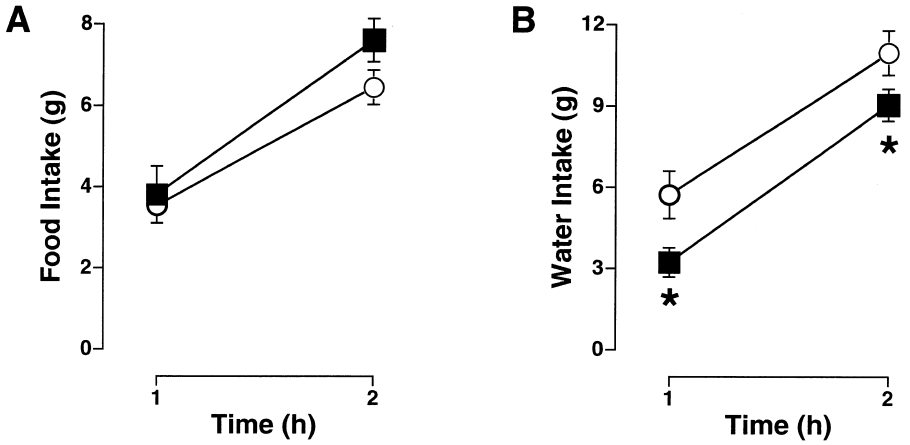

Fig. 3. Effects of MK801 (black squares) or vehicle (white circles) on cumulative 1- and 2-h powdered chow intake (A) or water intake (B) after MK801 (black squares) or vehicle (white circles).

measured at 1-h intervals (although a significant increase in final 2-h intakes was detected by *t* test). The small difference seen in Experiment II may have been due to low frequency of measurement (e.g., measuring only every hour): if MK801 increases the size of the first and second meals only, then the increase in food intake may not be detectable when integrated over 2 h. Conversely, the increase in initial meal duration may be caused by decreased rate of eating due to motor deficits. The dose of MK801 used, however, was not atypical of previously published experiments and no gross effects on rats' behavior were observed (e.g., ataxia or head swaying).

Rats in this study had ad lib access to rodent chow until 25 min before testing, and thus were not significantly food-deprived. Because it is likely that the rats ate very little during the preceding light phase, however, potentially they were being tested at the end of their longest intermeal interval of the day. Additional experiments will be required to dissociate the effects of prior deprivation, prior feeding, and circadian phase. Other pharmacological manipulations of feeding have been shown to be sensitive to prior deprivation and circadian phase. Exogenous pancreatic glucagon, for example, decreases meal size in deprived and nondeprived rats during the light phase, but only decreases meal size at dark onset if rats are food-deprived (Geary et al., 1987). Alternatively, the controls of food intake may be differentially modulated by testing rats with scheduled, daily test meals versus testing under free-feeding conditions.

Additionally, MK801 decreased licking to water in this experiment. Burns and Ritter (Burns and Ritter, 1997) have previously demonstrated that MK801 has no effect on water intake by water-deprived rats. It is not clear if MK801 differentially affects water and food intake in scheduled short-term tests of non water-deprived rats. It is possible that rats do not drink appreciable amounts during test meals conducted in the middle of the light period (especially when the test meal is an aqueous solution). Therefore, any effect of MK801 on water intake may be masked by the conditions of the test.

MK801's modulation of feeding and drinking across the first two meals is consistent with the drug's pharmacokinetics. In the rat, MK801 reaches peak concentrations in the plasma and brain within 10 to 30 min (Vezzani et al., 1989), and has an elimination half-life of 1–2 h (Hucker et al., 1983; Vezzani et al., 1989). The second bouts of feeding and drinking all occurred within 90 min of the injection time; thus, the effects on ingestion were contemporaneous with the peak levels of MK801.

Because MK801 was administered systemically, and because MK801 crosses the blood–brain barrier (Vezzani et al., 1989), we cannot conclude that the drug acted at any specific site of action to increase meal size. NMDA receptors are distributed throughout the periphery and the brain in sites known to play a role in regulating ingestion, e.g., in the gut (Burns et al., 1994), the sensory neurons of the vagus (Shigemoto et al., 1992), central relays of visceral sensory information such as the NTS (Qian et al., 1997), and forebrain sites implicated in the control of food intake (Van den Pol et al., 1994). MK801 may modulate visceral afferent information directly or indirectly; when visceral afferents are destroyed by systemic capsaicin or subdiaphragmatic vagotomy, the action of MK801 to increase intake of 15% sucrose in deprived rats is attenuated or eliminated (Burns and Ritter, 1998). MK801 may modulate visceral afferent information centrally, because fourth-ventricular or site-specific injections into the NTS of small amounts of MK801 replicate the effects of larger systemic doses on sucrose intake in deprived rats (Treece et al., 1998).

It is not known whether MK801 acts at the same sites through the same mechanisms to increase food intake during spontaneous meals as during test meals, but this is a plausible hypothesis. Our finding that an NMDA antagonist increases meal length and decreases water intake in non-deprived, freely feeding rats supports the hypothesis that endogenous activation of NMDA receptors regulates meal size not only under scheduled test conditions but also during spontaneous, nocturnal meals of rats.

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