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Effects of agmatine on the escalation of intravenous cocaine and fentanyl self-administration in rats

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Abstract

Escalation of drug intake reliably occurs when animals are allowed extended self-administration access. As a form of plasticity, escalation of drug intake may be accompanied by neuroadaptive changes that are related to the transition from controlled use to addiction. The purpose of the present experiment was to examine the effects of agmatine (decarboxylated L-arginine) on the escalation of intravenous (iv) fentanyl and cocaine self-administration in rats. Subjects were allowed 12 h of daily access to fentanyl (2.5 μg/kg) or cocaine (0.2 mg/kg) under a fixed-ratio (FR) 1 schedule of reinforcement for 30 days. Animals self-administering fentanyl were distributed into three groups: (1) low-dose agmatine (10 mg/kg) throughout self-administration; (2) high-dose agmatine (30 mg/kg) throughout self-administration; and (3) high-dose agmatine after significant escalation (Day 18) of drug intake had occurred. Animals in a fourth group were pretreated with a high dose of agmatine throughout 30 days of cocaine self-administration. Both doses of agmatine, when given throughout self-administration, significantly decreased the escalation of responding that occurred for fentanyl but not cocaine. In the group that received agmatine after significant escalation had occurred, fentanyl-maintained responding was not significantly altered. These data indicate that agmatine attenuates the escalation of fentanyl self-administration if administered before the escalation begins and may mediate neuroadaptive events related to chronic opioid self-administration.

Keywords: Agmatine; Cocaine; Escalation; Fentanyl; Intravenous; Self-administration; NMDA antagonist; NOS inhibitor; Sensitization; Tolerance

1. Introduction

The transition from controlled drug use to drug addiction occurs when chronic drug exposure results in excessive intake and difficulty controlling consumption. Understanding this transition, typically exemplified by an escalating pattern of drug intake, may help to provide future prevention and treatment strategies for drug addiction in humans. Laboratory animal models of escalating drug intake can result in stable or escalating patterns of drug-taking behavior by varying daily access time (Ahmed and Koob, 1998, 1999; Ahmed et al., 2000; Koob and Le Moal, 2001) or number of opportunities to self-administer drug per hour (Fitch and Roberts, 1993; Lynch and Roberts, 2001). If rats are given short access (1 h/day) to cocaine or heroin self-administration, low and stable patterns of responding emerge and continue for long periods; however, when given longer access (6–11 h/day) to drug self-administration, rats significantly escalate their drug intake over time (Ahmed and Koob, 1998). In addition, if the number of opportunities to self-administer cocaine per hour is restricted (1–2 per hour), rats will maintain stable responding, but binge patterns and disruption of circadian rhythmicity emerge as opportunities increase to 4 per hour (Fitch and Roberts, 1993). After a history of escalated drug intake, rats show stress-induced reinstatement of drug-seeking behavior for longer periods compared to rats that did not show escalation (Ahmed and Koob, 1999; Ahmed et al., 2000). Thus, the changes that occur during the transition from low and stable patterns of self-administration to high and uncontrolled self-administration may be critical mediators in developing and maintaining addiction. It is important to test the feasibility of this transition model of addiction for evaluating treatments that may potentially prevent escalation of drug intake.

Two biological neuroadaptation processes, sensitization and tolerance, allow an organism to adaptively respond to

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changes in the internal or external environment and may partially account for the transition from controlled drug use to drug addiction. Sensitization and tolerance may both be demonstrated in certain situations depending upon the dose and temporal factors used in drug pretreatment (Celerier et al., 2001; Koob and Le Moal, 2001). Although tolerance to the reinforcing effects of self-administered cocaine has been previously reported by demonstrating a rightward shift in the dose–response curve using a multi-dosing method (Emmett-Oglesby et al., 1993), a more recent account indicates that the development of tolerance to the reinforcing effects of cocaine can only be demonstrated under some chronic dosing regimens (Schenk and Partridge, 1997). Additionally, Deroche et al. (1999) reported no signs of tolerance to cocaine self-administration using conditioned place preference and cocaine-induced runway performance.

In contrast, sensitization to the reinforcing effects of drugs has been shown in all phases of drug addiction, and it appears to be a long-lasting phenomenon (Robinson and Berridge, 1993, 2000, 2001; Schenk and Partridge, 1997). For example, prior exposure to a wide range of potentially addictive drugs enhances the subsequent acquisition of self-administration (Campbell and Carroll, 2000; Carroll and Lac, 1998; Mendrek et al., 1998; Schenk and Partridge, 2000; Schenk et al., 1990). Prior exposure can also increase the reinforcing efficacy of a drug by increasing the highest fixed-ratio (FR) completed or break point under a progressive-ratio (PR) schedule (Lorrain et al., 2000). Reinstatement of drug-seeking behavior has also been associated with the expression of locomotor sensitization (De Vries et al., 1998). Thus, both sensitization and tolerance mechanisms may underlie escalating patterns of drug intake.

Several pharmacological agents have been implicated in neuroadaptations that can mediate the development and expression of locomotor and self-administration sensitization (Haracz et al., 1995; Herman et al., 1995; Khanna et al., 1998; Semenova et al., 1999; Trujillo, 1995, 2000; Trujillo and Akil, 1991, 1995). Both the *N*-methyl-D-aspartate (NMDA) receptor and nitric oxide synthase (NOS) systems have been shown to be critical mediators of a wide range of neuroadaptive events (Galea et al., 1996; Koob and Le Moal, 2001; Koob and Weiss, 1992; Trujillo, 1995). For example, NMDA antagonists such as dizocilpine can modulate the development and expression of locomotor and self-administration sensitization in rats (Robinson and Berridge, 2000; Schenk et al., 1993). However, low-affinity NMDA antagonists such as memantine and MRZ 2/579 are more effective than MK-801 in blocking the acquisition of intravenous (iv) morphine self-administration (Semenova et al., 1999). Thus, investigation of low-affinity NMDA antagonists may provide important information about the escalation of drug self-administration. A goal of this experiment was to use a pharmacological agent that may modify plasticity and to determine whether it would prevent escalation of drug self-administration.

Agmatine (decarboxylated L-arginine), an endogenous cationic amine, was initially isolated in the mammalian central nervous system in 1994, and it is a constituent in bacteria, invertebrates and plants (Li et al., 1994). Endogenously, agmatine may play a role as a neurotransmitter or neuromodulator, and it acts by antagonizing the NMDA receptor, inhibiting NOS and binding to 5-HT, alpha2-adrenoceptors and imidazoline binding sites (Galea et al., 1996; Reis and Regunathan, 1999, 2000; Trujillo and Akil, 1995). Recently, agmatine has been demonstrated to have direct involvement in blocking tolerance to opioid analgesia (Kolesnikov et al., 1996) and acute- and chronic-spinal morphine tolerance (Fairbanks and Wilcox, 1997). Furthermore, agmatine can potentiate the analgesic effect of morphine (Yesilyurt and Uzbay, 2001) and inhibit ethanol- and morphine-withdrawal syndromes (Aricioglu-Kartal and Uzbay, 1997; Uzbay et al., 2000), and it has neuroprotective roles in persistent pain and neuronal injury models (Fairbanks et al., 2000). The properties of agmatine, such as low affinity for the NMDA receptor coupled with NOS inhibition properties, suggest that it may be useful in mediating neuroadaptive events such as the escalation of drug self-administration.

The purpose of the present study was to examine the effects of agmatine on the escalation of intravenous self-administration of drugs from two different pharmacological classes, cocaine and fentanyl. Animals self-administering fentanyl were distributed into three groups: (1) low-dose agmatine (10 mg/kg) throughout self-administration; (2) high-dose agmatine (30 mg/kg) throughout self-administration; and (3) high-dose agmatine after significant escalation (Day 18) of drug intake had occurred. Animals in a fourth group self-administered cocaine and received a high dose of agmatine throughout self-administration.

2. Materials and methods

2.1. Subjects

Fifty-seven adult male Wistar rats (Harlan Sprague Dawley, Madison, WI), weighing between 375 and 425 g at the start of the experiment were used as subjects. Rats were initially pair-housed for at least 3 days in plastic cages with free access to food and water. After cannulation, rats were individually housed in operant test chambers for the duration of the experiment. All rats had free access to ground Purina Rat Chow (Purina Mills, Minneapolis, MN) and water for 3 days while recovering from surgery and adapting to the operant chambers. Subsequently, subjects received free access to water and daily rations of 20 g food, which maintained them at 85% of their free-feeding body weight and ensured an adequate responding range to test the effects of a pharmacotherapy. Use of the animals for this protocol was approved by the University of Minnesota Institutional Animal Care and Use Committee (protocol

number 9904A00343). Laboratory facilities were accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and principles of laboratory animal care (National Research Council, 1996) were followed.

2.2. Apparatus

Operant chambers were octagonally shaped with alternating stainless-steel and Plexiglas walls. Stainless-steel walls contained a drinking spout insertion, recessed food jar compartment and two levers for self-administration (Coulbourne Instruments, Lehigh Valley, PA). Stimulus lights were located above each lever. A 4.76-W house light was constantly illuminated at the top of the chamber, except for a 30-min timeout period before each daily session. Each chamber was housed in a sound-attenuating cubicle with an exhaust fan for ventilation. An infusion pump (Fluid Metering, Oyster Bay, NY, model rhsyockc) and a 500-ml drug reservoir were mounted on the outside of the cubicle that connected to a swivel via Tygon tubing (1.52 mm o.d., 0.51 mm i.d., Fisher Scientific, Springfield, NJ) mounted at the top of the chamber. A spring covered tether (model C313CS, Plastics One, Roanoke, VA) was attached to the swivel and to the rats' cannula connector (model C323G, Plastics One) and to a silastic catheter (0.94 mm o.d., 0.508 mm i.d., Fisher Scientific), which was held in place by a covance infusion harness (Harvard Apparatus, Holliston, MA). Med-PC interfacing and PC-compatible computers were used for data collection and behavioral programming (Med Associates, St. Albans, VT).

2.3. Drugs

Fentanyl HCl and cocaine HCl were provided by the National Institute on Drug Abuse (Research Triangle Institute, Research Triangle Park, NC). Drugs were mixed in a sterile saline (0.09% NaCl) solution, and dose was determined by infusion duration (1 s/100 g of body weight); thus, initial infusion durations ranged from 3.8 to 4.3 s. The infusion volumes ranged from 0.112 to 0.128 ml based on body weight. The dose for fentanyl was 2.5 μg/kg/infusion, and the cocaine dose was 0.2 mg/kg/infusion. These doses were selected based on previous research as low to moderate (Campbell and Carroll, 2000) to obtain escalation while avoiding potentially toxic effects of long-session and long-term self-administration access. Drug solutions were made every few weeks and were refrigerated. They were added daily to the intravenous reservoir at room temperature. Agmatine sulfate was purchased from Sigma-Aldrich (St. Louis, MO) and was dissolved in sterile saline.

2.4. Procedure

Rats were anesthetized with ketamine (90 mg/kg), pentobarbital (10 mg/kg) and atropine (0.02 mg/kg) at the time of surgery. Chronic indwelling silastic catheters were implanted in the right jugular vein with the tip terminating at the opening of the right atrium. The free end was led subcutaneously to a medial incision made 1 cm caudal to the scapulae and connected to the harness via the cannula connector. After 3 days of recovery from surgery, the self-administration procedure began. Food was taped to the left lever for 3 days, and three priming infusions were given at the start of the following two sessions to facilitate stable responding. Responding on the left lever under an FR 1 schedule resulted in drug infusion and stimulus light illumination for the infusion duration. Responding on the right lever had no consequence, but was recorded. Daily 12-h sessions were conducted from 10 a.m. to 10 p.m. If, after the initial 5 days, a subject had not begun lever pressing, three priming infusions were given at 10 a.m. until the rat self-administered a minimum of 20 infusions per session. The experimental conditions for the four groups are summarized in Table 1.

Group LF (Low-Dose Agmatine–Fentanyl) self-administered fentanyl (2.5 μg/kg/infusion) and received agmatine (10 mg/kg) or saline given intravenously (9:30 a.m., 12:30 p.m., and 3:30 p.m.) throughout the treatment phase (Days 6–30). The agmatine group ($n=8$) received a total of 30 mg/kg daily and the saline group ($n=8$) received equal amounts of saline. Group HF (High-Dose Agmatine–Fentanyl) self-administered fentanyl (2.5 μg/kg/infusion) and agmatine (30 mg/kg) or saline was injected intravenously (8:00 a.m. and 2:00 p.m.) during the treatment phase. The agmatine group ($n=7$) received 60 mg/kg daily and the control group ($n=13$) received equal amounts of saline. In Group DF (Delayed Agmatine–Fentanyl), rats ($n=9$) self-administered fentanyl (2.5 μg/kg/infusion), and agmatine (30 mg/kg) was injected intravenously (8:00 a.m. and 2:00 p.m.) from Day 18 to Day 30. These days were chosen based on the initial groups (LF and HF) that showed a large divergence in the intake pattern (between the agmatine and saline groups) beginning at Day 18. In Group HC (High-Dose Agmatine–Cocaine), cocaine (0.2 mg/kg/infusion) was self-administered and agmatine (30 mg/kg) or saline was given intravenously (8:00 a.m. and 2:00 p.m.) during the treatment phase. The agmatine group ($n=6$) received a total of 60 mg/kg daily and the control group ($n=6$) received equal amounts of saline.

A short nonsystematic follow-up study with Group HC was conducted to examine the effects of multiple high doses

Table 1
Experimental design

Group	Drug self-administered	Agmatine dose (mg/kg)	Treatment days	Treatment regimen
LF	2.5 μg/kg fentanyl	10	6–30	tid
HF	2.5 μg/kg fentanyl	30	6–30	bid
DF	2.5 μg/kg fentanyl	30	18–30	bid
HC	0.2 mg/kg cocaine	30	6–30	bid

of agmatine (60, 100 and 200 mg/kg iv) on behavioral and operant performance. Briefly, four animals in Group HC that successfully completed the 30-day study were given two of three doses (60, 120 or 200 mg/kg bid) of agmatine for a period of 5–10 days, and the effect on cocaine-maintained infusions was monitored.

Daily food (g/kg) and water intake were measured over the 30-day test period in all experiments. Body weights and pump calibration were checked weekly. Catheter patency was confirmed weekly by an immediate loss of the righting reflex after a sodium methohexital (5 mg/kg iv) injection. If a catheter was not patent, the animal's data from the previous week were excluded from data analysis.

2.5. Data analysis

Groups were compared for mean daily infusions over 30 days using the Friedman repeated-measures analysis of variance. Groups were examined separately for differences in the initial 5-day baseline period and for the treatment period (Days 6–30). Post hoc comparisons between groups were made with Bonferroni-corrected Student's t tests. Significance was defined as $P < .05$.

3. Results

No significant differences were found between groups for food and water intake, or body weights in all experiments. The remaining results are presented below for each experimental group.

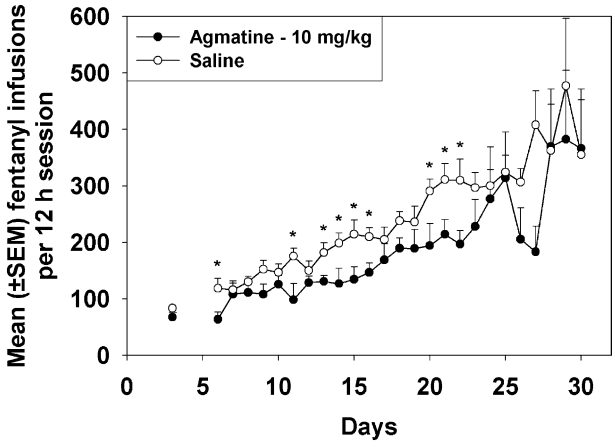

Fig. 1. Mean (±S.E.M.) number of fentanyl infusions (2.5 μg/kg/infusion, FR 1) obtained in the groups pretreated with agmatine (10 mg/kg iv) [Group LF] and saline over the 30-day experimental period. (* Indicates a significant between-group difference, $P < .05$; on specific days based on Bonferroni-corrected t tests.) The 5-day baseline is represented on the left with unconnected points. Connected points indicate the 25-day agmatine and saline treatment period. Closed circles indicate agmatine pretreatment, and open circles indicate saline pretreatment.

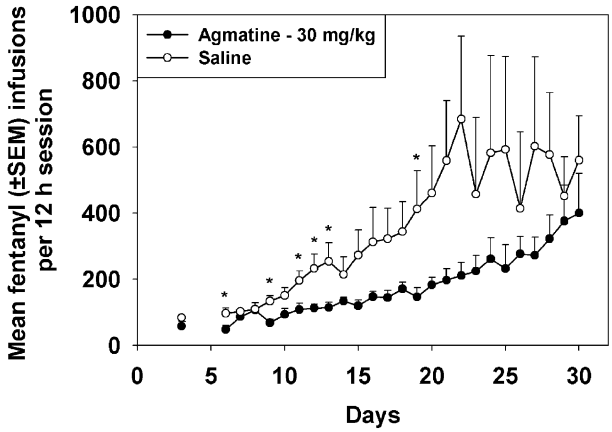

Fig. 2. Mean (±S.E.M.) number of fentanyl infusions (2.5 μg/kg/infusion, FR 1) obtained by agmatine (30 mg/kg iv) [Group HF] and saline over the 30-day experimental period. (* Indicates a significant between-group difference, $P < .05$; on specific days based on Bonferroni-corrected t tests.) Disconnected points on the left side indicate the 5-day baseline. Connected points signify the 25-day agmatine and saline treatment period. Closed circles indicate agmatine pretreatment, and open circles indicate saline pretreatment.

3.1. Low-dose agmatine (Days 6–30)–fentanyl (LF)

Fig. 1 depicts the mean number of fentanyl (2.5 μg/kg) infusions per 12-h session during the 30-day testing period. There were no differences between fentanyl and saline infusions during the 5-day baseline period; however, there was a significant main effect of treatment ($F = 5.87$; $df = 1$; $P < .05$), and there were significant increases in infusions in both groups over Days 6–30 ($F = 18.5$; $df = 24$; $P < .05$). Bonferroni-corrected Student's t tests revealed between-groups differences (indicated by asterisks) on single day means for Days 6, 11, 13–16, 20–22 and 27. The low-dose

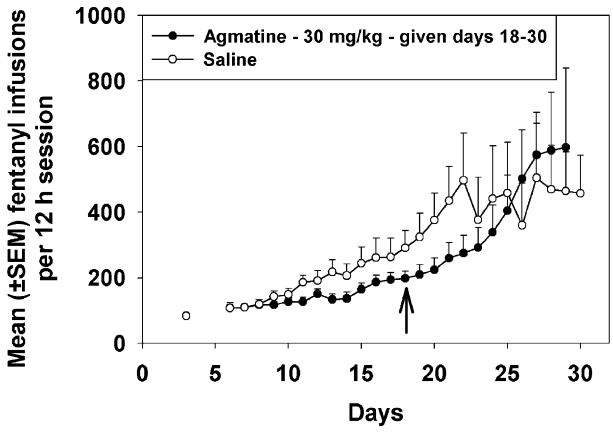

Fig. 3. Mean (±S.E.M.) number of fentanyl infusions (2.5 μg/kg/infusion, FR 1) obtained by the agmatine (30 mg/kg iv) [Group DF] and are plotted with the saline groups from HF and LF. Agmatine was administered from Day 18 to Day 30 for DF as indicated by the arrow. The 5-day baseline is represented on the left with disconnected points. Connected points indicate the 25-day self-administration period. Closed circles indicate agmatine pretreatment, and open circles indicate saline pretreatment.

agmatine significantly attenuated fentanyl-maintained responding, but it did not completely prevent the escalation from occurring.

3.2. High-dose agmatine (Days 6–30)–fentanyl (HF)

Fig. 2 shows the mean number of fentanyl (2.5 μg/kg) infusions per 12-h session during the 30-day testing period. During the 5-day pretreatment baseline period, fentanyl self-administration did not differ between agmatine and saline groups. There was a significant main effect of treatment ($F = 5.31$; $df = 1$; $P < .05$), and both groups displayed significant escalation over Days 6–30 ($F = 6.23$; $df = 24$; $P < .05$). Bonferroni-corrected Student's t tests revealed between-groups differences (indicated by asterisks) on single day means for Days 6, 9, 11–13 and 19. Animals were removed from the study if adverse effects due to excess fentanyl intake were observed. The high-dose agmatine also significantly attenuated fentanyl-maintained responding, but it did not completely prevent the escalation from occurring.

3.3. Delayed high-dose agmatine (Days 18–30)–fentanyl (DF)

In Group DF, the effects of administering agmatine (30 mg/kg bid) to animals from Day 18 to Day 30 were examined. Day 18 was chosen as the delayed period because it was 1 day prior to the last significant difference between animals in Group HF. Thus, attenuation of responding due to agmatine may have maintained the between group difference. Fig. 3 shows the mean number of fentanyl (2.5 μg/kg) infusions over 12-h sessions when agmatine was given to animals from Day 18 to Day 30. These data are plotted against the control animals' data from group LF and HF combined, as they were not significantly different, and thus allowed for more similar numbers in the two groups. Significant increases in infusions per session occurred in the collapsed saline groups and DF agmatine group during Days 6–18, and there was no significant main effect due to treatment. When agmatine was administered after escalation occurred, there was no attenuation of the fentanyl-reinforced responding, and the escalation was similar to the saline-treated controls.

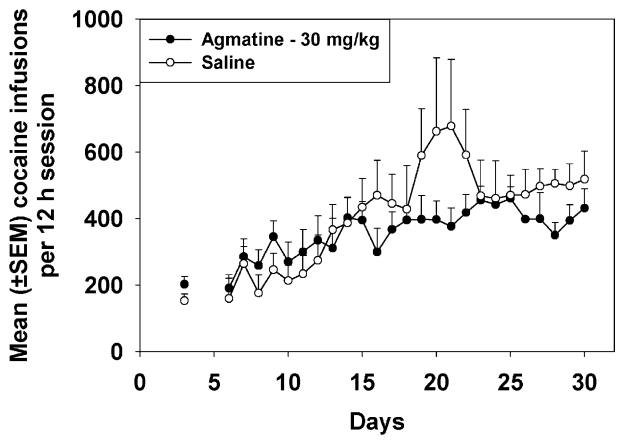

Fig. 4. Mean (±S.E.M.) number of cocaine infusions (0.2 mg/kg/infusion, FR 1) obtained by agmatine (30 mg/kg iv) [Group HC] and saline group over the 30-day experimental period. The 5-day baseline is represented on the left with disconnected points. Connected points indicate the 25-day agmatine and saline treatment period. Closed circles indicate agmatine pretreatment, and open circles indicate saline pretreatment.

3.4. High-dose agmatine (Days 6–30)–cocaine (HC)

Fig. 4 illustrates the mean number of cocaine infusions (0.2 mg/kg/injection) per 12-h session during a 30-day testing period. There were no significant differences over the 5-day pretreatment baseline period between the cocaine and saline groups. Significant increases in infusions occurred over Days 6–30 ($F = 5.97$; $df = 24$; $P < .05$) for both groups; however, there was no significant main effect of treatment. Treatment with agmatine had no effect on the escalation of cocaine self-administration.

4. Discussion

The present study showed that agmatine attenuates the escalation of fentanyl self-administration when administered before the escalation of intake occurs. This result is consistent with previous reports showing that the NMDA receptor and NOS systems can modulate neuroadaptive events such as sensitization and tolerance to opioids and stimulants (Celerier et al., 2001; Dambisya and Lee, 1996; Deroche et al., 1999; Galea et al., 1996; Herman et al., 1995; Trujillo, 2000). An additional finding of the present experiment was that both doses (10 and 30 mg/kg) of agmatine had a similar effect on fentanyl escalation, and agmatine doses up to 200 mg/kg did not affect established responding for cocaine. In fact, the few studies with systemic agmatine have indicated dose-dependent effects in the range of 10–50 mg/kg. Side effects have been reported only at 300–600 mg/kg (Onal and Soykan, 2001; Uzbay et al., 2000). It appears that agmatine can have pronounced central effects when administered systemically at low doses, but dose-related effects were not revealed in the current study. Nevertheless, a potential interpretation of the attenuation of escalation in the current study could be due to the direct effect of the drugs on rate of responding. Although not directly examined via effects of agmatine on a nondrug reinforcer, multiple lines of indirect evidence appear to rule out any nonspecific effects of agmatine. First, the distribution of infusions over the 12-h sessions was examined in the agmatine- and saline-treated rats. The pattern of responding in the agmatine group remained constant throughout the daily sessions and was comparable to the saline group. Secondly, a brief follow-up study with animals in group HC showed no stereotypic movement, ataxic movement or significant effects on cocaine-main-

tained responding when three higher doses of agmatine (60, 120 and 200 mg/kg) were administered (data not shown). Furthermore, there was no difference in food or water intake between animals receiving agmatine or saline throughout the study. Inactive lever responding was low or absent in all rats and was not specifically affected by agmatine. Thus, it is unlikely that agmatine had any major nonspecific effects in the present study.

The self-administration model of escalation used in the current study resulted in significant escalating patterns of drug intake for the 30-day test period. These data support previous work in demonstrating that rats will readily increase their drug intake over time when exposed to relatively low drug doses and longer access time (6–11 h/day) (Ahmed and Koob, 1999; Ahmed et al., 2000). Ahmed et al., (2000) reported that self-administration models similar to the one used in the present study may provide a useful model of the transition from drug use to drug addiction. The present results extend previous work by demonstrating a pharmacological intervention that attenuates the escalation of drug self-administration. Agmatine may have preferentially attenuated fentanyl-maintained responding over cocaine-maintained responding due to characteristics of cocaine, agmatine or their combination. It was previously demonstrated that low-affinity NMDA antagonists (e.g. MRZ 2/579, memantine) inhibited the acquisition of intravenous morphine self-administration more effectively than dizocilpine (Semenova et al., 1999), and agmatine may have selectively affected acquisition of opioid self-administration. Alternatively, the dose of cocaine may have been too high to be affected by agmatine pretreatment. For example, previous work has shown that medications are more effective when the self-administered cocaine doses are lower (Carroll et al., 1990) versus higher (Porrino et al., 1989).

The underlying causes of escalation of drug intake remain to be fully understood. Two neuroadaptive processes, sensitization and tolerance, are presumed to have roles in the escalation process. Further work is needed to determine how agmatine may be affecting these processes and their role in fentanyl self-administration. It appears that tolerance to the effects of agmatine did not develop as the distribution of infusions was constant throughout the study. Further, agmatine did not block or attenuate cocaine self-administration in the present study, although NMDA antagonists have been shown to block cocaine-induced locomotor sensitization (Pulvirenti et al., 1991; Trujillo, 2000).

Agmatine is known to act through pathways other than the NMDA receptor. In addition, further characterization of other phases of addiction (e.g. acquisition, relapse) may provide more sensitive measures whereby agmatine can modulate cocaine self-administration or locomotor activity. For example, a recent examination found no effects of L-NAME, a NOS inhibitor, in the acquisition phase of cocaine self-administration, but found that L-NAME can modulate extinction and relapse phases (Orsini et al., 2002). An extensive parametric analysis of shifts in the dose–response curve would have yielded additional data concerning whether sensitization or tolerance is occurring to cocaine and fentanyl self-administration; however, the use of higher self-administration doses in the present design that produces escalation is precluded by toxicity resulting from the long sessions and duration of the study.

Ahmed and Koob (1998) addressed this problem by imposing days off to prevent significant weight loss in animals self-administering cocaine with 6 h of daily access. Further work using a modified design would be needed to confirm that agmatine was blocking sensitization or tolerance to self-administered fentanyl. A dose–response curve using a PR schedule would be useful for directly measuring the effects of agmatine on the reinforcing efficacy of cocaine and fentanyl self-administration (Richardson and Roberts, 1996).

Endogenously, agmatine may play a role as a neurotransmitter/neuromodulator (Reis and Regunathan, 1999), as it acts by antagonizing the NMDA receptor, inhibiting NOS, binding to 5-HT, alpha2-adrenoceptors and imidazoline binding sites (Galea et al., 1996; Reis and Regunathan, 2000; Trujillo and Akil, 1995). Clinical limitations of NMDA antagonists have been primarily linked to the behavioral, psychotomimetic and toxicological side effects of these drugs (Herman et al., 1995). Agmatine has an affinity for the NMDA receptor that is 30 to 500,000 times lower than clinically (dextromethorphan, ketamine, memantine) and experimentally (aminoguanidine, ifenprodil, dizocilpine, LY235959) used NMDA antagonists (Elliott et al., 1995; Fairbanks et al., 2000). Thus, agmatine may be a potentially useful pharmacotherapy. According to the incentive sensitization theory (Robinson and Berridge, 1993), successful pharmacotherapies will need to address the neuroadaptive processes that initially lead to addiction, and in order to be successful, medications need to prevent the expression of neural sensitization in behavior (Robinson and Berridge, 1993, 2000, 2001).

Independently of the NMDA system, inhibition of NOS has been implicated in a diverse range of neuroplasticity relating to drugs of abuse including cocaine (Collins et al., 2001), ethanol (Koob et al., 1998) and morphine (Dambisya and Lee, 1996). Furthermore, mice lacking the neuronal nitric oxide synthase (nNOS) gene have been shown to be resistant to cocaine-induced behavioral sensitization (Itzhak et al., 1998), methamphetamine-induced dopaminergic neurotoxicity and locomotor sensitization (Itzhak, 1997). Taken together, it appears that agmatine may play a role in modulating behavioral neuroadaptations that could be mediated through multiple neurotransmitter, receptor and feedback loops.

In summary, results from the present study indicate that agmatine attenuates the escalation of fentanyl (2.5 μg/kg) self-administration, if administered before the escalation occurs. Agmatine did not affect the escalation of cocaine (0.2 mg/kg) self-administration. These data suggest that agmatine is acting to mediate neuroadaptive changes in-

volved with the escalation of drug self-administration. Further investigation of the endogenous compound agmatine is necessary to fully understand its mechanism of action and its role in modifying neuroplastic actions associated with the reinforcing effects of drugs of abuse.

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