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Agmatine prevents LPS-induced spatial memory impairment and hippocampal apoptosis

Asadollah Zarifkar [a], Samira Choopani [b], Rasoul Ghasemi [c], Nasser Naghdi [b], Amir Hossein Maghsoudi [c], Nader Maghsoudi [c], Karim Rastegar [a,*], Maryam Moosavi [a]

[a] Neuroscience Research Center and department of Physiology, Shiraz University of Medical sciences, Shiraz, Iran
[b] Department of physiology and pharmacology, Pasteur institute of Iran, Tehran, Iran
[c] Neuroscience Research Center, Shahid Beheshti University, M.C, Tehran, Iran

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ABSTRACT

Neuroinflammation is associated with a number of neurodegenerative diseases. It is known that lipopolysaccharide (LPS) treatment induces neuroinflammation and memory deterioration. Agmatine, the metabolite of arginine by arginine decarboxylase, is suggested to be a neuroprotective agent. The aim of this study was to explore if agmatine can prevent LPS-induced spatial memory impairment and hippocampal apoptosis. Adult male Wistar rats (200–250 g) were trained in water maze for 4 days (3 days in hidden platform and the last day in visible platform task). Saline, LPS (250 μg/kg/ip) or (and) agmatine (5 or 10 mg/kg) were administered 4 h before every training session. LPS treatment impaired water maze place learning while agmatine co-administration prevented it. Also western blot studies revealed that LPS induces hippocampal caspase-3 activation while agmatine treatment prevented it.

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1. Introduction

Neuroinflammation is implicated in several neurodegenerative diseases, like, Alzheimer's disease, autism, Down syndrome, HIV dementia and demyelinating diseases (McGeer and McGeer, 1998; Akiyama et al., 2000; Vargas et al., 2005; Fischer-Smith et al., 2004; Mhatre et al., 2004) and may contribute to learning and memory deficits associated with these disorders. During early stages of Alzheimer's disease the greatest degree of neuroinflammation is found within temporal lobe regions involved in learning and memory (Cagnin et al., 2001). The brain is also vulnerable to constitutive defense responses, such as systemic inflammation. The systemic inflammation leads to generation of circulating cytokines, which impacts central nervous system (CNS) and causes neuroinflammation (Perry, 2004). It has been shown that intraperitoneal injection of lipopolysacharid (LPS) — a cell wall component of gram-negative bacteria-induces neuroinflammation, hippocampal apoptosis, cognitive impairment, learning deficits and even beta amyloid plaques generation in the hippocampus (Lee et al., 2008; Shaw et al., 2001).

Agmatine is a polycationic amine synthesized via decarboxylation of L-arginine by arginine decarboxylase (ADC). It was long been known to as an intermediate in polyamine metabolism of various bacteria, plants and a range of invertebrates (Tabor and Tabor 1984). Later it was discovered that agmatine, ADC and agmatinase are expressed in mammalian tissues (Li et al., 1994; Raasch et al., 1995). In mammalian brains agmatine exist in some regions such as hypothalamus, hippocampus, cortex, locus ceruleus, Raphe nucleus and forebrain (Halaris and Plietz, 2007). It seems that agmatine meets most of the criteria of a neurotransmitter/neuromodulator in the brain. It is synthesized, stored and released from specific network of neurons, is inactivated by energy dependent reuptake mechanism, is degraded enzymatically and binds with high affinity to α_2 adrenergic and imidazoline (I_1) receptors (Reis and Regunathan, 2000).

Exogenously administered to rodents, agmatine reverses pain induced by inflammation, neuropathy and spinal cord injury (Fairbanks et al., 2000). Agmatine has also been reported to have some neuroprotective effects against MPTP neurotoxicity (Gilad et al., 2005), spinal cord ischemia (Gilad and Gilad, 2000) and restraint-induced structural changes in the brain (Zhu et al., 2008). In PC12 cells, cerebellum and cultured hippocampal cell, it prevents glutamate and NMDA neurotoxicity (Zhu et al., 2003; Olmos et al., 1999; Wang et al., 2006). Additionally it is known to exert antidepressant, anxiolytic, anti-tumor cell proliferative and anticonvulsive effects (Halaris and Plietz, 2007).

Considering the deteriorative effects of LPS on memory and suggested neuroprotective effect of agmatine, this study was designed to investigate if agmatine can prevent memory loss and hippocampal apoptosis caused by LPS.

* Corresponding author. Neuroscience Research Center and Physiology Department, Shiraz University of Medical sciences, Shiraz, Iran. Tel./fax: +98 711 2302026.
E-mail addresses: rastgark@gmail.com (K. Rastegar), marmoosavi@sums.ac.ir (M. Moosavi).

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2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 200–250 g were used. The animals were maintained at room temperature (25 ± 2 ºC) under standard 12–12 h light–dark cycle with lights on at 7:00 AM. Food and water were available ad libitum except for the times of experiments. Animal care was according to the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Materials

LPS and agmatine sulphate were purchased from Sigma, USA, Western blot antibodies (caspase-3, beta-actin and secondary HRP-conjugated) were purchased from Cell Signaling Technology Company, USA. ECL advanced reagent kit and PVDF membrane were purchased from Amersham Bioscience, UK. Other reagents were obtained from usual commercial sources.

2.3. Drug administration

LPS and agmatine were administered intraperitoneally 4 h before each block session of training (Lee et al., 2008). The animals were divided into groups of 8 and received saline (ip) as vehicle, LPS 250 µg/kg), agmatine (5 and 10 mg/kg) and a combination of LPS (250 µg/kg) and agmatine (5 or 10 mg/kg).

2.4. Behavioral testing

2.4.1. Morris water maze apparatus

The water maze used has been described extensively (Moosavi et al., 2007). Briefly, it consisted of a dark circular pool (140 cm in diameter and 55 cm high) filled with water (20 ± 1 ºC) to a depth of 25 cm. A transparent Plexiglas platform (11 cm diameter) was located 1.5 cm below the water surface in the centre of one of the arbitrarily designed north-east (NE), south-east (SE), south-west (SW), or north-west (NW) orthogonal quadrants. The platform provided the only escape from the water. Many extra-maze cues such as racks, a window, a door, bookshelves, and pictures on the walls surrounded the room where the water maze was housed. These were kept in fixed positions in the swimming pool to allow the rat to locate the hidden escape platform. The position of the animal was monitored by a camera that was mounted above the centre of the pool. The camera signal was digitized and fed to a computerized tracking system that monitored and stored the position of the rat every 100 ms, thus the time required (escape latency), the traveled distance to reach the platform and the swimming speed were recorded.

2.4.2. Procedure

The rats were trained in a protocol consisting of 4 days training session. During the first three days an invisible platform, submerged about 1.5 cm below water surface was put in the center of southwest quadrant. The platform position was fixed during those 3 days. A block session consisted of four trials with four different starting positions. Each rat was placed in the water facing the wall of the tank at one of the four designated starting points (north, east, south and west) and allowed to swim and find the hidden platform. During each trial, the rat was given 90 s to find the hidden platform. After mounting the platform, the animals were allowed to remain there for 20 s until the start of the next trial. After completion of training, the animal was dried by a towel and returned to its home cage. On day 4 the hidden platform was removed and a visible platform-covered by a piece of aluminum foil and not submerged in water-was placed in another position (southeast quadrant) to test rat motivation and sensorimotor coordination.

2.5. Tissue preparation

Immediately after the completion of behavioural tests, the animals were decapitated after deep anesthesia with CO_2 inhalation. The hippocampi were quickly isolated on ice and were transferred to liquid nitrogen and then stored in −80 °C.

2.6. Western blot analysis

The hippocampi were homogenized on ice in cold lysis buffer containing 50 mM Tris/HCl (pH 7.5), 2 mM phenylmethylsulfonyl fluoride (PMSF), 100 M sodium orthovanadate, 10 g/ml aprotinin and 10 g/ml leupeptin. The lysates were centrifuged at 13,000 *g* for 15 min at 4 °C to remove debris. Samples with equal amounts of protein were then separated by 12% polyacrylamide gel electrophoresis, and transferred to PVDF membranes. After blocking in 2% ECL advanced blocking reagent kit, the membranes probed with primary antibodies (caspase-3 and β-actin) overnight in 4 ºC. After washing, the membranes were incubated for 2 h at room temperature with horseradish peroxidase-conjugated anti-rabbit antibody. Blots were revealed by ECL advanced kit.

2.7. Data analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparison. All results have been shown as means ± SEM. In all statistical comparisons, $P<0.05$ considered as significant difference.

3. Results

Fig. 1 shows the results obtained from pre-training administration of saline, LPS or (and) agmatine administration on water maze spatial learning and memory. Fig. 1A shows the escape latency to the hidden platform during days 1–3 of training. One way ANOVA of the escape latency showed significant differences between groups in days 1–3 (day 1: P value = 0.0014, $F(5,42) = 4.830$; day 2: P value < 0.0001 $F(5,42) = 17.550$; day3: P value < 0.0001, $F(5,42) = 16.911$). Post hoc analysis by Tukey's test showed that LPS treatment significantly increased escape latency in all training days. Co-administration of agmatine with LPS in doses 5 and 10 mg/kg prevented LPS-induced escape latency increment. Agmatine administration in both doses by itself did not change escape latency in comparison to saline treated group. Fig. 1B shows the results obtained from pre-training administration of saline, LPS or (and) agmatine administration on traveled distance to the hidden platform during days 1–3 of training. One way ANOVA of the traveled distance showed significant differences between groups in days 1–3 (day 1: P value = 0.0030, $F(5,42) = 4.309$; day 2: P value < 0.0001 $F(5,42) = 11.148$; day3: P value < 0.0001, $F(5,42) = 10.422$). Post hoc analysis by Tukey's test showed that LPS treatment significantly increased the traveled distance in all training days. Co-administration of agmatine with LPS in doses 5 and 10 mg/kg prevented LPS-induced traveled distance increment. Agmatine administration in both doses by itself did not change traveled distance in comparison to saline treated group. Fig. 1 C shows the learning pattern of the animals treated with saline, agmatine or (and) LPS. This figure shows that there is a negative linear correlation between escape latency and the training sessions in all the groups. This means that all groups have learnt the platform location; however LPS administration has slowed down learning speed from the first learning session.

The effect of pre-training saline, LPS or (and) agmatine administration on escape latency to the visible platform on day 4 of training is depicted in Fig. 2. One way ANOVA of the escape latency to the visible platform showed no difference between groups (P value = 0.8151, $F(5,42) = 0.4441$).

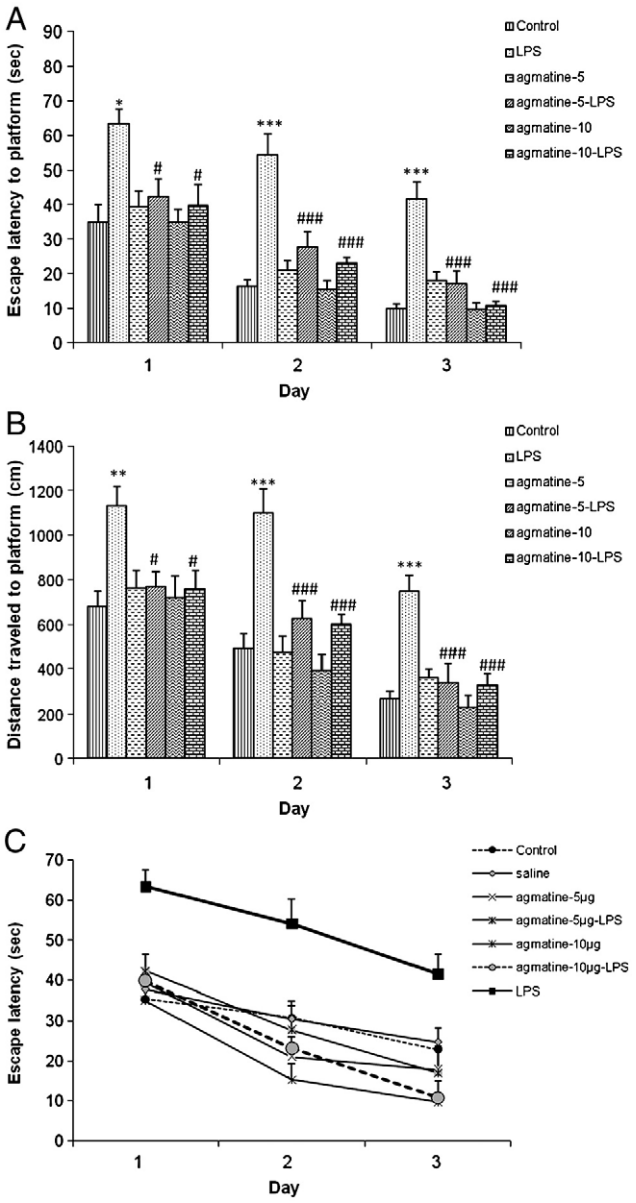

Fig. 1. The effect of saline, LPS or (and) agmatine administration on water maze spatial learning and memory. A) The escape latency to the hidden platform during days 1–3 of training. B) The traveled distance to the hidden platform during days 1–3 of training. C) The learning patterns of the animals treated by saline, agmatine or (and) LPS during training sessions. Data are represented as mean ± SEM. $^{**}P<0.01$ and $^{***}P<0.001$ represent the difference between LPS and saline treated groups. $\#P<0.05$ and $\#\#\#P<0.001$ represent the difference between LPS and agmatine treated groups.

Western blot analysis showing effects of saline, LPS or (and) agmatine administration on activated caspase-3 protein in the hippocampi of rats is depicted in Fig. 3. Antibody against activated (cleaved) caspase-3, as an indicator of apoptosis, detected two bands at 19 and 17 kDa. LPS treatment induced caspase-3 activation. Agmatine co-treatment in doses 5 and 10 mg/kg suppressed the LPS-induced activation of caspase-3.

4. Discussion

The current study confirmed that LPS significantly impairs water maze learning and memory, as the escape latency and the traveled distance to the hidden platform were increased. The LPS-induced impairments were completely reversed by concurrent intraperitoneal administration of agmatine in doses 5 and 10 mg/kg. Since LPS and/or

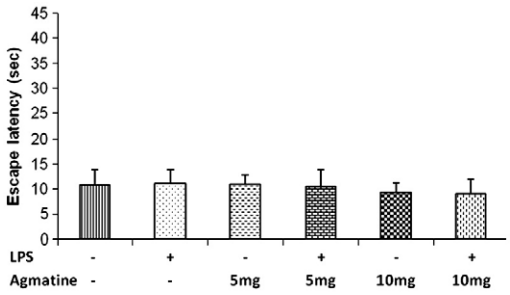

Fig. 2. The effect of saline, LPS or (and) agmatine administration on escape latency to the visible platform during day 4 of training. Data are represented as mean ± S.E.M.

agmatine did not affect the swimming speed of the animals (data not shown) and their ability to find the visible platform(in cued version of water maze) the observed effects are unlikely to be associated with changes in motivation or sensorimotor coordination and therefore can be attributed to spatial performance. Systemic injection of agmatine – without LPS – 4 h before training did not affect water maze performance, suggesting that the improvement described, is not agmatine's direct effect on memory. This is compatible with our previous study showing that exogenously administered agmatine does not affect water maze place learning (unpublished results). Since agmatine can cross blood–brain-barrier (Piletz et al., 2003) and the hippocampus is the critical structure involved in spatial learning and memory, it is speculated that agmatine protects the hippocampus from LPS-induced hippocampal function deterioration.

Our western blot studies showed that LPS treatment induces hippocampal caspase-3 activation as a marker of apoptosis, which was also prevented by agmatine treatment. Possibly the antiapoptotic effect of agmatine protected the hippocampal function against LPS damage. However more research is needed to elucidate this relationship.

Several putative mechanisms have been implicated in LPS-induced hippocampal damage. The neuroinflammation caused by LPS results in increased levels of glutamate (Fine et al., 1996; Espey et al., 1998); then it is possible that a significant portion of NMDA channels exist without Mg^{2+} block, allowing excessive calcium entry into postsynaptic neurons (Wenk et al., 2006). The selective antagonism of NMDA receptors by memantine has been reported to reduce neuroinflammation (Rosi et al., 2006). Agmatine which carries the guanidine group blocks heterometric NMDA receptor channels (Yang and Reis 1999, Askalany et al., 2005; Wang et al., 2006). However the NMDA channel inhibition caused by agmatine is weaker compared to memantine. As even memantine, the

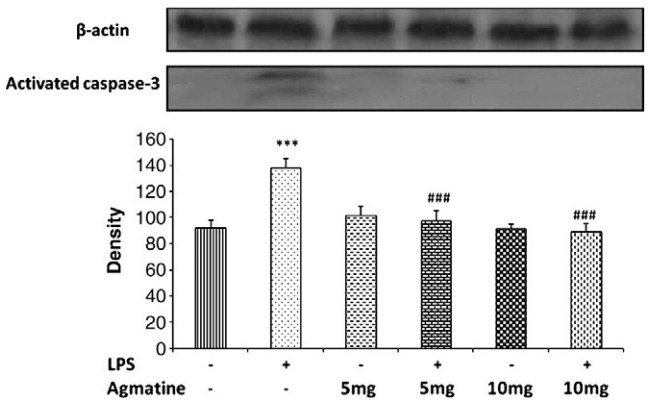

Fig. 3. Western blot analysis showing effects of saline, LPS or (and) agmatine administration on activated caspase-3 protein in the hippocampi of rats. Western immunoblots probed with antibodies against cleaved (activated) caspase-3 and β-actin. $^{***}P<0.001$ represent the difference between LPS and saline treated groups. $\#\#\#P<0.001$ represent the difference between LPS and agmatine treated groups.

potent inhibitor of NMDA channels could not reverse LPS-induced memory impairment completely (Rosi et al., 2006); it seems that NMDA receptor-mediated events are not solely responsible for LPS-induced cognitive deficits and also agmatine's protective effect.

Recently it is reported that systemic LPS injection elevates amyloidogenesis through activation of β and γ secretase and thereby elevates Aβ 1–42 levels in the hippocampus (Lee et al., 2008). Aβ 1–42 plays a central role in the pathogenesis of Alzheimer's disease and Aβ oligomeres were the principal toxins leading to neuronal death and neurite breakage (Estus et al., 1997; Mattson et al., 1998; Butterfield, 2003). Aβ triggers increasing calcium influx thorough different pathways (Hölscher, 1998, 2005; Zhong et al., 2009). Calcium overload in the mitochondria leads to caspase 3 activation and ultimately neuronal death (Pivovarova et al., 2004; Tardif et al., 2005). Therefore modulation of voltage-gated calcium channels (VGCC) can be considered as another possible mechanism responsible for LPS-induced hippocampal damage. Agmatine has been reported to block VGCC with a high potency in cultured rat hippocampal neurons (Wang et al., 2003; Zheng et al., 2004). Then this property of agmatine might also contribute to its neuroprotective effect.

LPS also is suggested to increase iNOS activation and thereby nitric oxide (NO) production (Yamada et al., 1999; Semmler et al., 2005). NO acts as an intracellular signaling molecule and a neurotoxin depending on its concentration (Dawson & Snyder, 1994; Garthwaite et al., 1988). It can damage DNA and irreversibly modify proteins such as tyrosine nitration or thiol oxidation, which are common pathogenic mechanisms in several neurodegenerative diseases (Schopfer et al., 2003). Also, NO-derived peroxynitrite can cause irreversible injury to mitochondria, release of cytochrome C and caspase-3 activation (Pacher et al., 2007) which eventually results in neuronal apoptosis. Agmatine has been shown to inhibit iNOS (Galea et al., 1996); then it can be speculated that agmatine prevent LPS-induced hippocampal cell apoptosis and memory loss via iNOS inhibition too.

Compatible with our results, it has been revealed that agmatine can prevent apoptosis induced by hypoxia in retinal cell ganglia (Hong et al., 2007, 2009) and also against glucocorticoid toxicity in cultured hippocampal neurons (Zhu et al., 2006). Perhaps the most significant finding of the present study was that the LPS-induced learning and memory deterioration was completely counteracted by agmatine administration, the effect that has not been seen by the currently used drugs treating Alzheimer's disease such as memantine (Rosi et al., 2006).

In conclusion our study showed for the first time, that agmatine can reverse the memory impairment and the hippocampal apoptosis resulted from LPS treatment. As neuroinflammation is involved in brain dementia and Alzheimer's disease, agmatine seems to act as a possible neuroprotective substance in these disorders. More investigations are necessary to know agmatine's protective effect and its cellular and molecular mechanisms.

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References

Akiyama, H., Arai, T., Kondo, H., Tanno, E., Haga, C., Ikeda, K., 2000. Cell mediators of inflammation in the Alzheimer disease brain. Alzheimer Dis. Assoc. Disord. 14, S47–53.

Askalany, A.R., Yamakura, T., Petrenko, A.B., Kohno, T., Sakimura, K., Baba, H., 2005. Effect of agmatine on heteromeric N-methyl-D-aspartate receptor channels. Neurosci. Res. 52, 387–392.

Butterfield, D.A., 2003. Amyloid beta-peptide [1-42]-associated free radical-induced oxidative stress and neurodegeneration in Alzheimer's disease brain: mechanisms and consequences. Curr Med Chem. 10, 2651–2659.

Cagnin, A., Brooks, D.J., Kennedy, A.M., Gunn, R.N., Myers, R., Turkheimer, F.E., Jones, T., Banati, R.B., 2001. In-vivo measurement of activated microglia in dementia. Lancet 358, 461–467.

Dawson, T.M., Snyder, S.H., 1994. Gases as biological messengers: nitric oxide and carbon monoxide in the brain. J Neurosci. 14, 5147–5159.

Espey, M.G., Kustova, Y., Sei, Y., Basile, A.S., 1998. Extracellular glutamate levels are chronically elevated in the brains of LP-BM5-infected mice: a mechanism of retrovirus-induced encephalopathy. J Neurochem. 71, 2079–2087.

Estus, S., Tucker, H.M., van Rooyen, C., Wright, S., Brigham, E.F., Wogulis, M., Rydel, R.E., 1997. Aggregated amyloid-beta protein induces cortical neuronal apoptosis and concomitant "apoptotic" pattern of gene induction. J Neurosci. 17, 7736–7745.

Fairbanks, C.A., Schreiber, K.L., Brewer, K.L., Yu, C.G., Stone, L.S., Kitto, K.F., Nguyen, H.O., Grocholski, B.M., Shoeman, D.W., Kehl, L.J., Regunathan, S., Reis, D.J., Yezierski, R.P., Wilcox, G.L., 2000. Agmatine reverses pain induced by inflammation, neuropathy, and spinal cord injury. Proc Natl Acad Sci U S A. 97, 10584–10589.

Fine, S.M., Angel, R.A., Perry, S.W., Epstein, L.G., Rothstein, J.D., Dewhurst, S., Gelbard, H.A., 1996. Tumor necrosis factor alpha inhibits glutamate uptake by primary human astrocytes. Implications for pathogenesis of HIV-1 dementia. J Biol Chem 271, 15303–15306.

Fischer-Smith, T., Croul, S., Adeniyi, A., Rybicka, K., Morgello, S., Khalili, K., Rappaport, J., 2004. Macrophage/microglial accumulation and proliferating cell nuclear antigen expression in the central nervous system in human immunodeficiency virus encephalopathy. Am. J. Pathol. 164, 2089–2099.

Galea, E., Regunathan, S., Eliopoulos, V., Feinstein, D.L., Reis, D.J., 1996. Inhibition of mammalian nitric oxide synthases by agmatine, an endogenous polyamine formed by decarboxylation of arginine. Biochem. J. 15, 247–249.

Garthwaite, J., Charles, S.L., Chess-Williams, R., 1988. Endothelium-derived relaxing factor release on activation of NMDA receptors suggests role as intercellular messenger in the brain. Nature 336, 385–388.

Gilad, G.M., Gilad, V.H., Finberg, J.P., Rabey, J.M., 2005. Neurochemical evidence for agmatine modulation of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) neurotoxicity. Neurochem. Res. 30, 713–719.

Gilad, G.M., Gilad, V.H., 2000. Accelerated functional recovery and neuroprotection by agmatine after spinal cord ischemia in rats. Neurosci. Lett. 296, 97–100.

Halaris, A., Plietz, J., 2007. Agmatine : metabolic pathway and spectrum of activity in brain. CNS Drugs. 21, 885–900.

Hölscher, C., 2005. Development of beta-amyloid-induced neurodegeneration in Alzheimer's disease and novel neuroprotective strategies. Rev. Neurosci. 16, 181–212.

Hölscher, C., 1998. Possible causes of Alzheimer's disease: amyloid fragments, free radicals, and calcium homeostasis. Neurobiol Dis. 5, 129–141.

Hong, S., Kim, C.Y., Lee, J.E., Seong, G.J., 2009. Agmatine protects cultured retinal ganglion cells from tumor necrosis factor-alpha-induced apoptosis. Life Sci. 84, 28–32.

Hong, S., Lee, J.E., Kim, C.Y., Seong, G.J., 2007. Agmatine protects retinal ganglion cells from hypoxia-induced apoptosis in transformed rat retinal ganglion cell line. BMC Neurosci. 8, 81.

Lee, J.W., Lee, Y.K., Yuk, D.Y., Choi, D.Y., Ban, S.B., Oh, K.W., Hong, J.T., 2008. Neuro-inflammation induced by lipopolysaccharide causes cognitive impairment through enhancement of beta-amyloid generation. J Neuroinflammation. 29, 5–37.

Li, G., Regunathan, S., Barrow, C.J., Eshraghi, J., Cooper, R., Reis, D.J., 1994. Agmatine: an endogenous clonidine-displacing substance in the brain. Science 263, 966–969.

Mattson, M.P., Partin, J., Begley, J.G., 1998. Amyloid beta-peptide induces apoptosis-related events in synapses and dendrites. Brain Res. 807, 167–176.

McGeer, E.G., McGeer, P.L., 1998. The importance of inflammatory mechanisms in Alzheimer disease. Exp. Gerontol. 33, 371–378.

Mhatre, M., Floyd, R.A., Hensley, K., 2004. Oxidative stress and neuroinflammation in Alzheimer's disease and amyotrophic lateral sclerosis: common links and potential therapeutic targets. J Alzheimers Dis. 6, 147–157.

Moosavi, M., Naghdi, N., Maghsoudi, N., Zahedi Asl, S., 2007. Insulin protects against stress-induced impairments in water maze performance. Behav. Brain Res. 176, 230–236.

Olmos, G., DeGregorio-Rocasolano, N., Paz Regalado, M., Gasull, T., Assumpció Boronat, M., Trullas, R., Villarroel, A., Lerma, J., García-Sevilla, J.A., 1999. Protection by imidazol(ine) drugs and agmatine of glutamate-induced neurotoxicity in cultured cerebellar granule cells through blockade of NMDA receptor. Br. J. Pharmacol. 127, 1317–1326.

Pacher, P., Beckman, J.S., Liaudet, L., 2007. Nitric oxide and peroxynitrite in health and disease. Physiol. Rev. 87, 315–424.

Perry, V.H., 2004. The influence of systemic inflammation on inflammation in the brain: implications for chronic neurodegenerative disease. Brain Behav. Immun. 18, 407–413.

Piletz, J.E., May, P.J., Wang, G., Zhu, H., 2003. Agmatine crosses the blood–brain barrier. Ann. N. Y. Acad. Sci. 1009, 64–74.

Pivovarova, N.B., Nguyen, H.V., Winters, C.A., Brantner, C.A., Smith, C.L., Andrews, S.B., 2004. Excitotoxic calcium overload in a subpopulation of mitochondria triggers delayed death in hippocampal neurons. J Neurosci. 24, 5611–5622.

Raasch, W., Regunathan, S., Li, G., Reis, D.J., 1995. Agmatine is widely and unequally distributed in rat organs. Ann. N. Y. Acad. Sci. 763, 330–334.

Reis, D.J., Regunathan, S., 2000. Is agmatine a novel neurotransmitter in brain? Trends Pharmacol. Sci. 21, 187–193.

Rosi, S., Vazdarjanova, A., Ramirez-Amaya, V., Worley, P.F., Barnes, C.A., Wenk, G.L., 2006. Memantine protects against LPS-induced neuroinflammation, restores behaviorally-induced gene expression and spatial learning in the rat. Neuroscience. 142, 1303–1315.

Schopfer, F.J., Baker, P.R., Freeman, B.A., 2003. NO-dependent protein nitration: a cell signaling event or an oxidative inflammatory response? Trends Biochem. Sci. 28, 646–654.

Semmler, A., Okulla, T., Sastre, M., Dumitrescu-Ozimek, L., Heneka, M.T., 2005. Systemic inflammation induces apoptosis with variable vulnerability of different brain regions. J Chem Neuroanat. 30, 144–157.

Shaw, K.N., Commins, S., O'Mara, S.M., 2001. Lipopolysaccharide causes deficits in spatial learning in the wate rmaze but not in BDNF expression in the rat dentate gyrus. Behav. Brain Res. 124, 47–54.
Tabor, C.W., Tabor, H., 1984. Polyamines. Annu. Rev. Biochem. 53, 749–790.
Tardif, K.D., Waris, G., Siddiqui, A., 2005. Hepatitis C virus, ER stress, and oxidative stress. Trends Microbiol. 13, 159–163.
Vargas, D.L., Nascimbene, C., Krishnan, C., Zimmerman, A.W., Pardo, C.A., 2005. Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann. Neurol. 57, 67–81.
Wang, W.P., Iyo, A.H., Miguel-Hidalgo, J., Regunathan, S., Zhu, M.Y., 2006. Agmatine protects against cell damage induced by NMDA and glutamate in cultured hippocampal neurons. Brain Res. 1084, 210–216.
Wang, Z.M., Sun, G.Q., Wang, Z.A., He, R.R., 2003. Effects of agmatine on neuronal discharges in rat hippocampal CA1 area. Sheng Li Xue Bao. 55, 717–721.
Wenk, G.L., Parsons, C.G., Danysz, W., 2006. Potential role of N-methyl-D-aspartate receptors as executors of neurodegeneration resulting from diverse insults: focus on memantine. Behav Pharmacol. 17, 411–424.
Yamada, K., Komori, Y., Tanaka, T., Senzaki, K., Nikai, T., Sugihara, H., Kameyama, T., Nabeshima, T., 1999. Brain dysfunction associated with an induction of nitric oxide synthase following an intracerebral injection of lipopolysaccharide in rats. Neuroscience. 88, 281–294.
Yang, X.C., Reis, D.J., 1999. Agmatine selectively blocks the N-methyl-D-aspartate subclass of glutamate receptor channels in rat hippocampal neurons. J. Pharmacol Exp. Ther. 288, 544–549.
Zheng, J.Q., Weng, X.C., Gai, X.D., Li, J., Xiao, W.B., 2004. Mechanism underlying blockade of voltage-gated calcium channels by agmatine in cultured rat hippocampal neurons. Acta Pharmacol Sin. 25, 281–285.
Zhong, S.Z., Ge, Q.H., Li, Q., Qu, R., Ma, S.P., 2009. Peoniflorin attenuates Abeta((1–42)) mediated neurotoxicity by regulating calcium homeostasis and ameliorating oxidative stress in hippocampus of rats. J Neurol Sci. 280, 71–78.
Zhu, M.Y., Piletz, J.E., Halaris, A., Regunathan, S., 2003. Effect of agmatine against cell death induced by NMDA and glutamate in neurons and PC12 cells. Cell. Mol. Neurobiol. 23, 865–872.
Zhu, M.Y., Wang, W.P., Bissette, G., 2006. Neuroprotective effects of agmatine against cell damage caused by glucocorticoids in cultured rat hippocampal neurons. Neuroscience. 141, 2019–2027.
Zhu, M.Y., Wang, W.P., Cai, Z.W., Regunathan, S., Ordway, G., 2008. Exogenous agmatine has neuroprotective effects against restraint-induced structural changes in the rat brain. Eur. J. NeuroSci. 27, 1320–1332.