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Antidepressant-like effect of agmatine and its possible mechanism

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Abstract

In mammalian brain, agmatine is an endogenous neurotransmitter and/or neuromodulator, which is considered as an endogenous ligand for imidazoline receptors. In this study, the antidepressant-like action of agmatine administered p.o. or s.c. was evaluated in three behavioral models in mice or rats. Agmatine at doses 40 and 80 mg/kg (p.o.) reduced immobility time in the tail suspension test and forced swim test in mice or at dose 20 mg/kg (s.c.) in the forced swim test. Agmatine also reduced immobility time at 10 mg/kg (p.o.) or at 1.25, 2.5 and 5 mg/kg (s.c.) in the forced swim test in rats. These results firstly indicated that agmatine possessed an antidepressant-like action. With 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay and lactic dehydrogenase (LDH) assay, 1, 10 and 100 μM agmatine or a classical antidepressant, 2.5 and 10 μM desipramine, protected PC12 cells from the lesion induced by 300 μM *N*-methyl-D-aspartate (NMDA) treatment for 24 h. Using high-performance liquid chromatography with electrochemical detection (HPLC-ECD), it was found that the levels of monoamines including norepinephrine, epinephrine, dopamine or 5-hydroxytryptamine (5-HT) in PC12 cells decreased after the treatment with 200 μM NMDA for 24 h, while in the presence of 1 and 10 μM agmatine or 1 and 5 μM desipramine, the levels of norepinephrine, epinephrine or dopamine were elevated significantly while 5-HT did not change. Moreover, norepinephrine, 5-HT or dopamine had the same cytoprotective effect as agmatine at doses 0.1, 1 and 10 μM. In the fura-2/AM (acetoxymethyl ester) labeling assay, 1 and 10 μM agmatine, 1 and 5 μM desipramine or monoamines norepinephrine, 5-HT at doses 0.1 and 1 μM attenuated the intracellular Ca^{2+} overloading induced by 200 μM NMDA treatment for 24 h in PC12 cells. In summary, we firstly demonstrated that agmatine has an antidepressant-like effect in mice and rats. A classical antidepressant, desipramine, as well as agmatine or monoamines protect the PC12 cells from the lesion induced by NMDA treatment. Agmatine reverses the NMDA-induced intracellular Ca^{2+} overloading and the decrease of monoamines (including norepinephrine, epinephrine or dopamine) contents in PC12 cells, indicating that agmatine's antidepressant-like action may be related to its modulation of NMDA receptor activity and/or reversal of the decrease of monoamine contents and Ca^{2+} overloading induced by NMDA.

Keywords: Agmatine; Antidepressant; PC12 cell; Monoamine; Ca^{2+}

1. Introduction

Accumulating data indicate that there is a close relationship between antidepressant action and *N*-methyl-D-aspartate (NMDA) receptor activity. Firstly, antidepressants down-regulate NMDA receptor function. Chronic antidepressant treatment, which is required for clinical improvement, reduces the reactivity/function of the glutamate/NMDA receptor complex in rodent brain (Nowak, 2001). Chronic antidepressant treatment also "down-regulates" (reduced density/affinity) NMDA receptors in the cerebral cortex (Nowak, 2001). Secondly, NMDA receptor antagonists, such as MK801 (dizocilpine, (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine), amantadine or Zn^{2+}, always possess antidepressant-like effects in animal models (Kroczka et al., 2000). Moreover, subjects with depression show significant improvement of depressive symptoms within 72 h after intravenous treatment with ketamine, an NMDA receptor antagonist (Berman et al., 2000).

Agmatine has long been known to be a constituent of bacteria, plants and a range of invertebrates, and has been viewed as a precursor of putrescine. In mammalian brain, agmatine is an endogenous ligand at imidazoline receptors, to which it binds with high affinity (Yang and Reis, 1999; Reis and Regunathan, 2000). Exogenously administered to rodents, agmatine decreases the hyperalgesia accompanying inflammation, normalizes the mechanical hypersensitivity (allodynia/hyperalgesia) produced by chemical or mechanical nerve injury, and reduces autotomy-like behavior and lesion size after excitotoxic spinal cord injury. This suggests

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a unique antiplasticity and neuroprotective role for agmatine in processes underlying persistent pain and neuronal injury (Fairbanks et al., 2000). In cultured hippocampal neurons studied by whole-cell patch clamp, extracellularly applied agmatine produces a voltage-and concentration-dependent block of NMDA but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainite currents. This effect is independent of agmatine's affinity at imidazoline receptors (Yang and Reis, 1999). Furthermore, NMDA hyperalgesia relies on nitric oxide synthase activation and agmatine inhibits all isoforms of nitric oxide synthase (Fairbanks et al., 2000). We speculate that NMDA receptor block and nitric oxide synthase inhibition mediates agmatine's neuroprotective action.

The block by agmatine on NMDA receptors suggests a potential antidepressant-like action. In this study, the antidepressant-like effect of agmatine was observed on three depressive animal models and the possible mechanisms of action of agmatine were also studied in PC12 cells.

2. Materials and methods

2.1. Reagents and drugs

Agmatine (white powder), imipramine, desipramine, 5-hydroxytryptamine (5-HT), dopamine, norepinephrine, epinephrine, MK801, 1-octanesulfonic acid sodium salt (OSA) and 3,4-dihydroxybenzylamine (DHBA) were Sigma (USA) products. NMDA was from ACROS ORGANICS (USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was purchased from Merck (USA); fura-2/AM (acetoxymethyl ester) was obtained from Fluka (Sweden); the lactic dehydrogenase (LDH) assay kit was from Promega (USA).

2.2. Animals

Male Kunming mice weighing 18–22 g and male Wistar rats weighing 220–260 g (Experimental Animal Center, AMMS, Beijing) were used. They were housed and maintained on a 12-h light and dark cycle in a temperature-controlled room (22–24 °C) with free access to food and water. Imipramine and agmatine were dissolved in distilled water; agmatine was administered in volumes of 20 ml/kg (p.o.) or 10 ml/kg (s.c.) in mice and 2 ml/kg (p.o.) or 1 ml/kg (s.c.) in rats. Distilled water alone was administered as a control. Experiments followed a protocol approved by the local animal Ethics Committee and the Local Government. All experiments were in accordance with the recommendations of the European Union regarding animal experimentation (Directive of the European Council 86/609/EC).

2.3. Tail-suspension test

Mice were pretreated with agmatine (10–160 mg/kg p.o.) or distilled water once daily for 3 days. The test was performed 1 h after the last administration. Imipramine (10 mg/kg i.p.) was administered only once 1 h prior to the test. The mice were suspended by the tail to the edge of a shelf 75 cm above the floor. The tail was secured to the shelf by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded only during the last 4 min of the total 6-min test period. Mice were considered immobile only when they hung passively and completely motionless.

2.4. Forced swim test

Mice were placed in individual glass cylinders (20 cm height × 10 cm diameter) containing 10 cm of water at 22–25 °C for 6 min. The duration of immobility was scored during the last 4 min of the 6-min test period. A mouse was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water. Mice were given agmatine (10–80 mg/kg p.o. or 5–40 mg/kg s.c.) or distilled water once daily for 3 days. The last dose was given 1 h before the test. Imipramine (10 mg/kg i.p.) was administered only once 1 h prior to testing. In a variant of this procedure for rat experiments, the animals were placed in clear glass cylinders (40 cm height × 18 cm diameter) filled with water (22–25 °C) to a depth of 23 cm for 15 min. The rats were then dried and returned to their home cages. Almost all rats had immobility time of more than 90 s during the last 5 min in the training session and the animals that passed the criterion were used in the next test session. Before the test session on the following day, the rats were given agmatine (2.5–20 mg/kg p.o. or 1.25–10 mg/kg s.c.) or distilled water at 24, 5 and 1 h, respectively, prior to the test. Imipramine (10 mg/kg i.p.) was administered only once, 1 h before testing. In the test session, the rats were exposed to the cylinders as described above.

2.5. Spontaneous motor activity

Mice were given agmatine (10–80 mg/kg p.o.) or distilled water once daily for 3 days. The last dose was given 1 h before the test. The mice were placed individually in an open field (35 × 30 × 22 cm) fitted with a black rubber floor. Four mice were always tested in one period. The mice were placed in the open field and allowed to habituate to the environment for 10 min. Subsequently, the Vidiomex-V image analytic system (Columbus, USA) displayed and recorded spontaneous movements automatically in the next 10 min. The parameters observed included traveling distance, time of ambulation, time of rest and average speed.

2.6. PC12 cells culture and evaluation of cell viability

The pheochromocytoma cells (PC12) were kindly presented by Dr. You Wan, Beijing University. The cells were seeded into 96-well plates at a density of 2×10^5/ml and cultured in the medium consisting of 90% Dulbecco's

Modified Eagle Medium (DMEM), 5% heat-inactivated horse serum, 5% fetal calf serum, 200 kU/l benzylpenicillin and 100 mg/l streptomycin in a humidified incubator with 5% CO_2, 37 °C for 3–4 days. The cells were incubated with serum-free low-glucose DMEM containing NMDA 300 μM and agmatine (1, 10 and 100 μM), desipramine (2.5 and 10 μM), monoamines (0.1–10 μM) or NMDA receptor non-competitive antagonist, MK801 5 μM, for 24 h. The medium was collected and LDH activity, which was quantified as an index of cell death was determined with a LDH assay kit. The reaction mixture was monitored at 340 nm with a spectrophotometer (Vital Scientific, Netherlands) and the LDH activity was calculated from the decrease of NADH absorbance resulting from the conversion of pyruvate to lactate. In the MTT assay, after two washes with D-Hanks, the cells were incubated with DMEM containing 0.5 mg/ml MTT for another 4 h at 37 °C. A total of 100 μl ten percent sodium dodecyl sulfate (SDS) was subsequently added to each well to dissolve formazan crystals (about 12–16 h). Absorbance at 570 nm ($A_{570\ nm}$ values) was detected with a Versamax spectrophotometer (Molecular Devices, USA).

2.7. Measurement of monoamines by high-performance liquid chromatography with electrochemical detection (HPLC-ECD)

PC12 cells were seeded into a 10-mm dish at a density of 2×10^5/ml and cultured for 3–4 days. The cells were incubated with serum-free low-glucose DMEM containing 200 μM NMDA and agmatine (1 and 10 μM) or desipramine (1 and 5 μM) for 24 h. The cells were harvested and washed with phosphate buffer solution (PBS), then ice-chilled with 120 μl of perchloric acid (0.2 M) containing 0.5 mM EDTA, and 0.05 mg/ml DHBA was added to the cell pellets. After ultrasonication, the samples were centrifuged at 7000 × *g* for 20 min at 4 °C. The supernatant was collected and analyzed with HPLC-ECD system (Shimadzu, Japan). HPLC-ECD conditions were a modified method of Wetherell et al. (1989). Briefly, separations were performed using a 150 × 4.6 mm ODS C_{18} column. The mobile phase consisted of 0.1 M Na_2HPO_4, 0.8 mM OSA, 0.5 mM EDTA and 10% (v/v) methanol, and was adjusted to pH 3.63 with phosphoric acid. Column temperature was 40 °C, flow rate 1.0 ml/min and the detector was set at a potential of +0.75 V relative to a Ag/AgCl reference electrode. The working standard solution was prepared in 0.2 M perchloric acid containing 0.5 mM EDTA and 0.05 mg/ml DHBA was stored at −20 °C.

2.8. Measurement of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in PC12 cells

The $[Ca^{2+}]_i$ in PC12 cells was monitored by fluorometry, using the Ca^{2+}-sensitive dye, fura-2/AM. The cells were seeded in a 24-well plate at the density 2×10^5/ml and cultured for 3–4 days. The medium was replaced with serum-free DMEM containing agmatine (1 and 10 μM), desipramine (1 and 5 μM), 5-HT (0.1 and 1 μM) or norepinephrine (0.1 and 1 μM) in the presence of 200 μM NMDA, and the cells were then cultured for another 24 h. For fura-2/AM loading, cells were collected and incubated with the complete medium containing 5 μM fura-2/AM at 37 °C for 45 min. Subsequently, the cells were washed and resuspended again with cold balanced salt solution (BSS) buffer (130 mM NaCl, l5.4 mM KCl, 1.8 mM $CaCl_2$, 5.5 mM glucose, 20 mM HEPES, pH 7.4) containing 0.2% bovine serum albumin. The cells were incubated at 37 °C for another 5 min just prior to measurement. $[Ca^{2+}]_i$ was determined by alternating excitation wavelengths of between 340 and 380 nm with emission at 510 nm, using a fluorescence spectrophotometer (F-4500, HITACH, Japan) and the data were analyzed with customized software provided by F-4500. The ratio of fluorescence intensities excited by 340 or 380 nm was calculated after subtraction of the background fluorescence.

2.9. Statistical analysis

The results were expressed as means and standard error. Significance of differences between groups was evaluated with a one-way analysis of variance (ANOVA) and Duncan's test.

3. Results

3.1. Antidepressant-like effects of agmatine in mice

In the tail-suspension test, pretreatment of the mice once daily for 3 days with agmatine at doses of 40 or 80 mg/kg (p.o.) produced significant reductions in the duration of immobility, by 29% or 33%, respectively (Fig. 1). Treatment with agmatine at the dose of 40 mg/kg (p.o., Fig. 2A) or 20

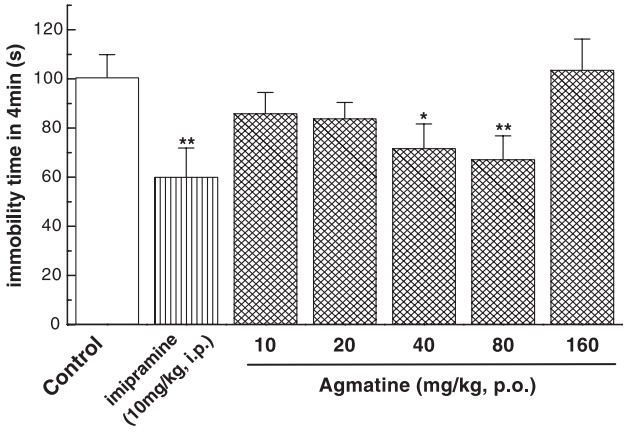

Fig. 1. Effect of agmatine on immobility time in the tail suspension test in mice. In agmatine groups, the mice were treated with distilled water or agmatine (p.o.) once a day for 3 days. The test was performed 1 h after the last administration of agmatine. In the positive control, imipramine was given only once (i.p.) 1 h prior to the test. Data were expressed as means ± S.E.M. *$P<0.05$, **$P<0.01$ vs. control.

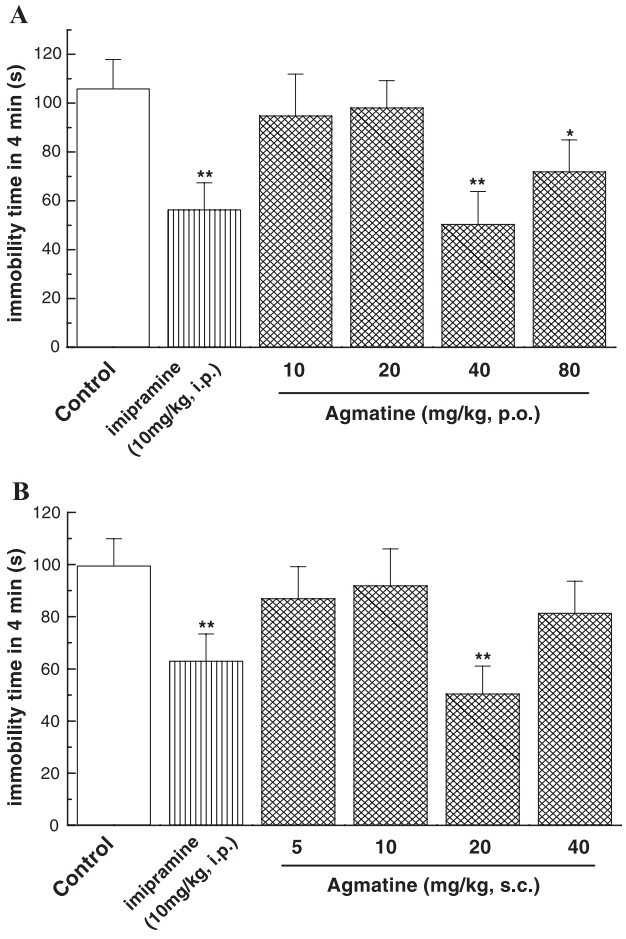

Fig. 2. Effects of agmatine on immobility time in the forced swim test in mice. In agmatine groups, the mice were treated with distilled water or agmatine (p.o.: A or s.c.: B) once a day for 3 days. The tests were performed 1 h after the last administration of agmatine. In the positive control, imipramine was given only once (i.p.) 1 h prior to the test. Data were expressed as means ± S.E.M. $*P<0.05$, $**P<0.01$ vs. corresponding control.

mg/kg (s.c., Fig. 2B) for 3 days also reduced the immobility time by 52% or 52%, respectively, in the forced swimming test in mice. In these tests, the effects of agmatine were dose-dependent with a "U-shaped" trend. Acute treatment (only once) with agmatine prior to the tests had no effects on the immobility time (results not shown). Imipramine (10 mg/kg i.p.) reduced the immobility time by 37–47% in these experiments.

3.2. Antidepressant-like effects of agmatine in rats

After 10 mg/kg (p.o., Fig. 3A) or 1.25, 2.5 and 5 mg/kg (s.c., Fig. 3B) agmatine was administered 24, 5 and 1 h prior to the test, respectively (three times total), the immobility time in the forced swimming test in rats was also significantly reduced by 34% (p.o.) or 21%, 19% and 33% (s.c.), respectively. These effects also showed the "U-shaped" trend and dose-dependence. Imipramine (10 mg/kg i.p.) reduced the immobility time by 37–39% in these tests.

3.3. Effect of agmatine on the spontaneous motor activity in mice

In order to determine whether agmatine really has an antidepressant-like action, we have to find whether agmatine has excitatory or inhibitory actions on the central nervous system. The 10–80 mg/kg (p.o.) agmatine had no effect on spontaneous motor activity in mice as shown in Table 1, indicating that agmatine had no excitatory or inhibitory action in the central nervous system at least at the doses of 10–80 mg/kg (p.o.). All these results indicate that agmatine has an antidepressant-like effect in mice and rats.

3.4. Protective effect of agmatine on PC12 cells from the lesion induced by NMDA

After the treatment of PC12 cells with 300 μM NMDA for 24 h, the $A_{570\ nm}$ values decreased and LDH activity increased markedly compared with the corresponding control, indicating that the cells were impaired or that some were

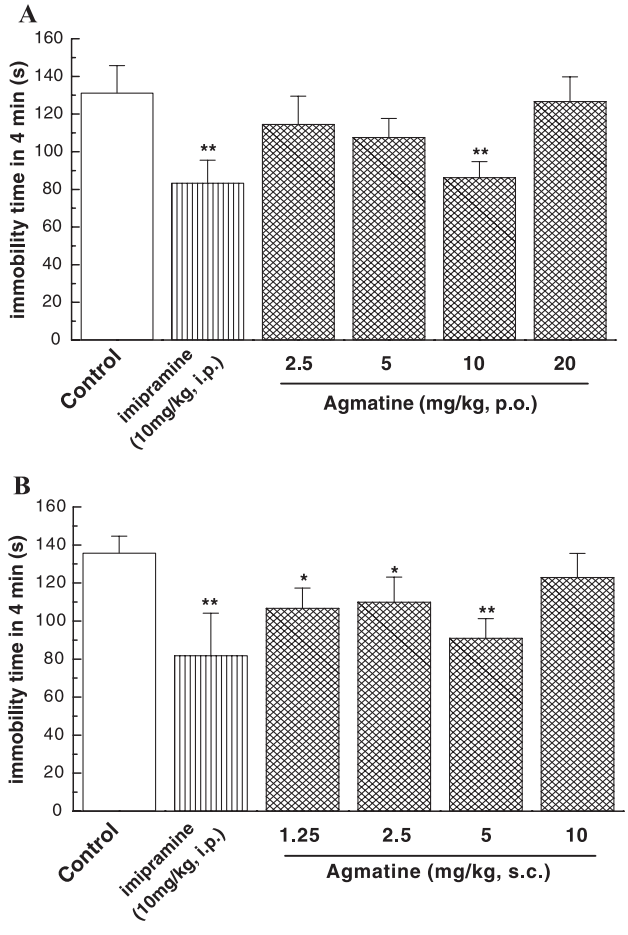

Fig. 3. Effects of agmatine on immobility time in the forced swim test in rats. In agmatine groups, the rats were treated with distilled water, agmatine (p.o.: A or s.c.: B) at 24, 5 and 1 h, respectively, prior to the tests. In the positive control, imipramine was given only once (i.p.) 1 h before the tests. Data were expressed as means ± S.E.M. $*P<0.05$, $**P<0.01$ vs. corresponding control.

Table 1
Effects of agmatine on spontaneous motor activity in mice

Groups	Doses (mg/kg)	Traveling distance (cm)	Time of ambulation (s)	Time of rest (s)	Average speed (cm /s)
Control	–	844 ± 129	361 ± 33	237 ± 33	2.2 ± 0.2
Agmatine	10	912 ± 158	376 ± 30	222 ± 30	2.3 ± 0.2
	20	771 ± 95	346 ± 28	252 ± 28	2.2 ± 0.1
	40	845 ± 50	373 ± 14	225 ± 15	2.3 ± 0.1
	80	714 ± 99	344 ± 25	254 ± 25	2.1 ± 0.1

Mice were treated with distilled water or agmatine (p.o.) once a day for 3 days. The test was performed 1 h after the last administration of agmatine. Data were expressed as means ± S.E.M.

dead. Agmatine (1, 10 and 100 μM), desipramine (2.5 and 10 μM) or NMDA receptor non-competitive antagonist, MK801 (5 μM), reversed the changes of cell viability. Compared with their effects on the corresponding NMDA treatment group, the drugs as mentioned above increased the $A_{570\ nm}$ values and decreased LDH release significantly, indicating that agmatine, desipramine or MK801 could protect the cells from the lesion induced by NMDA (Figs. 4 and 5).

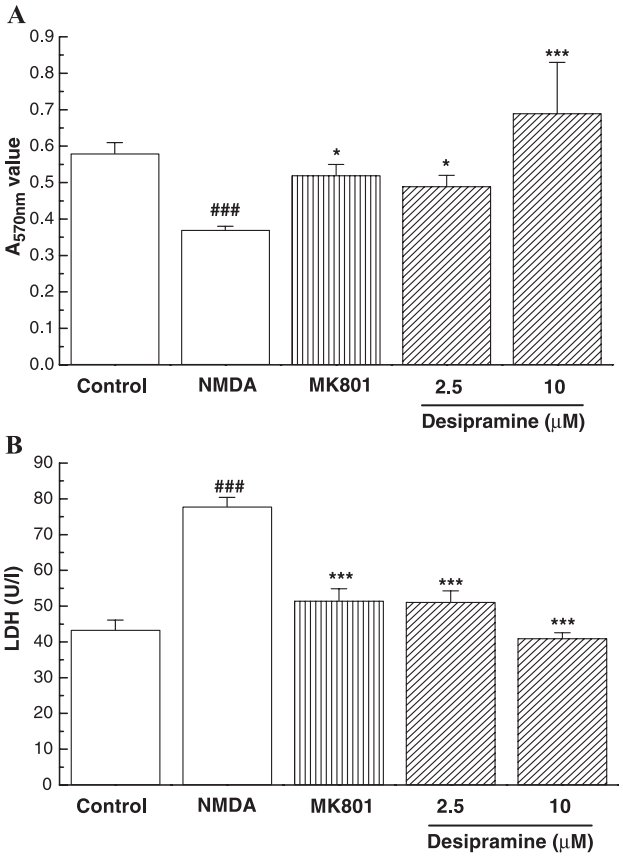

Fig. 4. Protective effect of desipramine or MK801 on the PC12 cells from the lesion induced by NMDA. Cells were exposed to NMDA 300 μM in the absence or presence of MK801 or desipramine for 24 h, cell viability was measured using a colorimetric MTT assay (A) and LDH assay (B). Data were expressed as means ± S.E.M. $^{###}P<0.001$ vs. corresponding control. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ vs. corresponding NMDA-treated groups.

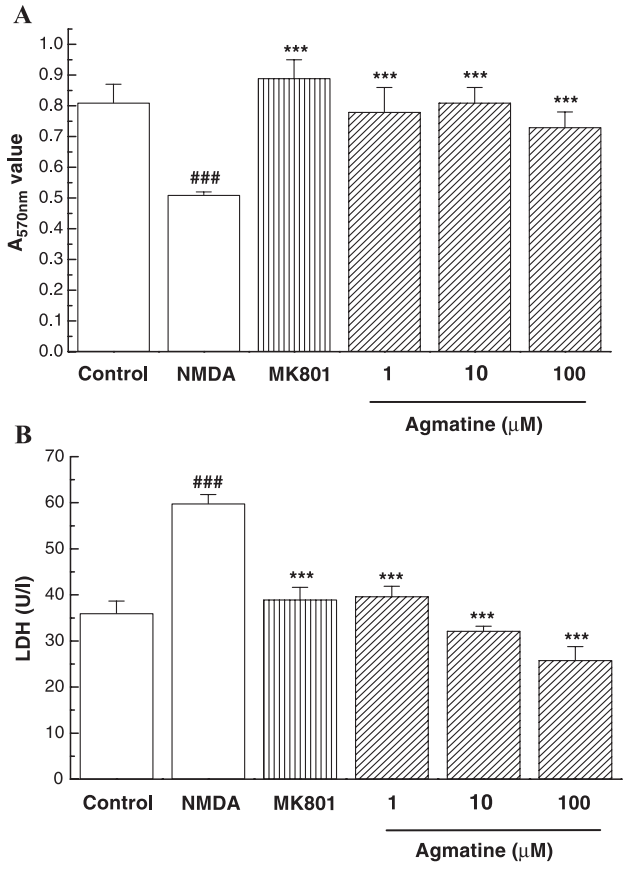

Fig. 5. Protective effect of agmatine or MK801 on the PC12 cells from the lesion induced by NMDA. Cells were exposed to NMDA 300 μM in the absence or presence of agmatine or MK801 for 24 h and the cell viability was measured using a colorimetric MTT assay (A) and LDH assay (B). Data were expressed as means ± S.E.M. $^{###}P<0.001$ vs. corresponding control. $^{***}P<0.001$ vs. corresponding NMDA-treated groups.

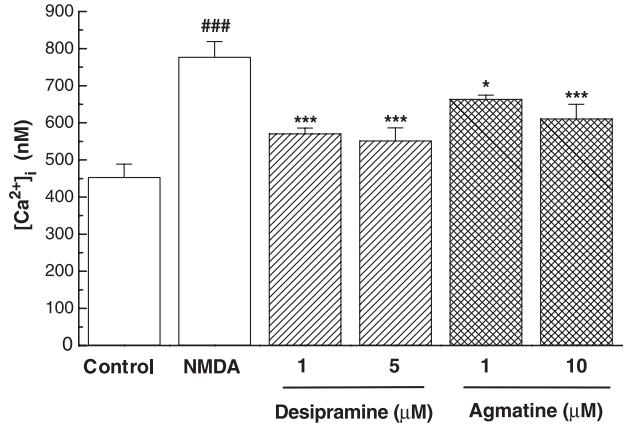

Fig. 6. Effect of agmatine or desipramine on the NMDA-induced $[Ca^{2+}]_i$ overloading in PC12 cells. Cells were exposed to NMDA 200 μM in the absence or presence of agmatine or desipramine for 24 h and $[Ca^{2+}]_i$ was detected using the sensitive indicator dye, fura-2/AM. Data were expressed as means ± S.E.M. $^{###}P<0.001$ vs. control. $^{***}P<0.001$ vs. NMDA-treated group.

Table 2
Effect of agmatine or desipramine on monoamine levels in the NMDA-treated PC12 cells

Treatments (μM)		Levels of monoamines (ng/g)			
		Norepinephrine	5-HT	Epinephrine	Dopamine
Control	–	1590.7 ± 115.3	255.8 ± 1.8	193.3 ± 32.7	51,992.4 ± 1051.2
NMDA	200	1088.6 ± 45.1[a]	178.2 ± 10.0[b]	102.2 ± 6.2[a]	4035.4 ± 129.6[b]
NMDA + desipramine	1	1680.3 ± 99.8[c]	180.8 ± 10.2	138.3 ± 13.3[d]	4432.6 ± 150.6
	5	1433.4 ± 119.5[c]	176.5 ± 11.0	178.8 ± 28.3[d]	5608.2 ± 214.0[d]
NMDA + agmatine	1	1328.0 ± 69.4[d]	193.1 ± 9.9	218.2 ± 39.2[d]	5559.3 ± 92.5[d]
	10	1351.8 ± 72.1[d]	158.5 ± 15.9	166.6 ± 13.1[d]	5659.7 ± 208.1[d]

Cells were exposed to 200 μM NMDA in the absence or presence of agmatine or desipramine for 24 h and the levels of monoamines were measured by HPLC-ECD. Data were expressed as means ± S.E.M.
[a] $P < 0.05$.
[b] $P < 0.001$ vs. control.
[c] $P < 0.001$ vs. NMDA-treated group.
[d] $P < 0.05$.

3.5. Effect of agmatine on the NMDA-induced $[Ca^{2+}]_i$ overloading in PC12 cells

After the treatment of PC12 cells with 200 μM NMDA for 24 h, $[Ca^{2+}]_i$ was obviously elevated compared with the control, while in the presence of 1 and 10 μM agmatine or 1 and 5 μM desipramine (Fig. 6), the NMDA-induced $[Ca^{2+}]_i$ overloading was attenuated. These results add the evidence that the cytoprotective action of agmatine or desipramine may be associated with their reduction of $[Ca^{2+}]_i$ overloading.

3.6. Effect of agmatine on the monoamine levels in NMDA-treated PC12 cells

Treatment with 200 μM NMDA for 24 h reduced the norepinephrine, 5-HT, dopamine and epinephrine contents in PC12 cells, which was consistent with the changes in the brains of depressive patients. In the presence of 1 and 10 μM agmatine or 1 and 5 μM desipramine, however, the levels of norepinephrine, dopamine and epinephrine were elevated significantly compared with those in the NMDA-treated group. The 5-HT content did not change, as shown in Table 2.

3.7. Effect of monoamines on the NMDA-induced lesion or $[Ca^{2+}]_i$ overloading in PC12 cells

After the treatment of PC12 cells with 300 μM NMDA for 24 h, the LDH release increased markedly compared with control, indicating that the cells were impaired or that some were dead. The monoamines, including norepinephrine (0.1, 1 and 10 μM), 5-HT (0.1, 1 and 10 μM) or dopamine (0.1 and 1 μM) possessed the same cytoprotective action against NMDA (Table 3). After the treatment of PC12 with 200 μM NMDA for 24 h, $[Ca^{2+}]_i$ was elevated compared with the control, while in the presence of norepinephrine or 0.1 and 1 μM 5-HT, the NMDA-induced $[Ca^{2+}]_i$ overloading was attenuated (Table 4).

These results suggest that monoamines are involved in the cytoprotection by agmatine or desipramine. Simultaneous studies demonstrated that 5-HT also antagonized the corticosterone-induced lesion and $[Ca^{2+}]_i$ overloading in PC12 cells, for which 0.1 μM was the most effective of

Table 3
Protective effects of monoamines on the PC12 cells from the lesion induced by NMDA

Treatments (μM)		LDH release (U/l)
Control		6.12 ± 0.64
NMDA	300	10.06 ± 0.84[a]
NMDA + 5-HT	0.1	4.49 ± 0.23[b]
	1	5.04 ± 0.54[c]
	10	5.14 ± 0.39[c]
NMDA + dopamine	0.1	3.64 ± 0.40[c]
	1	3.51 ± 0.37[c]
NMDA + norepinephrine	0.1	5.08 ± 0.50[c]
	1	4.29 ± 0.44[c]
	10	4.27 ± 1.43[c]

Cells were exposed to NMDA 300 μM in the absence or presence of monoamines for 24 h and cell viability was measured using a LDH assay. Data were expressed as means ± S.E.M.
[a] $P < 0.001$ vs. control.
[b] $P < 0.001$.
[c] $P < 0.001$ vs. NMDA-treated group.

Table 4
Effect of norepinephrine or 5-HT on the *N*-methyl-D-aspartate (NMDA)-induced $[Ca^{2+}]_i$ overloading in PC12 cells

Treatments (μM)		$[Ca^{2+}]_i$ (nM)
Control		511.19 ± 12.18
NMDA	200	624.13 ± 25.14[a]
NMDA + norepinephrine	0.1	375.99 ± 6.88[b]
	1	386.67 ± 13.53[b]
NMDA + 5-HT	0.1	390.41 ± 4.85[b]
	1	439.91 ± 9.66[b]

Cells were exposed to 200 μM NMDA in the absence or presence of norepinephrine or 5-HT for 24 h and $[Ca^{2+}]_i$ was detected using the sensitive indicator dye, fura-2/AM. Data are expressed as means ± S.E.M.
[a] $P < 0.001$ vs. control.
[b] $P < 0.001$ vs. NMDA-treated group.

doses including 0.01, 0.1, 1 and 10 μM, suggesting that the most effective dose for the cytoprotective action of 5-HT is about 0.1 μM. Norepinephrine and dopamine had the same cytoprotective actions against corticosterone for which the most effective dose was about 1 and 1–10 μM, respectively, further indicating that norepinephrine and dopamine might be involved in the cytoprotective effect of agmatine.

4. Discussion

It was reported that volumes of the double-side hippocampus were reduced in patients with major depression compared to healthy controls, and there was a positive correlation between hippocampus atrophy and the time course of the depression (Sapolsky, 2000a,b). Chronic psychosocial stress caused apical dendritic atrophy of hippocampal CA3 pyramidal neurons, which may be mediated by activation of the hypothalamic–pituitary–adrenal axis acting in concert with the endogenous excitatory amino acid release (Sapolsky, 2000a,b; Magarinos et al., 1996). Our results showed that three main kinds of antidepressants all protected the PC12 cells as well as primary cultured hippocampus neurons from the lesion induced by NMDA or corticosterone, while chlorpromazine or diazepam that had no such effect suggested that cytoprotective effect probably is the common pathway for action (unpublished). We also found that agmatine, desipramine, MK801 or monoamines incubated alone with PC12 cells at the doses indicated were without effect on cell viability at least over 24 h. Desipramine showed cytotoxicity on PC12 cells when its concentration exceeded 10 μM (e.g. 20 μM). Agmatine had no marked cytotoxicity even at concentrations up to 1 mM.

In fact, the excitatory amino acids accumulate in the brain of depressive patients and are capable of ultimately leading to the lesion of brain neurons (especially hippocampus neurons). Thus, region-specific damping of NMDA receptor function becomes an attractive strategy for discovering novel antidepressants (Skolnick, 1999; Pacher et al., 2001).

In the present study, we firstly demonstrated that agmatine possessed an antidepressant-like effect with dose-dependence and a "U-shaped" trend in mice or rats, which might be "NMDA receptor block based". There is evidence suggesting that many antidepressant drugs show inhibitory activity at the NMDA receptor and that NMDA antagonists have antidepressant profiles in preclinical models of depression (Petrie et al., 2000). MK801 and (±)-2-amino-7-phosphonopentanoic acid (AP7), two NMDA antagonists, administered to mice all mimicked the effect of clinically effective antidepressants in the forced swim test (Trullas and Skolnick, 1990). Agmatine itself can act as an antagonist of NMDA receptors, which suggests its antidepressant-like action. Analysis of the voltage dependence of the block suggests that the guanidine group of agmatine is the active moiety when blocking the NMDA channel. Moreover, it seems that, in hippocampal neurons, agmatine selectively modulates the NMDA subclass of glutamate receptor channels through an interaction between the guanidine group and the channel pore. So, we speculated that the guanidine group of agmatine is the active moiety of its antidepressant-like action.

Many NMDA receptor antagonists have antidepressant-like properties in behavioral despair procedures after acute (single-dose) treatment, while agmatine shows the same action after relatively chronic (3 days), but not acute treatment, suggesting that the site of action and modulation mechanism of agmatine in NMDA receptors are different from those of other NMDA receptor antagonists currently known (such as MK801, AP7, Zn^{2+}, etc.). Also, the chronic action of agmatine may also be associated with its relatively low bioavailability.

Although there has been no investigation that directly answers the question whether agmatine can penetrate the central nervous system after oral or parenteral administration, we think it can, because (1) central or peripheral administration of agmatine showed the same analgesic effect in our former studies (unpublished); (2) agmatine and L-arginine have the same transporter, and L-arginine can penetrate the central nervous system (Babal et al., 2000; Mahar Doan et al., 2000).

The plasma agmatine concentration is elevated significantly in depressed patients compared to that in healthy controls. There is related evidence that a change in plasma agmatine levels may lead to similar changes in platelet imidazoline I_1 receptors. Treatment with antidepressants normalizes plasma agmatine and imidazoline I_1 receptors levels (Halaris et al., 1999; Piletz et al., 1994). It was also hypothesized that brain agmatine was depleted in depressive patients (Piletz et al., 1994), a change similar to that in brain monoamine levels. Is there a close relationship between agmatine and monoamine? Our study found that a high concentration of NMDA induced a decrease of norepinephrine, 5-HT, epinephrine and dopamine in PC12 cells, which was consistent with the changes in the brain of depressive patients. Agmatine or desipramine reversed these changes (at least partly), which also supported the antidepressant-like effect of agmatine.

It is well known that $[Ca^{2+}]_i$ overloading may mediate the NMDA-induced lesion in neurons. Norepinephrine, 5-HT or dopamine also exert a cytoprotective effect and attenuate the $[Ca^{2+}]_i$ overloading induced by NMDA or corticosterone, as does desipramine or agmatine, which suggests that monoamine action may involve the down-regulation of NMDA receptor activity caused by agmatine or antidepressants. The monoamines and NMDA receptor are two closely related systems; their interaction probably underlies the antidepressant-like effect of agmatine. Another possibility is that the NMDA block by agmatine causes the increase of monoamine contents in PC12 cells and monoamines subsequently cascade the cytoprotective effect of agmatine. Further studies are needed.

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