Pharmacological Reports
2008, 60, 645–654
ISSN 1734-1140

Effects of agmatine on nicotine-evoked behavioral responses in rats

Magdalena Zaniewska[1], Andrew C. McCreary[2], Gulay Sezer[3], Edmund Przegaliński[1], Małgorzata Filip[1]

[1]Laboratory of Drug Addiction Pharmacology, Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences, Smętna 12, PL 31-343 Kraków, Poland

[2]Solvay Pharmaceutical Research Laboratories, CJ van Houtenlaan 36, 1380 DA Weesp, The Netherlands

[3]Department of Pharmacology, Faculty of Medicine, University of Erciyes, 38039 Kayseri, Turkey

Correspondence: Magdalena Zaniewska, e-mail: zaniew@if-pan.krakow.pl

Abstract:
Agmatine [2-(4-aminobutyl)guanidine], is a novel endogenous ligand at α_2-adrenoceptors, imidazoline and N-methyl-D-asparate receptors, as well as a nitric oxide synthase inhibitor. The present study tested whether agmatine (5–40 mg/kg, *sc*) modulated the locomotor, sensitizing, and discriminative stimulus effects of nicotine in male Wistar rats. Agmatine (10–40 mg/kg) affected neither the basal locomotor activity, nor the nicotine (0.4 mg/kg, *sc*)-evoked hyperactivation. A challenge with saline or nicotine (0.4 mg/kg, *sc*) on day 10 to rats treated repeatedly (for 5 days) with nicotine (0.4 mg/kg, *sc*; and exposed to experimental chambers), resulted in the expression of nicotine-evoked conditioned hyperlocomotor response or behavioral sensitization. Given on day 10, agmatine at a dose of 40 mg/kg (but not 20 mg/kg) attenuated nicotine-induced conditioned hyperactivity. However, when this dose was administered to the nicotine-sensitized rats, agmatine failed to alter the effect of the challenge dose of nicotine. In rats trained to discriminate nicotine (0.4 mg/kg, *sc*) from saline in a two-lever water-reinforced fixed ratio 10 task, agmatine (20 or 40 mg/kg) did not substitute for the training dose of nicotine. In combination studies, pretreatment with agmatine (5–40 mg/kg) did not affect the nicotine (0.4 mg/kg) discrimination and the fixed dose of agmatine (20 mg/kg) did not change the effects of the lower doses of nicotine (0.05–0.2 mg/kg).
Our pharmacological analyses indicate that agmatine does not affect the locomotor, sensitizing, or subjective effects of nicotine. However, these data do show an inhibitory effect of agmatine over the expression of nicotine-induced conditioned hyperlocomotion.

Key words:
agmatine, nicotine, drug discrimination, locomotor activity, rat, sensitization

Abbreviations: ANOVA – analysis of variance, CPP – conditioned place preference, FR – fixed ratio, L-NAME – N^G-nitro--arginine-methyl-ester, nAChRs – nicotinic acetylcholine receptors, NMDARs – N-methyl-D-asparate receptors, NOS – nitric oxide synthase

Introduction

Nicotine is the major psychoactive constituent of tobacco smoke that elicits positive subjective [48] and

reinforcing effects in humans [21], and its repeated administration to laboratory animals produces self-administration [11], behavioral sensitization [31], conditioned place preference (CPP) [52], and discriminative stimulus effects [46].

Nicotine exerts its effects mainly through a direct interaction with nicotinic acetylcholine receptors (nAChRs) [36], which are widely distributed throughout the brain and periphery [23]. However, the psychoactive actions of nicotine are mediated centrally *via* the activation of nAChRs. In addition to nAChRs, certain neurotransmitter systems such as glutamatergic or adrenergic systems appear to regulate the behavioral effects evoked by nicotine [19]. In fact, it was shown in preclinical studies that antagonists of N-methyl-D-asparate receptors (NMDARs) or α_2-adrenoceptors, as well as nitric oxide synthase (NOS) inhibitors, may participate in the attenuation of the behavioral (e.g. hyperactivity, behavioral sensitization, CPP, or withdrawal) effects of nicotine [1, 33, 35, 49–51, 66]. Furthermore, nicotine has been demonstrated to interact with NMDARs and it blocks receptor-induced responses [2].

Recently, the endogenous polyamine, agmatine, has been identified in the rat brain and periphery [15, 16, 22, 34, 42–44, 67]. This putative neurotransmitter shows an interesting receptor binding profile and regulates a variety of receptor and enzyme functions. Of note, the affinity of agmatine at α_2-adrenoceptors (K_i = 4 μM) [27, 32, 41] and imidazoline receptors (K_i = 1 μM) [27, 40, 41, 68] is in the low micromolar range. Agmatine also antagonizes NMDARs (K_i = 14.8 μM) [8, 20, 45, 63] and possesses NOS inhibitory activity [9, 12, 18].

Among its different physiological actions, agmatine has also been implicated in processes related to addiction [6, 39, 54]. Interestingly, exogenously administered agmatine reduces tolerance to morphine and symptoms associated with morphine abstinence syndrome [4, 7, 29, 30, 62], as well as the behavioral (locomotor) and biochemical (FosB and dynorphin expression or extracellular dopamine release) expression of morphine sensitization in rats [57, 58]. On the other hand, agmatine potentiates the effects of morphine-induced CPP [55, but in contrast see 57] and analgesia in rodents [25, 28, 47, 64]. In addition, an inhibitory effect of agmatine on ethanol withdrawal symptoms has also been demonstrated in rats [56].

In light of these reports, we expected that agmatine might also influence nicotine-induced responses, but until now this hypothesis has not been investigated. Therefore, we tested whether agmatine modulated the behavioral effects of acute nicotine, nicotine-induced sensitization, conditioned locomotor activity, or discriminative stimulus effects in rats.

Materials and Methods

Animals

Male Wistar rats (derived from the licensed animal breeder T. Górzkowska, Warszawa, Poland) weighing 180–280 g at the beginning of the experiment were used. The animals were housed 7–8 per cage (locomotor activity studies) or 2 per cage (drug discrimination studies) in a colony room maintained at 21 ± 1°C and 40–50% humidity under a 12-h light-dark cycle (the lights were on at 06:00 h). Rodent chow and water were available *ad libitum*, except for in the drug discrimination studies, where water that an animal received was restricted to that given during daily training sessions (5–6 ml/rat per session), after the test sessions (15 min), and on weekends (36 h). All the experiments were conducted during the light phase of the light-dark cycle (between 08:00 and 14:00 h), and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the approval of the Bioethics Commission (compliant with the Polish Law of August 21, 1997).

Drugs

The following drugs were used: agmatine sulfate (1-amino-4-guanidinobutane sulfate; Sigma-Aldrich Chemicals, St. Louis, MO, USA) and (–)-nicotine bitartrate (Sigma-Aldrich Chemicals, St. Louis, MO, USA). Agmatine and nicotine were dissolved in 0.9% saline. In case of nicotine, the pH was adjusted to 7.0 using 20% NaOH. All doses and pretreatment times of the drugs are in agreement with previously published studies [3, 65]. The agmatine doses refer to the weight of the respective salt, while doses of nicotine are expressed as that of the free base. Both agmatine and nicotine were administered in a volume of 1 ml/kg subcutaneously (*sc*).

Locomotor activity studies

Apparatus and measurements

The locomotor activity was recorded individually for each animal in Opto-Varimex cages (Columbus Instruments, Columbus, OH, USA) linked on-line to a compatible IBM-PC. Each cage (43 × 44 × 25 cm) was surrounded with a 15 × 15 array of photocell beams located 3 cm from the floor surface as reported previously [17, 38]. Interruptions of the photobeams resulted in horizontal locomotor activity, defined as a distance traveled, and was expressed in cm. Measurements of locomotor activity began immediately after saline or nicotine injection, and were recorded and divided into 15 min time intervals for a total of 60 min. Agmatine was administered 30 min before the injection of saline or nicotine.

Acute treatment (basal and nicotine-induced locomotor activity)

Before locomotor activity was recorded, rats were habituated in the experimental cages for 120 min/day for 2 days before the start of the experiment, and on the test day for 30 min before the start of experiment. Locomotor activity was recorded in animals which received either saline (1 ml/kg) or agmatine (10–40 mg/kg) combined with either saline or nicotine (0.4 mg/kg).

Nicotine repeated treatment (expression of nicotine-evoked conditioned locomotor activity and sensitization)

Rats were given repeated pairings of a distinct test environment (experimental chamber) with either nicotine (0.4 mg/kg) or saline (1 ml/kg) for 5 days. Rats remained in their home cages during days 6–9 of the experiment. Animals were then challenged on day 10, with saline (expression of conditioned locomotor activity) or nicotine (0.4 mg/kg, expression of nicotine sensitization) in experimental chambers. Agmatine (20 and 40 mg/kg) was given on day 10 of the experimentation before injection of saline (conditioned locomotor activity) or nicotine (nicotine sensitization).

Nicotine discrimination

Apparatus

Commercially available, two-lever operant chambers (MedAssociates, St. Albans, VT, USA) were used. Each chamber was equipped with a water-filled dispenser mounted equidistantly between two response levers on the wall and housed in a light- and sound-proof cubicle (MedAssociates). Illumination came from a 28 V house light, while ventilation and masking noise were supplied with a ventilation fan. A computer with MedState software was used to program and record all the experimental events.

Nicotine discrimination procedure

Standard two-lever, water-reinforced drug discrimination procedures were utilized [65]. Drug naive rats (n = 8) were trained to discriminate nicotine (0.4 mg/kg) from saline (1 ml/kg). Both nicotine and saline were administered 15 min before the start of the training session. Daily sessions lasted 15 min and were conducted on Mondays through Fridays. In the initial "errorless training" phase, only the stimulus-appropriate (drug or saline) lever was present. Training began under a fixed ratio (FR) 1 schedule of water reinforcement and the FR requirement was incrementally adjusted until all the animals were responding reliably under the FR 10 schedule for each experimental condition. For half of the rats, right-lever responses were reinforced after nicotine administration, whereas left-lever responses were reinforced after saline administration; the conditions were reversed for the remaining rats. During the training phase, nicotine and saline were administered irregularly with the restriction that neither condition prevailed for more than three consecutive sessions. After responses were stabilized, discrimination training was initiated and both levers were presented simultaneously during 15 min sessions. The rats were trained to respond on the stimulus-appropriate (correct) lever in order to obtain water reinforcement, and there were no programmed consequences of responding on the incorrect lever. This phase of training continued until the performance of all the trained rats met the criterion (defined as mean accuracies of at least 80% correct for 10 consecutive sessions).

When the rats achieved the accuracy criterion, test sessions were initiated and conducted once or twice

per week with training sessions intervening during the remaining days. The rats were required to maintain accuracies of at least 80% correct for the saline and nicotine maintenance sessions which immediately preceded a test. During test sessions, the animals were placed in the chambers and upon completion of 10 responses on either lever, a single reinforcer was delivered and the house lights were turned off. The test sessions were terminated after 15 min if the rats did not complete 10 responses on either lever. Then the rats were removed from the chamber, returned to the colony, and allowed free access to water for 15 min beginning 15–30 min after the end of each test.

Several pharmacological manipulations were performed during the test sessions. In substitution (generalization) tests, the rats were examined for lever responses after various doses of the training drug (nicotine), or doses of agmatine (20 and 40 mg/kg). In combination (antagonism or potentiation) tests, agmatine (5–40 mg/kg) was administered prior to nicotine (0.4 mg/kg), or a fixed dose of agmatine (20 mg/kg) was given before different doses of nicotine (0.05–0.2 mg/kg). Agmatine was given at 30 min, and nicotine at 15 min before tests.

Data analyses

Locomotor activity

The data are expressed as a mean horizontal locomotor activity (± SEM) for the 60 min observation period and for the 15 min time intervals. The acute treatment data were analyzed using two-way analysis of variance (ANOVA) for the factors of pretreatment (0 mg/kg and different doses of agmatine), treatment (0 and 0.4 mg/kg nicotine), and the pretreatment × treatment interaction, followed by a *post-hoc* Newman-Keuls test used to evaluate the treatment group effects. The nicotine-repeated treatment data were analyzed using a one-way ANOVA, followed by *post-hoc* Dunnett's tests applied to evaluate the treatment group effect.

Nicotine discrimination

The average sessions that were needed for the training are presented with SEM. During training sessions, accuracy was defined as the percentage of correct responses to total responses before the delivery of the first reinforcer. During the test sessions, performance was expressed as the percentage of nicotine-appropriate responses to total responses before the delivery of the first reinforcer. Response rates (responses per s) were evaluated during training and test sessions (as a measure of behavioral disruption). For the training sessions, the response rate was calculated as the total number of responses emitted on either lever before completion of the first FR 10 on the stimulus appropriate lever divided by the number of seconds taken to complete that FR 10. During test sessions, the response rate was calculated as the total number of responses before the completion of 10 responses on either lever divided by the number of seconds necessary to complete the FR 10. The data from all animals during test sessions were used.

A drug was considered to substitute for nicotine if it evoked at least 80–100% (maximum) of the drug-appropriate lever responses. The Student's *t*-test for repeated measures was used to compare the percentage of drug-lever responses and the response rate during test sessions with the corresponding values for either the previous saline or nicotine session (substitution and combination tests). A two-way ANOVA for repeated measures for the factors of pretreatment (0 mg/kg or 20 mg/kg agmatine), treatment (0 mg/kg and different doses of nicotine), and the pretreatment × treatment interaction was used to find out whether the percentage of nicotine-lever responses and the response rates observed for several doses of nicotine differed in the presence *vs*. absence of a fixed dose of agmatine (combination tests). All comparisons were made with an experiment wise type I error rate (α) set at 0.05.

Results

Locomotor activity studies

Acute treatment

Nicotine (0.4 mg/kg) in combination with saline during the 60 min observation period significantly increased the rats' basal locomotor activity by approximately 8-fold compared to the group that received saline in combination with saline (Fig. 1A).

An overall effect of the treatment [$F(1, 51) = 102.07$, $p < 0.001$] was seen for the total (60 min) locomotor

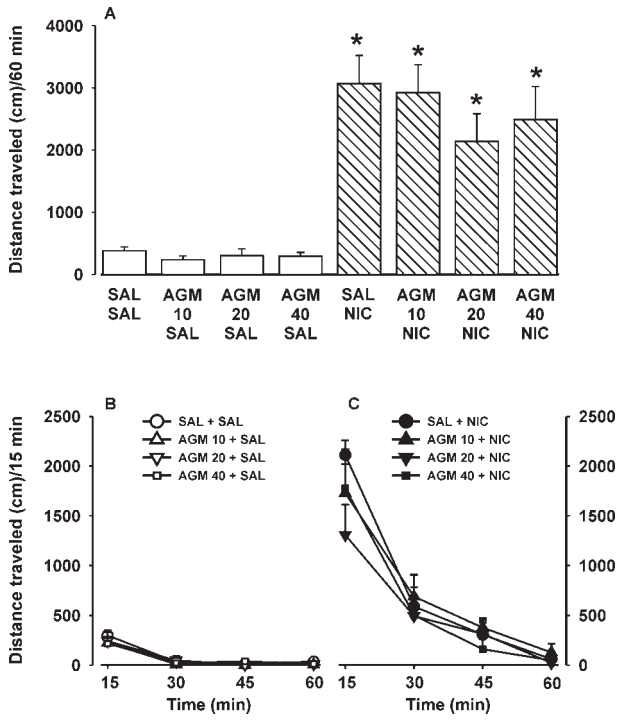

Fig. 1. Effects of agmatine (AGM) on the basal and acute nicotine (NIC; 0.4 mg/kg)-stimulated locomotor activity in rats. All the data represent the horizontal locomotor activity means (± SEM) of data from 7–8 rats. **(A)** Total (60 min session) horizontal activity mean after administration of saline (SAL) or AGM (10–40 mg/kg) followed by injection of SAL (white bars) or NIC (hatched bars). * $p < 0.01$ *vs.* SAL + SAL group. The time course of horizontal locomotor activity plotted in 15 min time intervals across the 60 min session is depicted for basal **(B)** and NIC-stimulated **(C)** locomotor activity

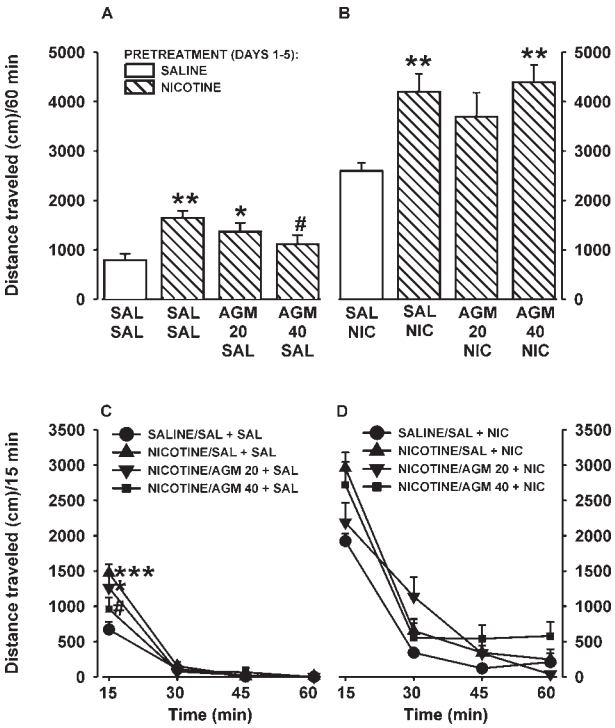

Fig. 2. Effects of agmatine (AGM) on the expression of nicotine (NIC; 0.4 mg/kg)-evoked conditioned locomotor activity **(A)** and sensitization **(B)**. Rats were treated repeatedly (days 1–5) with saline (SAL; white bars) or NIC (0.4 mg/kg; hatched bars). All the data represent the horizontal locomotor activity means (± SEM) of data from 6–8 rats. A: On day 10, the animals were given a challenge dose of SAL + SAL or AGM (20 or 40 mg/kg) + SAL. * $p < 0.05$, ** $p < 0.01$ *vs.* SAL-treated and SAL + SAL-challenged group; # $p < 0.05$ *vs.* NIC-treated and SAL + SAL-challenged group. B: On day 10, the animals were given a challenge dose of SAL + NIC (0.4 mg/kg) or AGM (20 or 40 mg/kg) + NIC (0.4 mg/kg). ** $p < 0.01$ *vs.* SAL-treated and SAL + NIC-challenged group. The time course of horizontal locomotor activity plotted in 15 min time intervals across the 60 min session is depicted for (NIC)-evoked conditioned locomotor activity **(C)** and sensitization **(D)**. For C: * $p < 0.05$, *** $p < 0.001$ *vs.* SAL-treated and SAL + SAL-challenged group; # $p < 0.05$ *vs.* NIC-treated and SAL + SAL-challenged group

activity in groups pretreated with agmatine (10–40 mg/kg), while neither a significant effect of a pretreatment [$F(3, 51) = 1.24$] nor pretreatment × treatment interaction [$F(3, 51) = 0.42$] was observed. Agmatine (10–40 mg/kg) did not change either the rats' basal locomotor activity or acute nicotine response (Fig. 1A). When the separate 15 min time intervals were examined for the effects of agmatine on basal locomotion, a significant effect of the treatment [$F(3, 78) = 30.96$, $p < 0.001$] was seen. In contrast, no significant effect of the pretreatment [$F(3, 26) = 0.20$] or of the pretreatment × treatment interaction [$F(9, 78) = 0.50$] was observed (Fig. 1B). Again, when the separate 15 min time intervals were examined for the effects of agmatine on acute nicotine administration, a significant effect of treatment [$F(3, 75) = 83.40$, $p < 0.001$] was observed, while there was no significant effect of pretreatment [$F(3, 25) = 0.82$] or pretreatment × treatment interaction [$F(9, 75) = 1.07$] (Fig. 1C).

Nicotine repeated treatment

On day 10, the saline challenge of rats treated repeatedly (days 1–5) with nicotine (0.4 mg/kg) significantly enhanced the total locomotor activity compared to the effect of saline-treated (days 1–5) animals (approximately 2-fold) in experimental chambers (conditioned locomotor activity; Fig. 2A).

On day 10, when agmatine (40, but not 20 mg/kg) was given to nicotine-treated rats, a significant decrease in total locomotor activity was observed in comparison to nicotine-treated and saline-challenged rats [$F(3, 25) = 5.15$, $p < 0.01$] (Fig. 2A). Locomotor activity scorings during the 0–15 [$F(3, 25) = 5.48$,

$p < 0.01$], but not 16–30 [$F(3, 25) = 0.35$], 31–45 [$F(3, 25) = 0.66$], or 46–60 [$F(3, 25) = 1.18$] min time intervals showed an inhibitory effect of agmatine (40 mg/kg) on saline challenge in nicotine treated rats (Fig. 2C).

On day 10, administration of a challenge dose of nicotine (0.4 mg/kg) to animals that received repeated (days 1–5) nicotine (0.4 mg/kg) treatments produced a significant (approximately 1.6-fold) increase in the total locomotor activity compared to the effect of acute nicotine injection to saline-treated (days 1–5) animals (nicotine sensitization; Fig. 2B).

On day 10, agmatine (20 and 40 mg/kg) given in combination with nicotine (0.4 mg/kg), produced no alteration in total locomotor activity as compared to nicotine treated and nicotine challenged rats [$F(3, 20) = 3.72$, $p < 0.05$] (Fig. 2B). Similarly, locomotor activity scorings during the 15 min time intervals (0–15 min: [$F(3, 20) = 2.99$]; 16–30-min: [$F(3, 20) = 2.54$]; 31–45 min: [$F(3, 20) = 1.11$]; 46–60-min: [$F(3, 20) = 2.83$) did not demonstrate any significant effect of agmatine on nicotine challenge in nicotine treated rats (Fig. 2D).

Nicotine discrimination

Acquisition of the nicotine (0.4 mg/kg) *vs*. saline discrimination was reached in an average of 29 ± 4 sessions.

Substitution studies

Administration of nicotine (0.025–0.4 mg/kg) to rats produced a dose-dependent increase in the nicotine-appropriate responses (Fig. 3B), whereas saline administration resulted in < 10% of nicotine-lever responses (Fig. 3A). The drug-lever responses after doses of 0.025 and 0.05 mg/kg of nicotine were significantly different from the preceding nicotine training session ($p < 0.001$), and these lower doses of nicotine did not fully substitute for the nicotine training dose (0.4 mg/kg) (Fig. 3B). Response rates for all the test doses of nicotine and saline did not differ from those obtained during the immediately preceding nicotine or saline maintenance sessions (Fig. 3).

At the doses tested, agmatine (20 and 40 mg/kg) (Tab. 1) or its vehicle (saline) evoked no drug-appropriate lever responses when given alone, which indicated no substitution for the nicotine training dose (0.4 mg/kg). None of the doses tested (Tab. 1) nor the vehicle (saline) affected the response rates of animals as compared to previous nicotine and saline training sessions.

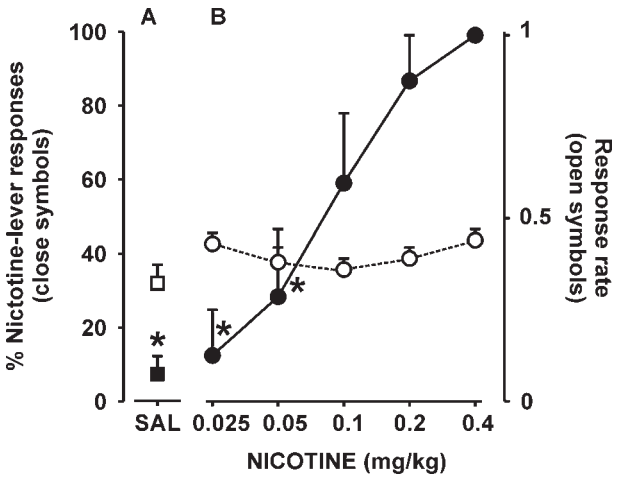

Fig. 3. Substitution studies with nicotine in rats trained to discriminate nicotine (0.4 mg/kg) from saline (SAL). Symbols show the mean percentage of nicotine-lever responses (± SEM; closed symbols) and the mean number of responses/s (± SEM; open symbols). Performance is shown after injection of SAL **(A)** (1 ml/kg; squares) or nicotine **(B)** (0.025–0.4 mg/kg; circles). All the data points represent the means of data from 7–8/7–8 rats [n/N, number of rats (n) completing the FR 10 on either lever out of the number of rats tested (N)]. * $p < 0.001$ *vs.* preceding nicotine training session

Tab. 1. Substitution studies with agmatine in rats trained to discriminate nicotine (0.4 mg/kg) from saline

Drug (mg/kg)	% Nicotine-lever responses	Response rate (responses/s)
Saline	7.39 ± 4.81*	0.32 ± 0.05
Agmatine (20)	0.00 ± 0.00*	0.55 ± 0.08
Agmatine (40)	0.00 ± 0.00*	0.48 ± 0.08

* Denotes performance during test sessions which were significantly different ($p < 0.001$) from the preceding nicotine maintenance sessions that indicated no substitution for nicotine

Combination studies

Pretreatment with agmatine (5–40 mg/kg) in combination with nicotine (0.4 mg/kg) altered neither the nicotine-lever responses [$F(4, 29) = 1.16$], nor the response rates [$F(4, 29) = 0.81$] (Fig. 4A).

A fixed dose of agmatine (20 mg/kg) administered together with lower doses of nicotine (0.05–0.2 mg/kg) did not change the nicotine-lever responses (overall

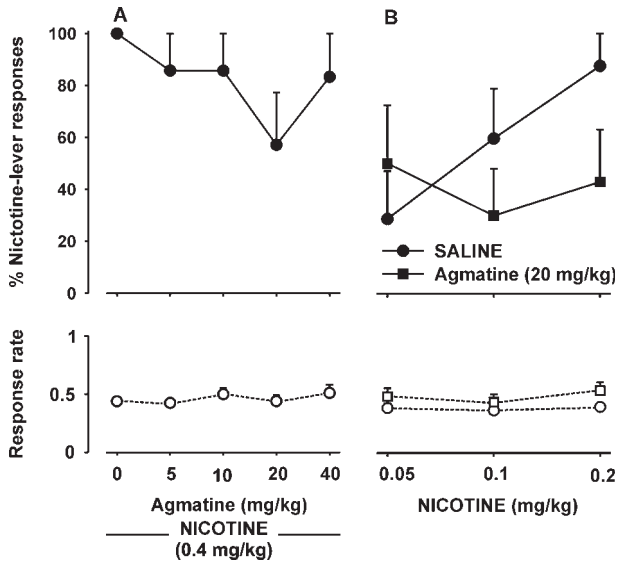

Fig. 4. Combination studies with agmatine in rats trained to discriminate nicotine (0.4 mg/kg) from saline. **(A)** Effects of agmatine (5–40 mg/kg) on the nicotine (0.4 mg/kg)-induced discriminative stimulus effects. Performance is shown after injection of nicotine preceded by injection of agmatine (circles). **(B)** Effects of agmatine (20 mg/kg) on the nicotine (0.05–0.2 mg/kg)-induced discriminative stimulus effects. Performance is shown after injection of saline (circles), or agmatine (squares) in combination with nicotine. All the data points represent the means of data from 6–8/6–8 rats. For more details see Figure 3

effects of pretreatment [F(1, 13) = 2.04], treatment [F(2, 26) = 1.16], or pretreatment × treatment interaction [F(2, 26) = 1.87] were not observed), and response rates were similarly unaffected (overall effects of a pretreatment [F(1, 13) = 4.01], treatment [F(2, 26) = 1.32], or pretreatment × treatment interaction [F(2, 26) = 0.40] were not observed) (Fig. 4B).

Discussion

Agmatine is an endogenous drug that has been established to act *via* several receptors, including α_2-adrenoceptors [27] and imidazoline receptors [27, 41], and displays inhibitory actions at NMDARs [8, 20, 45, 63] and NOS [9]. Based on such a binding and functional profile, agmatine seems to be a good candidate for the attenuation of the behavioral effects of several addictive substances.

In the present study, we found that exogenously administered agmatine displayed little potency in affecting acute or repeated nicotine treatments in rats. In fact, agmatine administered only at the highest dose (40 mg/kg) attenuated the nicotine-induced conditioned locomotor activity, without affecting either the locomotor (acute and sensitizing) or discriminative stimulus effects of nicotine.

Specifically, using agmatine at a dose-range of 10–40 mg/kg caused no changes in either basal locomotion or hyperactivation to acute nicotine (0.4 mg/kg) treatment.

In the next set of experiments, when the rats were given nicotine (0.4 mg/kg; unconditioned stimulus) paired with the environment (conditioned stimulus; experimental chambers) for 5 days and were challenged with the same dose of nicotine on day 10 of the experiment, the locomotor activity of the animals was about two times higher than that observed in saline-treated animals challenged with nicotine (an expression of nicotine sensitization). Sensitization arises as a consequence of repeated intermittent nicotine administration and may be considered as a valid animal model of craving and relapse [13]. Here, we demonstrate that agmatine administered at a dose of 20 or 40 mg/kg before the challenge dose of nicotine does not counteract the expression of nicotine sensitization.

We have previously demonstrated the responses to a discriminative stimulus mediated by nicotine in rats [65], and now we have shown that agmatine does not influence this paradigm. In fact, agmatine (5–40 mg/kg) did not substitute for or change the expression of nicotine discrimination.

Presently, the lack of effect of agmatine on nicotine-induced locomotor or subjective effects is difficult to explain, but it is certain that the tested doses of this drug were in the correct concentration range and pretreatment time. To support this claim of efficacy, 10 mg/kg (*sc*) of agmatine was sufficient to enhance morphine analgesia and attenuate the morphine tolerance in mice [25]. Administration to rats, at similar dose ranges as those used here (20–40 mg/kg), demonstrated effective blockade of morphine [7] or ethanol withdrawal syndromes [56]. Similarly, the 30 min pretreatment time was shown to be sufficient in decreasing immobility time in the forced swim test and in enhancing the time spent in the open arms of the elevated plus maze test [3].

The other possible reason for the observed ineffectiveness of agmatine might be due to its relatively short duration of action (10–30 min) as reported by Roberts et al. [45]. However, detailed examinations of our locomotor activity scorings during the 0–15 or

16–30 min time intervals did not show any effect of agmatine on either acute nicotine injection or nicotine challenge in nicotine-treated rats. Similarly, the studied effect of agmatine in the nicotine discrimination paradigm was within the proposed time course of agmatine's action (≤ 15 min).

Additional evidence supporting that the lack of effect of agmatine on nicotine-evoked hyperactivity, expression of sensitization, and stimulus discrimination is not related to its pharmacokinetics is that agmatine administered at a dose of 40 mg/kg significantly reduced conditioned locomotion induced by nicotine. In the latter model, repeated (5 days) pairings of nicotine (0.4 mg/kg) with an environment (experimental chambers) evoked the expression of conditioned locomotor activity (i.e., the enhancement of activity occurs following saline injection in the group of rats treated repeatedly with nicotine, but not with saline). Here, we confirm that environmental factors have a major influence on the effects of nicotine [26]. Furthermore, these findings establish for nicotine a link with previously published data demonstrating the existence of conditioned hyperactivity in rodents in an environment previously paired with another drug of abuse, such as cocaine [10]. This model of conditioning allows the investigation of one of the withdrawal symptoms, craving, which in human addicts is evoked by the exposure to the conditioned stimulus associated with drug consumption [53]. Hence, the idea here being that nicotine is withdrawn, but the animals are tested in the experimental cages associated with previous nicotine administrations [14]. A question arises as to whether agmatine's effect on nicotine-associated cues results from its modulatory action on motivational (due to previous pairing of the drug with the environment) or emotional consequences of nicotine exposure (e.g., anxiety and depression). It must be noted that agmatine at the dose required to reduce the nicotine cue, did not affect the animals' basal locomotor activity or the response rate in the nicotine discrimination paradigm. Agmatine's effect does not seem to be related to an attenuation of motivational behavior, since it did not alter the subjective effects of nicotine (Fig. 4). However, studies examining the effect of agmatine on nicotine self-administration are missing from the literature. The fact that agmatine increased the caloric intake in satiated, but not hungry, rats [37] may account for its inhibitory effect on environmentally triggered cravings. To address this issue, further studies with food self-administration are needed. On the other hand, agmatine's anti-depressive-like and anxiolytic effects [3] may have some association with its attenuating effect on nicotine-evoked cravings.

Agmatine has been shown to be effective in attenuating the withdrawal symptoms of morphine [4, 7, 29, 30, 62]. Some earlier results demonstrated that imidazoline receptor antagonists abolish the attenuating effects of agmatine on naloxone-precipitated morphine withdrawal [30, 59–61]. Likewise, others reported that NOS might be a mediator of morphine abstinence symptoms [5, 29, 62]. In terms of the rewarding properties of morphine (tested in the CPP task), agmatine was proposed to act *via* a mechanism associated with NO and of α_2-adrenoceptors [24, 55].

It is difficult to conclude whether agmatine binds a specific target to mediate its effects on nicotine craving. Some literature indicates that NMDARs are key receptors in the attenuation of expression of nicotine-induced conditioned responses or CPP [33, 35]. In contrast, a nonselective NOS inhibitor, L-NAME [N^G-nitro-L-arginine methyl ester], has been shown to reduce nicotine abstinence signs [1]. However, to further explain the roles of NMDARs or NO signaling in the inhibitory actions of agmatine toward the nicotine-evoked conditioned hyperlocomotion will require additional studies.

In conclusion, the findings of the present study indicate that agmatine has a weak regulatory effect on the behavioral properties of nicotine.

Acknowledgments:
The authors wish to thank Mrs. E. Nowak and K. Wydra for providing technical assistance. This research was supported by the statutory funds from the Department of Pharmacology, Institute of Pharmacology, Polish Academy of Sciences (Kraków, Poland).

References:

1. Adams ML, Cicero TJ: Nitric oxide mediates mecamylamine- and naloxone-precipitated nicotine withdrawal. Eur J Pharmacol, 1998, 345, R1–R2.
2. Aizenman E, Tang LH, Reynolds IJ: Effects of nicotinic agonists on the NMDA receptor. Brain Res, 1991, 551, 355–357.
3. Aricioglu F, Altunbas H: Is agmatine an endogenous anxiolytic/antidepressant agent? Ann N Y Acad Sci, 2003, 1009, 136–140.
4. Aricioglu F, Means A, Regunathan S: Effect of agmatine on the development of morphine dependence in rats: potential role of cAMP system. Eur J Pharmacol, 2004, 504, 191–197.

5. Aricioglu F, Paul IA, Regunathan S: Agmatine reduces only peripheral-related behavioral signs, not the central signs, of morphine withdrawal in nNOS deficient transgenic mice. Neurosci Lett, 2004, 354, 153–157.
6. Aricioglu-Kartal F, Regunathan S: Effect of chronic morphine treatment on the biosynthesis of agmatine in rat brain and other tissues. Life Sci, 2002, 71, 1695–1701.
7. Aricioglu-Kartal F, Uzbay IT: Inhibitory effect of agmatine on naloxone-precipitated abstinence syndrome in morphine dependent rats. Life Sci, 1997, 61, 1775–1781.
8. Askalany AR, Yamakura T, Petrenko AB, Kohno T, Sakimura K, Baba H: Effect of agmatine on heteromeric N-methyl-D-aspartate receptor channels. Neurosci Res, 2005, 52, 387–392.
9. Auguet M, Viossat I, Marin JG, Chabrier PE: Selective inhibition of inducible nitric oxide synthase by agmatine. Jpn J Pharmacol, 1995, 69, 285–287.
10. Barr GA, Sharpless NS, Cooper S, Schiff SR, Paredes W, Bridger WH: Classical conditioning, decay and extinction of cocaine-induced hyperactivity and stereotypy. Life Sci, 1983, 33, 1341–1351.
11. Corrigall WA, Coen KM: Nicotine maintains robust self-administration in rats on a limited-access schedule. Psychopharmacology, 1989, 99, 473–478.
12. Demady DR, Jianmongkol S, Vuletich JL, Bender AT, Osawa Y: Agmatine enhances the NADPH oxidase activity of neuronal NO synthase and leads to oxidative inactivation of the enzyme. Mol Pharmacol, 2001, 59, 24–29.
13. DiFranza JR, Wellman RJ: A sensitization-homeostasis model of nicotine craving, withdrawal, and tolerance: integrating the clinical and basic science literature. Nicotine Tob Res, 2005, 7, 9–26.
14. DiFranza JR, Wellman RJ: Sensitization to nicotine: how the animal literature might inform future human research. Nicotine Tob Res, 2007, 9, 9–20.
15. Escribá PV, Ozaita A, Miralles A, Reis DJ, García-Sevilla JA: Molecular characterization and isolation of a 45-kilodalton imidazoline receptor protein from the rat brain. Brain Res Mol Brain Res, 1995, 32, 187–196.
16. Feng Y, Halaris AE, Piletz JE: Determination of agmatine in brain and plasma using high-performance liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl, 1997, 691, 277–286.
17. Frankowska M, Filip M, Przegaliński E: Effects of $GABA_B$ receptor ligands in animal tests of depression and anxiety. Pharmacol Rep, 2007, 59, 645–655.
18. Galea E, Regunathan S, Eliopoulos V, Feinstein DL, Reis DJ: Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem J, 1996, 316, 247–249.
19. George TP, O'Malley SS: Current pharmacological treatments for nicotine dependence. Trends Pharmacol Sci, 2004, 25, 42–48.
20. Gibson DA, Harris BR, Rogers DT, Littleton JM: Radioligand binding studies reveal agmatine is a more selective antagonist for a polyamine-site on the NMDA receptor than arcaine or ifenprodil. Brain Res, 2002, 952, 71–77.
21. Goldberg SR, Henningfield JE: Reinforcing effects of nicotine in humans and experimental animals responding under intermittent schedules of i.v. drug injection. Pharmacol Biochem Behav, 1988, 30, 227–234.
22. Halaris A, Plietz J: Agmatine: metabolic pathway and spectrum of activity in brain. CNS Drugs, 2007, 21, 885–900.
23. Kalamida D, Poulas K, Avramopoulou V, Fostieri E, Lagoumintzis G, Lazaridis K, Sideri A et al.: Muscle and neuronal nicotinic acetylcholine receptors. Structure, function and pathogenicity. FEBS J, 2007, 274, 3799–3845.
24. Khoshnoodi MA, Motiei-Langroudi R, Tahsili-Fahadan P, Yahyavi-Firouz-Abadi N, Ghahremani MH, Dehpour AR: Involvement of nitric oxide system in enhancement of morphine-induced conditioned place preference by agmatine in male mice. Neurosci Lett, 2006, 399, 234–239.
25. Kolesnikov Y, Jain S, Pasternak GW: Modulation of opioid analgesia by agmatine. Eur J Pharmacol, 1996, 296, 17–22.
26. Le Foll B, Goldberg SR: Control of the reinforcing effects of nicotine by associated environmental stimuli in animals and humans. Trends Pharmacol Sci, 2005, 26, 287–293.
27. Li G, Regunathan S, Barrow CJ, Eshraghi J, Cooper R, Reis DJ: Agmatine: an endogenous clonidine-displacing substance in the brain. Science, 1994, 263, 966–969.
28. Li J, Li X, Pei G, Qin BY: Analgesic effect of agmatine and its enhancement on morphine analgesia in mice and rats. Zhongguo Yao Li Xue Bao, 1999, 20, 81–85.
29. Li J, Li X, Pei G, Qin BY: Correlation between inhibitions of morphine withdrawal and nitric-oxide synthase by agmatine. Zhongguo Yao Li Xue Bao, 1999, 20, 375–380.
30. Li J, Li X, Pei G, Qin BY: Effects of agmatine on tolerance to and substance dependence on morphine in mice. Zhongguo Yao Li Xue Bao, 1999, 20, 232–238.
31. Miller DK, Wilkins LH, Bardo MT, Crooks PA, Dwoskin LP: Once weekly administration of nicotine produces long-lasting locomotor sensitization in rats via a nicotinic receptor-mediated mechanism. Psychopharmacology, 2001, 156, 469–476.
32. Molderings GJ, Menzel S, Kathmann M, Schlicker E, Göthert M: Dual interaction of agmatine with the rat α_{2D}-adrenoceptor: competitive antagonism and allosteric activation. Br J Pharmacol, 2000, 130, 1706–1712.
33. Murray JE, Bevins RA: Behavioral and neuropharmacological characterization of nicotine as a conditional stimulus. Eur J Pharmacol, 2007, 561, 91–104.
34. Otake K, Ruggiero DA, Regunathan S, Wang H, Milner TA, Reis DJ: Regional localization of agmatine in the rat brain: an immunocytochemical study. Brain Res, 1998, 787, 1–14.
35. Papp M, Gruca P, Willner P: Selective blockade of drug-induced place preference conditioning by ACPC, a functional NDMA-receptor antagonist. Neuropsychopharmacology, 2002, 27, 727–743.
36. Picciotto MR, Caldarone BJ, King SL, Zachariou V: Nicotinic receptors in the brain. Links between molecular biology and behavior. Neuropsychopharmacology, 2000, 22, 451–465.
37. Prasad A, Prasad C: Agmatine enhances caloric intake and dietary carbohydrate preference in satiated rats. Physiol Behav, 1996, 60, 1187–1189.

38. Przegaliński E, Filip M, Papla I, Siwanowicz J: Effect of serotonin (5-HT)$_{1B}$ receptor ligands on cocaine sensitization in rats. Behav Pharmacol, 2001, 12, 109–116.
39. Regunathan S: Agmatine: biological role and therapeutic potentials in morphine analgesia and dependence. AAPS J, 2006, 8, E479–E484.
40. Regunathan S, Youngson C, Raasch W, Wang H, Reis DJ: Imidazoline receptors and agmatine in blood vessels: a novel system inhibiting vascular smooth muscle proliferation. J Pharmacol Exp Ther, 1996, 276, 1272–1282.
41. Reis DJ, Li G, Regunathan S: Endogenous ligands of imidazoline receptors: classic and immunoreactive clonidine-displacing substance and agmatine. Ann N Y Acad Sci, 1995, 763, 295–313.
42. Reis DJ, Regunathan S: Agmatine: a novel neurotransmitter? Adv Pharmacol, 1998, 42, 645–649.
43. Reis DJ, Regunathan S: Agmatine: an endogenous ligand at imidazoline receptors is a novel neurotransmitter. Ann N Y Acad Sci, 1999, 881, 65–80.
44. Reis DJ, Regunathan S: Is agmatine a novel neurotransmitter in brain? Trends Pharmacol Sci, 2000, 21, 187–193.
45. Roberts JC, Grocholski BM, Kitto KF, Fairbanks CA: Pharmacodynamic and pharmacokinetic studies of agmatine after spinal administration in the mouse. J Pharmacol Exp Ther, 2005, 314, 1226–1233.
46. Rosecrans JA, Villanueva HF: Discriminative stimulus properties of nicotine: mechanisms of transduction. NIDA Res Monogr, 1991, 116, 101–116.
47. Ruiz-Durantez E, Llorente J, Ulibarri I, Pineda J, Ugedo L: Agmatine-morphine interaction on nociception in mice. Ann N Y Acad Sci, 2003, 1009, 133–136.
48. Russell MA: Subjective and behavioural effects of nicotine in humans: some sources of individual variation. Prog Brain Res, 1989, 79, 289–302.
49. Sahraei H, Falahi M, Zarrindast MR, Sabetkasaei M, Ghoshooni H, Khalili M: The effects of nitric oxide on the acquisition and expression of nicotine-induced conditioned place preference in mice. Eur J Pharmacol, 2004, 503, 81–87.
50. Shim I, Kim HT, Kim YH, Chun BG, Hahm DH, Lee EH, Kim SE, Lee HJ: Role of nitric oxide synthase inhibitors and NMDA receptor antagonist in nicotine-induced behavioral sensitization in the rat. Eur J Pharmacol, 2002, 443, 119–124.
51. Shoaib M, Benwell ME, Akbar MT, Stolerman IP, Balfour DJ: Behavioural and neurochemical adaptations to nicotine in rats: influence of NMDA antagonists. Br J Pharmacol, 1994, 111, 1073–1080.
52. Shoaib M, Stolerman IP, Kumar RC: Nicotine-induced place preferences following prior nicotine exposure in rats. Psychopharmacology, 1994, 113, 445–452.
53. Siegel S, Ramos BM: Applying laboratory research: drug anticipation and the treatment of drug addiction. Exp Clin Psychopharmacol, 2002, 10, 162–183.
54. Su RB, Li J, Qin BY: A biphasic opioid function modulator: agmatine. Acta Pharmacol Sin, 2003, 24, 631–636.
55. Tahsili-Fahadan P, Yahyavi-Firouz-Abadi N, Khoshnoodi MA, Motiei-Langroudi R, Tahaei SA, Ghahremani MH, Dehpour AR: Agmatine potentiates morphine-induced conditioned place preference in mice: modulation by α_2-adrenoceptors. Neuropsychopharmacology, 2005, 31, 1722–1732.
56. Uzbay IT, Yeşilyurt O, Celik T, Ergün H, Işimer A: Effects of agmatine on ethanol withdrawal syndrome in rats. Behav Brain Res, 2000, 107, 153–159.
57. Wei XL, Su RB, Lu XQ, Liu Y, Yu SZ, Yuan BL, Li J: Inhibition by agmatine on morphine-induced conditioned place preference in rats. Eur J Pharmacol, 2005, 515, 99–106.
58. Wei XL, Su RB, Wu N, Lu XQ, Zheng JQ, Li J: Agmatine inhibits morphine-induced locomotion sensitization and morphine-induced changes in striatal dopamine and metabolites in rats. Eur Neuropsychopharmacol, 2007, 17, 790–799.
59. Wu N, Su RB, Li J: Agmatine and imidazoline receptors: their role in opioid analgesia, tolerance and dependence. Cell Mol Neurobiol, 2008, 28, 629–641.
60. Wu N, Su RB, Liu Y, Lu XQ, Zheng JQ, Cong B, Li J: Modulation of agmatine on calcium signal in morphine-dependent CHO cells by activation of IRAS, a candidate for imidazoline I1 receptor. Eur J Pharmacol, 2006, 548, 21–28.
61. Wu N, Su RB, Xu B, Lu XQ, Liu Y, Zheng JQ, Piletz JE et al.: IRAS, a candidate for I1-imidazoline receptor, mediates inhibitory effect of agmatine on cellular morphine dependence. Biochem Pharmacol, 2005, 70, 1079–1087.
62. Yananli H, Gören MZ, Berkman K, Aricioğlu F: Effect of agmatine on brain L-citrulline production during morphine withdrawal in rats: a microdialysis study in nucleus accumbens. Brain Res, 2007, 1132, 51–58.
63. Yang XC, Reis DJ: Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J Pharmacol Exp Ther, 1999, 288, 544–549.
64. Yeşilyurt O, Uzbay IT: Agmatine potentiates the analgesic effect of morphine by an α_2-adrenoceptor-mediated mechanism in mice. Neuropsychopharmacology, 2001, 25, 98–103.
65. Zaniewska M, McCreary AC, Przegaliński E, Filip M: Effects of the serotonin 5-HT$_{2A}$ and 5-HT$_{2C}$ receptor ligands on the discriminative stimulus effects of nicotine in rats. Eur J Pharmacol, 2007, 571, 156–165.
66. Zarrindast MR, Homayoun H, Babaie A, Etminani A, Gharib B: Involvement of adrenergic and cholinergic systems in nicotine-induced anxiogenesis in mice. Eur J Pharmacol, 2000, 407, 145–158.
67. Zhao S, Wang B, Yuan H, Xiao D: Determination of agmatine in biological samples by capillary electrophoresis with optical fiber light-emitting-diode-induced fluorescence detection. J Chromatogr A, 2006, 1123, 138–141.
68. Zheng JQ, Weng XC, Gai XD, Li J, Xiao WB: Mechanism underlying blockade of voltage-gated calcium channels by agmatine in cultured rat hippocampal neurons. Acta Pharmacol Sin, 2004, 25, 281–285.

Received:
March 5, 2008; in revised form: August 19, 2008.