

The NMDA receptor antagonist MK-801 alters lipoprivic eating elicited by 2-mercaptoacetate

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Abstract

Eating behavior is controlled, at least in part, by levels of circulating metabolic fuels such as glucose and free fatty acids, and drugs that interfere with the availability of these fuels can elicit eating. One such drug is 2-mercaptoacetate (2MA), an inhibitor of fatty acid oxidation. Evidence also suggests that NMDA receptors may mediate some aspects of normal eating and satiety. The present study was conducted in order to determine whether NMDA receptors may play a role in feeding elicited by 2MA. Rats received intraperitoneal injections of either saline, 2MA, the non-competitive NMDA receptor antagonist MK-801 or a combined injection of 2MA and MK-801, and subsequent intake of a fat-enriched, mash diet was measured at 1, 2, 3 and 4 h post-injection. Results showed that cumulative food intake was significantly increased by 2MA alone, as compared to saline controls, with most of the 2MA-elicited eating occurring during the first hour post-injection. While MK-801 alone did not alter food intake, it did have a biphasic effect on feeding elicited by 2MA. MK-801 initially suppressed and later enhanced eating elicited by 2MA. Although it is unclear whether MK-801 is acting centrally, peripherally or both to alter 2MA-induced eating, these results implicate NMDA receptors and the neurotransmitter glutamate in the regulation of lipid-associated eating and satiety.

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1. Introduction

Eating is a complex behavior controlled by signals arising from both the central and peripheral nervous systems. Peripheral hunger/satiety signals include levels of circulating fuels such as glucose and free fatty acids, and drugs that interfere with glucose utilization and fat metabolism can alter food intake. One such drug is 2-mercaptoacetate (2MA). Recent experiments have shown that peripheral injections of 2MA can elicit eating for up to

4 h in mice and rats, especially when the animals are maintained on a fat-enriched diet [10,22,25]. 2MA is thought to elicit eating by inhibiting mitochondrial acyl-CoA dehydrogenase, interfering with fatty acid metabolism [24,25], and maintenance on a high fat diet leads to a larger dependence on fats for energy and therefore a more robust eating response to 2MA [13,24,25]. Studies indicate that 2MA may act peripherally, perhaps in the liver, to stimulate eating [23] since the eating response requires intact hepatic vagal sensory afferents [16,18,19]. Several brain regions have also been implicated in 2MA-elicited eating. Peripheral injections of 2MA increased fos protein expression in the dorsal motor nucleus of the vagus (DMNV), nucleus of the solitary tract (NTS), lateral parabrachial nucleus and the central nucleus of the amygdala [20], while lesions to many of these areas abolished lipoprivic feeding induced by 2MA [6,21]. Furthermore, as with the eating response, increased

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fos expression was eliminated by vagotomy [20]. 2MA injections also increased mRNA levels of the feeding-related peptide melanin concentrating hormone in the lateral hypothalamus (LH) [26], a brain region implicated in the control of eating [1,11,12,27,28,29].

In addition to levels of circulating metabolic fuels, gastric distention and the rate of gastric emptying are important factors for regulating hunger and satiety, and accumulating evidence suggests that *N*-methyl-D-aspartic acid (NMDA) receptors may be involved in processing these mechanoreceptive signals. Burns and Ritter [4] have shown that intraperitoneal injections of the non-competitive NMDA receptor antagonist MK-801 increased eating in rats that were food deprived or offered highly palatable foods. Similar effects are produced by MK-801 administration into the caudal brainstem suggesting involvement of central NMDA receptors [32]. However, peripheral NMDA receptors may also be important given that increased meal size after NMDA receptor blockade is abolished by vagotomy [5]. It has been suggested that NMDA receptor antagonism indirectly disrupts normal satiety signals arising from stomach by increasing the rate of gastric emptying leading to prolonged eating and increased meal size [7,8].

In the present experiment, we examined the effects of MK-801 on 2MA-elicited eating. While others have already shown that MK-801 enhances eating elicited by 2-deoxy-D-glucose (2DG) suggesting an interaction between glucose metabolism and mechanoreceptive cues in the control of eating [31], nothing is known about this in relation to lipoprivation. We hypothesized that NMDA receptor antagonism would enhance 2MA-elicited eating much like it does eating elicited by highly palatable foods, food deprivation and glucoprivation.

2. Methods

2.1. Subjects

Adult, male Sprague–Dawley rats weighing between 310 and 450 g were individually housed in cages and kept on a 12:12-h light–dark schedule in a temperature-controlled room. Rats were bred in the University of California, Riverside, Psychology Department vivarium and were descended from rats obtained through Charles River. The rats were maintained at least 10 days prior to the onset of the experiment on a fat-enriched, milk-mash diet consisting of Purina rat chow powder (45%), sucrose (9%), evaporated milk (32%) and corn oil (14%) with a total fat content of 20.7%.

2.2. Drugs and procedures

On the day of the experiment, rats received a single intraperitoneal injection (2 ml/kg) of either saline (0.9%) ($N=8$), 2MA (thioglycolic acid, Sigma, 70 mg/kg) ($N=9$),

MK-801 (dizocilpine, Research Biochemical International, Natick, MA, 0.1 mg/kg) ($N=6$) or 2MA in combination with MK-801 ($N=7$). Some rats received two injections separated by at least 10 days with the first injection always being a saline control. All drugs were made fresh and dissolved in chilled saline (0.9%). One hour prior to testing, rats were given a fresh batch of mash food, and food bowls were weighed at the time of injection and every hour for 4 h. The rats were given free access to water throughout the entire experiment, and all drug injections were given at least 6 h prior to the onset of the dark phase.

2.3. Statistical analysis

Cumulative and hourly food intakes were calculated. ANOVA was used to examine the differences between the means, followed by post-hoc comparisons using the least significant difference (LSD) method. A standard significance value of $p<0.05$ was used for all tests.

3. Results

As shown in Fig. 1, average cumulative food intake varied as a function of drug treatment and ANOVA revealed significant drug treatment effects at each of the 4 h examined [hour 1: $F_{(3,26)}=6.4$, hour 2: $F_{(3,26)}=7.3$, hour 3: $F_{(3,26)}=9.8$, hour 4: $F_{(3,26)}=9.2$, $p<0.05$]. Post-hoc multiple comparison tests showed that 2MA significantly increased

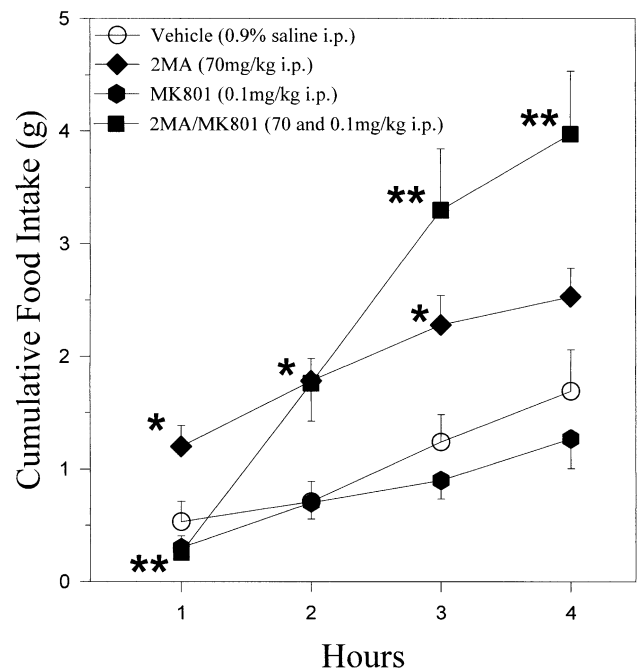


Fig. 1. Cumulative food intake in 4 h as a function of drug treatment. Single asterisk (*) indicates significant difference in cumulative food intake for 2MA vs. vehicle control at 1, 2 and 3 h post-injection, $p<0.05$. Double asterisk (**) indicates significant difference in cumulative food intake for 2MA/MK-801 vs. 2MA alone at 1, 3 and 4 h post-injection, $p<0.05$.

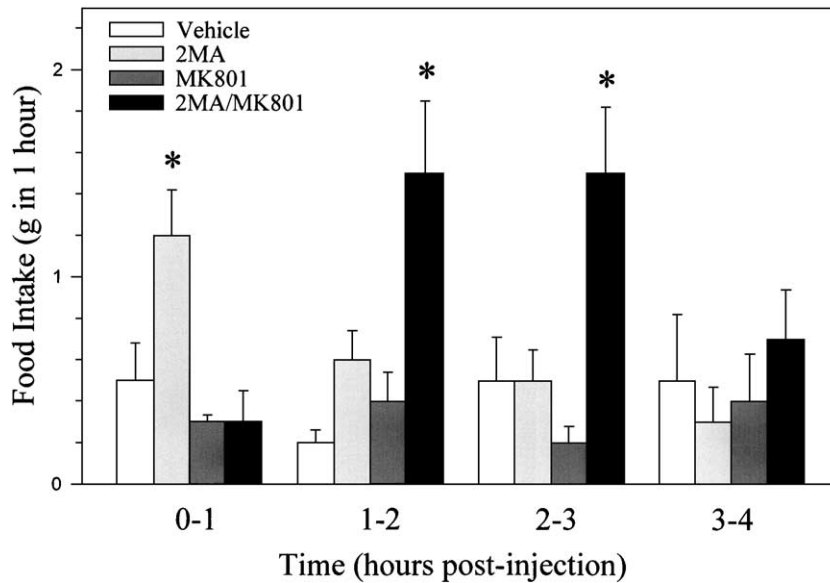


Fig. 2. Average hourly food intake as a function of drug treatment. Asterisk (*) indicates significant difference from all other groups at that hour, $p < 0.05$.

cumulative food intake over saline controls at 1, 2 and 3 h post-injection ($p < 0.05$), although most of the 2MA-elicited eating occurred in the first hour (see Fig. 2). Injections of MK-801 alone had no effect on eating compared to saline controls. Interestingly, MK-801 had a biphasic and synergistic effect on 2MA-elicited eating. Initially at hour 1, MK-801 suppressed 2MA-elicited eating; later at hours 3 and 4, it enhanced 2MA-elicited eating. Overall, during the 4-h test period, rats that received the combined injection of 2MA and MK-801 ate 56% more food (3.9 vs. 2.5 g) than rats receiving 2MA alone.

ANOVA also revealed significant effects of drug treatment on average hourly food intake at 1, 2 and 3 h post-injection [$F_{(3,26)}=6.4$, $F_{(3,26)}=8.5$, $F_{(3,26)}=6.9$, respectively, $p < 0.05$]. A breakdown of average hourly food intake in Fig. 2 shows that 2MA alone significantly increased eating only in the first hour and MK-801 alone had no effect at any time. However, in combination, MK-801 suppressed 2MA-elicited eating in the first hour and enhanced it during the second and third hours post-injection.

4. Discussion

Consistent with previous research [10,22,24], results from the present experiment show that intraperitoneal administration of 2MA elicits eating in rodents maintained on a fat-enriched diet. It has been suggested that 2MA elicits eating by blocking fatty acid metabolism which reduces the availability of fats as a source of energy [24,25], and that vagal sensory afferents, the amygdala and certain brainstem nuclei play an important role in 2MA-elicited eating since destruction of any of these structures abolishes the response [6,21]. Furthermore, consistent with Burns and Ritter [4],

the present study has shown that MK-801 alone does not elicit eating in satiated rats. A new finding, however, is that co-administration of MK-801 alters 2MA-elicited eating, initially by suppressing the response and later by enhancing it. Similarly, an enhancement, but not initial suppression, of 2DG-elicited eating by MK-801 has already been demonstrated by Sugimoto et al. [31]. The lack of an initial suppression of MK-801 on 2DG-elicited eating is not surprising given that a number of studies suggest that 2MA and 2DG elicit different patterns of macronutrient ingestion and likely affect feeding through different mechanisms [18,19,20,22,26].

The precise ways in which MK-801 alters 2MA-elicited eating are unknown, but the existence of both an initial suppression and a delayed enhancement suggests that two or more mechanisms are involved. The initial suppression of eating might be due to motor or other generalized impairments produced by MK-801, or alternatively by MK-801 acting centrally to suppress the activity of feeding-stimulatory neurocircuits. In support of a generalized motor impairment, Haggerty and Brown [15] have shown that acute, subcutaneous injections of MK-801 caused ataxia in rats at doses comparable to those used in the present experiment. However, motor disturbances may not be sufficient to account for the initial feeding suppression observed in the present study, given that a similar suppression of eating in response to 2DG was not reported by Sugimoto et al. [31] and that several other studies have demonstrated that MK-801 generally increases food intake under a variety of conditions [4,7,8,32,33]. As for a possible central mechanism of action for MK-801, Stanley and colleagues [11,12,27,28] have shown that LH administration of NMDA can elicit intense eating in rats and that the deprivation-induced and nocturnal eating, as well as NMDA-elicited eating, can be suppressed by the

competitive NMDA receptor antagonist D-AP5, suggesting a role for endogenous glutamate in eating stimulation [29]. Thus, the initial suppression of 2MA-elicited feeding by MK-801 might have resulted from blockade of feeding-stimulatory NMDA receptors in the LH. While this is a plausible mechanism, there is no evidence directly linking the observed feeding suppressive effects of MK-801 to actions in the LH. Indeed, it should be noted that MK-801 actually elicits eating when injected into other sites like the caudal hindbrain [32].

As to the feeding-stimulatory effect of MK-801, MK-801 may increase meal size by accelerating gastric emptying [7,8], perhaps by acting on NMDA receptors in the enteric nervous systems [3] or caudal brainstem [32]. The hypothesis is that the accelerated emptying of the gastric contents reduces mechanoreceptive satiety signals, resulting in continued eating. Applied to the present results, this would suggest that eating was elicited by lipoprivation and enhanced by the reduced satiety resulting from the increased rate of gastric emptying. This explanation would also be consistent with the findings of Sugimoto et al. [31] who demonstrated a late augmentation in glucoprivic eating by combining MK-801 with 2DG.

Interestingly, the eating response to MK-801 and presumably the accelerated gastric emptying may be mediated by neurons in the peripheral nervous system, central nervous system or both. NMDA receptor mRNA has been localized in the enteric nervous system suggesting the existence of peripheral NMDA receptors [3], and their role in eating is supported by that fact that peripherally administered MK-801 does not alter eating after the destruction of visceral sensory afferents [5]. In the central nervous system, the caudal hindbrain, particularly the DMNV and the NTS, may be of particular importance, since aspiration lesions of these regions abolished the eating produced by systemic MK-801 [33], although this could also be due to the disruption of vagal afferents as they enter the hindbrain. However, it is worth noting that MK-801 microinjected directly into the hindbrain can also lead to increased food intake, suggesting that MK-801's effects may not be restricted to the periphery [32]. This also suggests contrasting roles for NMDA-type glutamate receptors in various parts of the nervous system. Stimulating NMDA receptors in the LH of satiated rats leads to eating and antagonism of these receptors suppresses eating [11,12,27,28,29]. In contrast, antagonism of NMDA receptors in the caudal hindbrain [32] and periphery [4] of food-deprived rats or rats fed highly palatable foods leads to increases in food intake.

Regardless of the site(s) of action of MK-801, glutamate, and NMDA receptors in particular, may play a role in feeding elicited by 2MA. However, in addition to glutamate, several other neurotransmitters appear to be involved in lipoprivic eating including galanin [17,34], dopamine [2], serotonin [14], the adrenergics [14], nitric oxide [9] and the endogenous opioids [30].

In any event, the findings from the present study underscore the complexity of eating behavior with respect to the regulation and integration of hunger (lipoprivation) and satiety (rate of gastric emptying) signals, and provide further support for glutamate and NMDA receptor involvement in these processes. Finally, an understanding of the neurochemical substrates of hunger and satiety may one day lead to the development of drug therapies used to treat pathologies of eating such as anorexia, bulimia and obesity.

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