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Review

Neuroprotective offerings by agmatine

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ABSTRACT

Agmatine, an endogenous polyamine in CNS, is derived from arginine by dearboxylation. Like polyamines, agmatine has been studied for its neuroprotetive effects. At present, a large body of experimental evidences has been gathered that demonstrate the neuroprotective effects of agmatine. The neuroprotective effects have been observed in various CNS cell lines and animal models against the excitotocity, oxidative damage, corticosteroidid induced neurotoxicity, ischemic/hypoxic or oxygen-glucose deprivation toxicity, spinal cord injury and traumatic brain injury. The studies have been extended to rescue of retinal ganglion cells from toxicities. The mechanistic studies suggest that neuroprotection offered by agmatine can be assigned to its multimolecular biological effects. These include its action as glutamatergic receptor antagonist, α_2-adrenoceptor agonist, imidazoline binding site ligand, NOS inhibitor, ADP ribosylation inhibitor, and blocker of ATP-sensitive potassium and voltage-gated calcium channels, anti-apoptotic and antioxidant. Its action as regulator for polyamine synthesis, insulin release assists the neuroprotection.

The cumulative evidences of preclinical studies support the possible use of agmatine as an agent for neuronal damage and neurodegenerative diseases. However, it will be hasty to assert and promote agmatine as a novel therapeutic agent for neuroprotection. **The review is focused on the role of agmatine in different types and mechanisms of neural injuries.** The aspects of concern like dose range, pharmacokinetics of exogenous agmatine, levels of endogenous agmatine during events of injury etc. has to be addressed.

1. Introduction

Agmatine, a decarboxylated arginine, has been a known precursor for the synthesis of polyamines in plants and bacteria. Polyamines were found to exert neuroprotective effects in experimental models of neurotrauma (Gilad and Gilad, 1992). With the identification of agmatine and its biosynthetic activity in mammalian brain, it was hypothesized that agmatine might serve a neuroprotective role following neurotrauma. The hypothesis was upheld by its role of as endogenous ligand for imidazoline binding sites and its ability to interact with

Abbrevations: ADC, Arginine decarboxylase; Akt/protein kinase B, PI3K downstream effector protein; ARE, Antioxidant response element; ATF3, Activating transcription factor 3; Bax, Bcl-2 associated X protein; BCAO, Bilateral carotid artery occlusion; Bcl-2, B cell lymphoma 2; BMP, Bone morphogenetic protein; BrdU, Bromodeoxyuridine; CAST, Computer Assisted Stereological Toolbox; CE-T1WI, Contrast-enhanced T1-weighted images; CSO, Corticosterone; DWI, Serial diffusion-weighted images; DXM, Dexamethasone; eNOS, Endothelial nitric oxide synthase; ERK, Extracellular signal-regulated kinase; GCLc, Glutamate cysteine ligase, catalytic subunit; GFAP, Glial fibrillary acidic protein; Grp78, Glucose-regulated protein 78; GSTA2, Glutathione S-transferase α2; H & PI, Hoechst 33258 and propidium iodide; H&E, Hematoxylin and eosin; *hADC*, human hADC gene; HMGB1, high-mobility group box 1; HO-1, Heme oxygenase-1; Iba1, Calcium binding adaptor molecule 1; ICAM-1, Intercellular adhesion molecule 1; iNOS, Inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; Keap1, Kelch-like-ECH-associated protein 1; LDH, Lactate dehydrogenase; LPS, Lipopolysaccharide (*E. coli* 026:B6); MAP-2, Microtubule-associated protein-2 (MAP-2); MAPK, Mitogen associated protein kinase; MCAO, middle cerebral artery occlusion; MMPs, Matrix metalloproteinases; mPFC, Medial prefrontal cortex; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MT1-MMP, Membrane-type 1 matrix metalloproteinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NAME, Nω-nitro-L-arginine methyl ester; NeuN, Neuronal-specific nuclear protein; NF-κB, Nuclear factor-kappa B; NG2, Oligodendrocytes progenitor cells; NO, Nitric oxide; NQO1, NAD(P)H/quinone oxidoreductase; Nrf2, Nuclear factor (erythroid 2 derived)-like 2; OGD, Oxygen–glucose deprivation; Olig-2, Oligodendrocyte transcription factor-2; PI3K, Phosphatidylinositol- 3-kinase; PKC, Protein kinase C; RAGE, Receptor for advanced glycation end products; ROS, Reactive oxygen species; RT-PCR, Real-time PCR; T2WI, T2-weighted images; TBI, Traumatic brain injury; TGFβ-2, Transforming growth factor β-2; TLR, Toll-like receptor; TTC, Triphenyltetrazolium chloride; TUNEL, Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) nick end labeling assay; VEGF, Vascular endothelial growth factor; VEGFR2, VEGF receptor 2

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various neurotransmitter receptors, including nicotinic cholinergic receptors (nAchRs), α_2-adrenergic receptors (α_2-ARs), and N-methyl-D-aspartate receptors (NMDARs). By 1995, the first documentation on neuroprotective effects of agmatine was reported by Gilad and colleagues (Gilad et al., 1996a, 1996b). Thereafter, many detailed studies have evaluated the effects of agmatine employing several experimental models in animals or cell lines based on the simulation of neuronal damage arising from excitotoxicity, spinal cord injury, ischemia traumatic brain injury and others. These studies sought to understand the mechanism underling the protective effect of agmatine against nerve cell damage.

2. Effects of agmatine on neural injury

2.1. Glutamate induced excitotoxiciy

In seeking the neuroprotective role of agmatine along with cell line model of neurotoxicity, Gilad et al. examined its effects in animal models of ischemic injury (Gilad et al., 1996a, 1996b). The 3-day old primary cultures of cerebellar neurons from neonatal rat were challenged with 1 mM NMDA for 3 h and treated with agmatine (1–2000 µM). The cell survival after 24 h assessed by phase-contrast microscopy and trypan blue exclusion test proved the support of agmatine to neuron survival at concentrations between 10–100 µM. At higher concentrations it became toxic (apparent toxic dose TD_{50} = 700 µM), perhaps due to its inhibitory effects on cell proliferation (Fig. 1).

Neuroprotective potential of agmatine against NMDA challenged cerebellar cells studied by Gilad et al. was substantiated by Olmos et al. in the study designed to assess the effects of agmatine and several imidazoline drugs on glutamate induced necrosis and on apoptosis induced by low extracellular K^+ (5.6 mM) in primary cultures of neonatal rat cerebellar granule cells (Gilad et al., 1996a, 1996b; Olmos et al., 1999). Considering relatively low affinity of agmatine to NMDA receptor (Ki = 219 mM) and average brain concentrations of agmatine in the rat brain (1.5–8.5 µM), question arises whether endogenous agmatine could be acknowledged for the survival encouragement effects observed in vivo (Abe et al., 2000). Here, it can be assumed that efficacious concentrations of agmatine can be attained at the NMDA receptors due to non-uniform distribution of agmatine in the CNS and its release, if it occurs (Otake et al., 1998). Glutamate (1–100 µM) decreased the metabolism of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrzoliumbromide (MTT) into a reduced formazan product in cerebellar cells, loss of mitochondrial membrane potential, overstimulation of NMDA receptor leading to a Ca^{2+} overload and inability of the cells to maintain osmotic integrity leading to necrosis. The neurotoxicity induced by glutamate was blocked both by agmatine (100 or 500 µM) and dizocilpine (MK-801), a specific NMDA receptor antagonist, as well as other imidazoline drugs such as antazoline, cirazoline, idazoxan etc. This correlation was also noted in the affinity of agmatine and structural congeners for NMDA receptor and neuroprotection. This suggest that the mechanism of neuroprotection to be a direct action with NMDA receptor. This interpretation was supported by inability of agmatine and structural congeners to offer protection in low K^+ induced apoptotic neuronal death model in which NMDA receptors are not involved and the ability of agmatine to directly block glutamate-induced currents in HEK-293 cells transfected to express the NR1-1a/NR2C subunits of the NMDA receptor in concentration- and voltage-dependent manner in whole cell patch-clamp experiments. Looking at a disadvantage associated with using agmatine for chronic CNS disorders related to its transport through biological membranes, Gilad & Gilad patented novel derivatives of agmatine and other guanidines, hydrazine, polyamines and related compounds, referred to as 'polyaminoguanidine derivatives' for the treatment of neurotrauma and neurodegenerative diseases (Gilad and Gilad, 1997, 2000a, 2000b). The guanidino group of agmatine has been identified as the responsible moiety for blockade of the NMDA receptor channel by its interaction with a site located within the NMDA channel pore in rat hippocampal neurons (Yang and Reis, 1999). Agmatine applied extracellularly to cultured hippocampal neurons produced a voltage and concentration-dependent block of NMDA (but not AMPA or kainate) currents. Agmatine is unique to date among endogenous biogenic amines, as selectively exhibiting antagonist activity at non-glycine β sites of NMDA receptors. Stimulation of NMDA receptor results into fast mitochondrial Ca^{2+} uptake and I_2-BS imidazoline binding sites are mainly located on

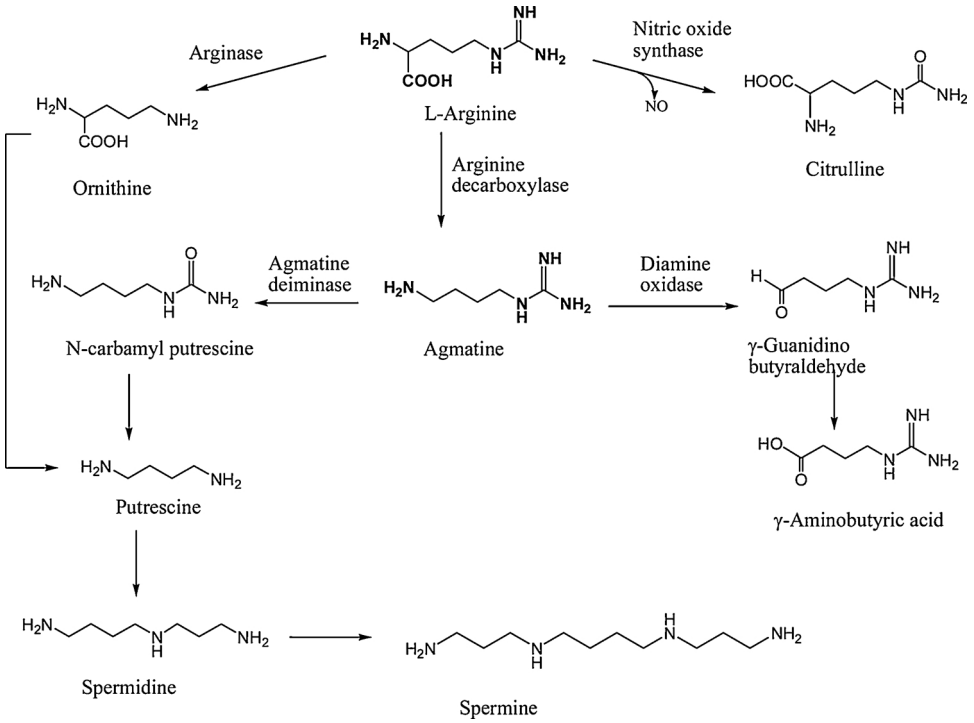

Fig. 1. Biosynthetic and metabolic pathway of agmatine.

Table 1
Neuroprotective effects of agmatine against excitotoxicity, oxidative damage.

Injury type, By	In	Agmatine	Observed activity Funtional recovery measurement	Observation	Reference
Excitotoxic; NMDA, 1 μM, 3 h	3-day primary rat cerebellum cell culture	1-2000 μM, 10-200 μM,	Phase-contrast microscopy, Trypan blue exclusion test	• Toxic dose of agmatine, TD_{50} = 700 μM • Agmatine exerted strong neuroprotection against the After 24	Gilad et al., 1996a
Excitotoxic; glutamate &/or NMDA, Low K + induced	Rat cerebellar granule cell cultures	100 or 500 μM	MTT assay	• Agmatine prevented glutamate induced neurotoxicity but did not protect cells against low K + induced apoptosis. • Effect mediated through NMDA receptor blockade	Olmos et al., 1999
Excitotoxic; glutamate 10 mM, or NMDA 100 mM or staurosporine or calcimycin 100 nM, 10 min	PC12 cells, Neonatal rat cortical neuron culture	10, 100 or 1000 μM for 24 h.	LDH assay	• Reduction in LDH release induced by glutamate, but not by staurosporine protein kinase blockade or calcimycin increase in cellular calcium	Zhu et al., 2003
Excitotoxic; NMDA or glutamate 100 or 200 μM, 1 h	12-day rat hippocampal neuron culture	1-100 μM, or MK801 (10 μM), arcaine, spermine, or putrescine (100 μM).	LDH assay, β-tubulin III staining, TUNEL assay	• Agmatine prevented neurotoxicity similar to MK801, arcaine, Spermine and putrescine failed to show this effect • Possible blockade of the NMDAR channels or potential anti-apoptotic property	Wang et al., 2006
Oxidative toxicity LPS	Microglia, neuron and co-culture from rat cerebral cortices	1–300 μM	MTT assay, Nitrite determination, iNOS expression	• Agmatine reduced neuronal loss induced by microglia-derived NO Agmatine inhibited LPS induced NO production but did not affect iNOS expression • Diphenyleneiodoium chloride prevented cell loss	Abe et al., 2000
Oxidative toxicity, LPS	BV2 microglia	100 μM	LDH assay, Nitrite determination	• Agmatine attenuated LPS-induced microglial death & nitrite production	Ahn et al., 2012
Oxidative toxicity LPS	Male ICR mice	100 μM	Iba1, iNOS, TNF-α, IL-1B expression	• Agmatine attenuates LPS-induced microglial damage by reducing expression of Iba1, iNOS, TNF-α, IL-1β	Ahn et al., 2012

mitochondrial membranes. It is likely that agmatine mediates neuroprotective effects by activation of I_2-BS to increase mitochondrial Ca^{2+} influx, and reduction in excessive cytosolic Ca^{2+} accumulation. However, no correlation was found between the potency of agmatine on rat brain I_2-BS and potency of protection against glutamate toxicity indicating that activation of I_2-BS was not involved in the neuroprotective effects of these drugs against glutamate-induced neurotoxicity (Table 1).

Contrary to the observation of neuroprotection by Olmos et al. agmatine was found to be neurotoxic in primary cultures of neonatal rat cerebellar cells in a high K^+ (27.5 mM) content in the medium but not when extracellular K^+ concentration was lower (10 mM) (Abe et al., 2003). Agmatine (200–800 μM) treatment to 7–8 day old primary culture of cerebellar granule neurons produced significant decrease in cell viability and cell death had begun after 6–12 h of agmatine addition that progressed gradually. The cell death was due to a significant increase in extracellular L-glutamate concentration ensued from exocytosis triggered by high concentrations of K^+. The pretreatment with botulinum toxin C, which is known to inhibit the exocytosis specifically, blocked agmatine-induced glutamate release and cell death. This glutamate toxicity was limited by NMDA receptor antagonists and enzymatic degradation of L-glutamate with glutamic pyruvic transaminase. The K^+ concentration in studies with cultured rat hippocampal neurons by Wang et al. was less than 6 mM where agmatine was neuroprotective against NMDA and glutamate toxicity (Wang et al., 2006). It can be suggested that whether agmatine exerts neuroprotection or neurotoxicity partly depends on the experimental conditions, including the concentrations of K^+ and agmatine applied. Though, no such ambiguity was reported further and NMDA receptor blocking was the most recurrent mechanism of neuroprotection.

Zhu et al. while describing effects on neuronal as well as PC12 cells derived from pheochromocytoma of rat adrenal medulla, attributed the neuroprotective effect of agmatine to blocking of NMDAR channels (Zhu et al., 2003). The possibility of nonspecific blockade of cation channels was ruled out with observation of inability of agmatine to reduce cell lysis [lactate dehydrogenase (LDH) release] induced with calcimycin - a calcium channel opener, and staurosporine - an apoptotsis inducer by intracellular actions. Seeking more compelling evidence for elucidating agmatine's neuroprotective role on different brain neurons and given its abundance in the hippocampus, addition of 100 μM agmatine into 12-day old primary cultured rat hippocampal neurons ablated the neurotoxicity induced by NMDA or glutamate. Agmatine decreased glutmate/NMDA induced cellular morphological changes, cell lysis and apoptosis. Arcaine, structurally similar analogue of agmatine, also prevented the neuronal damage. However, spermine and putrescine, the endogenous polyamine and metabolic products of agmatine without the guanidine moiety, failed to show this effect, affirming structural relevance for neuroprotection (Wang et al., 2006).

2.2. Glucocorticoid induced toxicity

The production of cortisol (in humans) and corticosterone (in rodents), in adrenal glands is regulated by integrated neuronal and endocrine functions of hypothalamic-pituitary-adrenal system (Frodl and O'Keane, 2013). These glucocorticoids alter the function of tissues and mobilize or store energy to meet the demands while coping with a stressful situation. On the other hand, overexposure to cortisol or corticosterone during prolonged periods of stress is harmful to brain by inducing apoptotic neuronal cell death (Lee et al., 2002). Neurons are the primary cells in the brain that suffer the cytotoxic effects of glucocorticoids. Particularly neuronal cells in the hippocampus are prone to death since it contains a high concentration of glucocorticoid receptors (Zunszain et al., 2011). The hippocampal neuronal damage by glucocorticoids is also well documented with several putative mechanisms. One of the proposed mechanisms is the glucocorticoid induced increase in the extracellular glutamate (Sapolsky et al., 1990). In

concert with these reports, Zhu et al. demonstrated protective effects of agmatine (100 μM) against cell damage caused by glucocorticoids, dexamethasone and corticosterone, in cultured rat hippocampal neurons (Zhu et al., 2006). Since glucocorticoids are the main hormones released during stress, these studies raised the possibility that agmatine plays a role in the homeostasis during stress, particularly with regard to physiological effects mediated by glucocorticoids and glutamate. To elaborate, in vivo for physiological effects as morphological changes induced by dexamethasone were investigated in hippocampus and PFC (Zhu et al., 2007). Seven day treatment with dexamethasone (10 or 50 μg/kg/day) failed to induce any morphological changes in neurons of hippocampus and PFC, however, its prolonged exposure of 21 days in higher dose produced noticeable structural changes. Simultaneous treatment with agmatine (50 mg/kg/day) prevented the morphological changes. Endogenous agmatine levels in the PFC, hippocampus, striatum and hypothalamus were increased after 7-day treatment with dexamethasone in a dose-dependent manner. On the contrary, 21-day treatment with glucocorticoid robustly reduced agmatine levels in these brain regions. Interestingly, treatment with glucocorticoids resulted in a similar change of arginine decarboxylase (ADC) protein levels in most brain areas to endogenous agmatine levels: an increase after 7-day treatment versus a reduction after 21-day treatment. It was suggested that stress-associated doses of glucocorticoids stimulated reactions of self-protection mechanisms. As a component of these mechanisms, increased endogenous agmatine levels in these brain areas contributed its neuronal protection at the initial stage. However, prolonged exposure to glucocorticoids exhausted endogenous agmatine stores and/or affected its biosynthesis, resulting in the reduced levels of endogenous agmatine.

Another mechanism of glucocorticoid toxicity is the induction of oxidative and inflammatory conditions in the brain accompanied by weakened antioxidant defense, lipid peroxidation, DNA damage, mitochondrial dysfunction, and abnormalities in monoaminergic systems, reduced neurogenesis and neuronal plasticity (Leonard and Maes, 2012). Based on this background, a study investigated the effects of agmatine on oxidative/nitrosative stress development on chlorpromazine-induced neuronal injury (Dejanovic et al., 2018). Subacute administration of chlorpromazine (38.7 mg/kg body weight i.p.) resulted in increased lipid peroxidation, nitric oxide concentration and superoxide anion production, while completely damaging the antioxidant defense system in the selective vulnerable brain regions as cerebral cortex, striatum, and hippocampus. However, the combined treatment with chlorpromazine and agmatine (75 mg/kg body weight i.p.) significantly attenuated the oxidative/nitrosative stress indices and restored the antioxidant defense to the control values in all of the examined brain regions. The master regulator of the antioxidant defense response is Nrf2 [nuclear factor (erythroid-2-derived)-like 2], a transcription factor, that activates a redox-sensitive gene regulatory network to maintain redox homeostasis in the brain and protect neurons against cell death (Johnson et al., 2008). Antioxidant defense that combats oxidative toxicity of glucocorticoids was focused by Freitas et al. to determine role of the transcription factor Nrf2 in neuronal protection afforded by agmatine (Freitas et al., 2015).

Corticosterone induced apoptotic cell death and increased ROS production in hippocampal neuronal HT22 cells were abolished in a concentration- and time-dependent manner by co-incubated agmatine (0.01–100 μM). The neuroprotective effect of agmatine was abolished by yohimbine (α_2-AR antagonist), ketanserin (5-HT_{2A} receptor antagonist), LY294002 (PI3K inhibitor), PD98059 (MEK1/2 inhibitor), tin (IV) protoporphyrin-IX dichloride (Heme oxygenase-1 [HO-1] inhibitor), and cycloheximide (protein synthesis inhibitor). Agmatine increased Akt and ERK phosphorylation and induced the transcription factor, Nrf2 and the proteins HO-1 and glutamate cysteine ligase catalytic subunit (GCLc). Induction of these proteins was prevented by yohimbine, ketanserin, LY294002 and PD98059. The study implicates Nrf2 induction via α_2-AR and 5-HT_{2A} receptors, Akt and ERK pathways, and HO-1 and GCLc expression in the neuroprotective effect of agmatine against corticosterone induced apoptotic cell death. The antioxidant activity of agmatine has also been observed against LPS-induced ROS accumulation in RAW 264.7 cells involving HO-1 expression induced by Nrf2 via PI3K/Akt pathway activation (Chai et al., 2016).

Agmatine afforded synergic protection against corticosterone induced cell death in HT22 cells in the combination of sub-effective concentrations (0.001 μM) with fluoxetine or imipramine or ketamine (0.01 μM) (Freitas et al., 2015; Tavares et al., 2018). While synergistic effect of agmatine and slow acting fluoxetine, imipramine was attributed to Nrf2 activation and the consequent up-regulation of antioxidant enzymes, synergistic effect of agmatine and fast acting ketamine was assigned to its ability to activate Akt and mTOR/S6 kinase signaling pathway, and increase the expression of synaptic proteins.

To test the hypothesis that the observed pathological impacts of chronic exposure to glucocorticoids are similar to the neuronal insults arising from chronic stress, the team used two different models viz. repeated restraint stress model and four point immobilization stress model (Zhu et al., 2008a, 2008b) The repeated restrain stress model, stress-induced induced structural changes in the mPFC, hippocampus of rats were prevented by simultaneous treatment with agmatine (50 mg/kg/day, i.p.). In restrained animals, the endogenous agmatine levels in mPFC, hippocampus, striatum and hypothalamus were significantly reduced as compared to controls accompanied by a significant increase of ADC protein levels. Moreover, administration of exogenous agmatine to restrained rats abolished increase of ADC protein levels (Zhu et al., 2008a). In four point immobilization stress model, repeatedly immobilized rats simultaneously treated with exogenous agmatine showed an almost normal morphology in the hippocampus and mPFC. The endogenous agmatine levels were found elevated (ranging from 92% to 265% of controls) accompanied by a significant increase of ADC protein levels in the same brain regions. The parallel increase in endogenous brain agmatine and ADC protein levels triggered by stress indicate that the endogenous agmatine system may play an important role in adaptation to stress as a potential self protection mechanism (Zhu et al., 2008b). The differences in observations in these studies might be due to the nature of stress and induction method employed. The stress induced by repeated immobilization is strong with higher-intensity than simple restraining and is accompanied by extended glucocorticoid secretion as a post-stress maintenance and anorexia (Marquez et al., 2002). Another possible reason can be a significantly high glutamate efflux caused by the repeated immobilization while there is no such measurement done in restraint stress study. The observation of noticeable structural alterations in hippocampus and mPFC but not in the striatum and hypothalamus may be attributed to agmatine's neuroprotective effects being consistent with regions that display robust synaptic plasticity. This is analogous to action of stress inducers as gluocorticoids and glutamate. Glucocorticoids exert effects throughout the brain, whereas exposure to glucocorticoids results in neuronal damage only in the hippocampus and mPFC. Although stress induced elevation in glutamate levels are seen in hippocampus, mPFC, striatum and hypothalamus, structural changes are found only in the hippocampus and mPFC (Table 2).

2.3. Drug induced neurotoxicity

In spontaneous pain-related grooming behavior model that arises following excitotoxicity in spinal cord by quisqualate, agmatine treatment delayed onset of excessive grooming behavior, reduced area of skin targeted for excessive grooming and grooming severity, and neuronal loss. The effect of agmatine on quisqualic acid (AMPA/metabotropic receptor agonist) induced excessive grooming has been seems to be themodulation of ongoing cellular events responsible for the progression of quisqualic acid induced excitotoxicity (Yu et al., 2003). The neurotoxicity arising due to chronic anticancer drugs as paclitaxel, cisplatin in primary neuron cells was counteracted by agmatine on both

Table 2
Neuroprotective effects of agmatine against glucocorticoid induced neurotoxicity.

Injury type, By	In	Agmatine	Observed activity Funtional recovery measurement	Observation	Ref
Neurotoxic; DXM 0.05, 0.5 or 5 mM &/or COS 1μM	12-day rat hippocampal neuron culture	100 μM (or arcaine, spermine or putrescine 100 μM)	LDH assay, β-tubulin III staining, TUNEL assay, Caspase-3 assay,	• Glucocorticoids increased LDH, caspase-3 activities TUNEL-positive cell numbers, caused morphological changes • GC induced effects prevented by agmatine, arcaine treatment but not by spermine or putrescine • Possible blockade of NMDAR & a potential anti-apoptotic	Zhu et al., 2006
Neurotoxic; DXM, 10 or 50 μg/kg/day, 7 or 21-days	Male Sprague–Dawley rats	50 mg/kg /day, i.p.	Agmatine & ADC levels	• DXM treatment 50 mg/kg/day of 7-day increased agmatine & ADC levels but of 21-day decreased agmatine & ADC levels in PFC, striatum, hippocampus, hypothalamus • Morphologic changes only with 21 days DXM treatment • Agmatine prevented effects induced by DXM	Zhu et al., 2007
Neurotoxic; COS 1μM	HT22 cell	100 μM	MTT assay, ROS estimation, Flow cytometry analysis, Phosphorylation of Akt, ERK, HO−1 & GCLc expression	• COS induced morphologic changes, increased ROS production, apoptosis • Agmatine abolished effects of COS • Effects of agmatine abrogated by LY294002, PD98059, SnPP, cycloheximide • Agmatine increased Akt & ERK phosphorylation, • Agmatine increased HO-1& GCLc expression that were inhibited by yohimbine ketanserin, LY294002, & PD98059 • Agmatine induced Nrf2 transcription mediated by α2-AR, 5-HT2A receptors, Akt & ERK signaling pathways	Freitas et al., 2015
Neurotoxic; 6 h of restraint stress daily for 21 days.	Sprague-Dawley rats	50 mg / kg / day, i.p.	β-tubulin III staining, Agmatine &ADC levels	• Restrain stress decreased agmatine & increased ADC levels in mPFC, hippocampus, striatum, hypothalamus • Exogenous agmatine abolished increase in ADC levels • Stress induced morphological changes in hippocampus, mPFC were reduced by agmatine	Zhu et al., 2008a
Neurotoxic; four-point immobilization for 2 hour daily for 7 days.	Sprague-Dawley rats	50 mg/kg/day, i.p.	β-tubulin III staining, Agmatine & ADC levels	• Repeated immobilization increased plasma CSO, glutamate levels & caused morphological changes in hippocampus, mPFC • Agmatine abolished morphological effects • Increased ADC & agmatine levels	Zhu et al., 2008b

acute (1 h) and chronic (24 h) exposure. Agmatine treatment 60 min prior to the exposure of paclitaxel and cisplatin, showed a stronger neuroprotective effect at lower dose against induced neurotoxicity. The neuroprotective effect of agmatine was demonstrated with increased cell viability, improved antioxidant and reduced oxidant capacity, decreased caspase 3, caspase 9 and TNF-α in neuron culture (Binnetoglu et al., 2019).

2.4. Ischemic toxicity

In vivo effects of agmatine were elucidated in rodents to endure global and focal ischemia induced by bilateral carotid artery occlusion or intraluminal middle cerebral artery occlusion (MCAO) (Gilad et al., 1996a, 1996b). Reduction of ischemic infarctions size and loss of cerebellar neurons was observed on the fourth day. The delayed neuronal injury sometimes requires a prolonged period to develop and hence Feng et al. extended the time from injury to brain assessment to 22 days in hypoxic brain injury. In hypoxic-ischemia model in rat pups with ligated carotid arteries and hypoxic exposure (8% oxygen) for 2.5 h, there was no reduction in the efficacy of agmatine. The cortical levels of endogenous agmatine in hypoxic rats were increased 2- to 3-fold as compared to normal control and administration of agmatine (100 mg/kg, i.p.) 5 min after hypoxic ischemia. Cortical levels of agmatine, measured 3 h after insult were 50-fold higher as compared to hypoxic ischemic rat brains without agmatine treatment (Feng et al., 2002). The weight deficit of the ischemic hemisphere as well as neuropathological damage score were attenuated by about 50% with this treatment. Since hypoxic-ischemic model did not immediately cause histopathological changes in either cerebral hemisphere, and yet the agmatine elevations were seen in both hemispheres, the upregulation of agmatine was in response to brain stress rather than tissue damage (Fairbanks et al., 2001).

Further, agmatine treatment (100 mg/kg, i.p.) accelerated the recovery of motor performance and prevented the loss of motoneurons in the spinal cord during the 17 day study in rat spinal cord ischemia-reperfusion paradigm by balloon occlusion of the abdominal aorta (Gilad and Gilad, 2000a, 2000b). The extensive experiments were designed to examine and compare changes in the activities of arginine decarboxylation (the existence of arginine decarboxylase was not characterized by that time, hence the term *arginine decarboxylation activity* was used) and ornithine decarboxylase (ODC) during development and after ischemic brain injury (Gilad et al., 1996a, 1996b). The arginine decarboxylation activity increased transiently during brain development and global forebrain ischemia in parallel to ODC activity and the conclusion was in favor of ODC activity for decarboxylation of arginine and ornithine. However, pronounced decarboxylation activity in the membrane fraction during brain maturation, and reduced ODC activity in cytosolic but increased in membrane fractions was observed. These observations are in agreement with later finding that arginine decarboxylase (ADC) is a membrane bound enzyme (Tabor and Tabor, 1984). As a result of pathophysiological events the rate limiting enzyme in the agmatine's biosynthesis was enhanced suggesting biological and pathophysiological significance of agmatine. The endogenous agmatine levels in the penumbra also increased during common carotid artery occlusion in a mouse model (Hong et al., 2003).

In ischemic insult nitric oxide (NO) is elevated that promotes neuronal damage. NO is generated by Nitric oxide synthase (NOS) mediated sequential oxidation of guanidinium group in L-arginine. The structural resemblance between L-arginine, and agmatine, a decarboxylated arginine, makes them competitive substrates for NOS and hence agmatine serves as inhibitor of NOS (Auguet et al., 1995; Galea et al., 1996). This suggests that agmatine may offer neuroprotection against ischemic injury by interfering NO signaling. The hypothesis was tested by Kim et al. evaluated the effects of agmatine on ischemia-like insults *in-vitro* using primary cultured cortical neurons and *in-vivo* against transient focal ischemia in adult mice (Kim et al., 2004, 2016). Agmatine treatment (100 μM) in primary cortical neuronal cultures before or at the start of oxygen-glucose deprivation (OGD), or upon reperfusion reduced neuronal death by 25% when agmatine was presented during OGD, and this protection was associated with a reduced production of NO, decreased neuronal NOS (nNOS) expression and without affecting inducible NOS (iNOS). For *in-vivo* studies, agmatine (100 mg/kg, i.p.) treatment before ischemia, at the start of MCAO, at the start of reperfusion or after reperfusion markedly reduced infarct area in MCAO mice and the number of nNOS immunopositive cells was correlated with neuroprotection. Interestingly, immunoreactivity for iNOS was reduced only when agmatine was administered before and at the onset of MCAO. During the initial phase of ischemia, enhanced NO generated by eNOS maintains cerebral blood flow, while NO derived from nNOS is neurotoxic. However, no significant effect on blood pressure and blood flow was detected in Laser-Doppler flowmetry, therefore, possibility of mediation of effects with vascular physiology was negated. The generation of iNOS and the continued production of NO can contribute to neuronal injury arising in the late term of cerebral ischemia (Iadecola, 1997).

In transient global ischemia induced by vessel occlusion with simultaneous reperfusion and agmatine (100 mg/kg, i.p.) administration demonstrated significant protection of hippocampal neurons in CA1, pyramidal neurons in cortical layers III–V of cerebral cortex after 6, 24, 48, and 72 h of reperfusion. In addition, agmatine effectively suppressed endoplasmic reticulum dysfunction, and increased the expression of glucose-regulated protein 78 (Grp78), inhibited production NO generation by decreasing the expression of nNOS and iNOS and inhibited peroxynitrite generation indicated by nitrotyrosine levels, a marker of the interaction between NO and superoxide to form peroxynitrite (Mun et al., 2009, 2010a, 2010b). Evidence obtained in recent years has demonstrated that endoplasmic reticulum-mediated cell death plays an important role in cerebral ischemia (Ouyang and Giffard, 2012; Nakka et al., 2010).

Astrocytes, a type of glial cell in the CNS, play crucial roles during neuronal healing (Fitch et al., 1999)· The responses of astrocytes in ischemia are ambivalent including protection of neurons (storage of glycogen and possibly its provision to neighboring neurons as an alternative energy substrate of glucose, take up glutamate released by neurons in the early stage) and promotion of neuronal injury by releasing the accumulated glutamate in the late stage or propagation of the damaging molecules through their gap-junctions. Astrocytes were probed for their response to agmatine treatment (Lee et al., 2009). Primary cortical astrocyte culture was subjected to OGD model (less than 0.1% O_2 for 4 h) and followed by restoration (glucose at concentration of 5.5 mM for 20 h). Monitoring with LDH assay, annexin V by flow cytometric and Hoechst 33258-propidium iodide double nuclear staining, illustrated protective effect of agmatine on astrocytes promoted cell viability. This neuroprotective ability of agmatine in astrocytes may be connected to facilitation of activation and translocation of nuclear factor-kappa B (NF-κB). The effect of agmatine on NF-κB activation was opposite to the earlier study on retinal ganglionic cell where agmatine suppressed the phosphorylation and nuclear translocation of NF-κB (Hong et al., 2007). The specific role of NF-κB probably is tissue- or cell type-specific and may also depend on the types of stimuli.

Microglial activity is crucial in maintaining homeostasis against various neuronal injuries. Reactive microglia secretes neurotoxic proinflammatory cytokines (TNF-α, IL-1β) glutamate, free radicals and release large amount of NO (Chao et al., 1995). Excessive microglial activation and NO production destroy damaged neurons as well as healthy neurons. The control of NO production may be beneficial for neuroprotective effects in ischemia-induced oxidative toxicity (Iadecola et al., 1995). Microglial activation by lipopolysaccharide (LPS), a bacterial endotoxin, induces oxidative toxicity. LPS (*E.coli* 026:B6, 1 mg/ml) activation of microglial cells from rat cortical cell culture was assessed by the accumulation of nitrite in the culture supernatants

indicating production of NO through expression of iNOS (Abe et al., 2000). Although, agmatine had no effect on the expression of iNOS, it significantly suppressed the LPS-induced NO production in a concentration-dependent manner. In co-cultures of rat cortical neurons and microglia, LPS caused significant loss of neuron viability. The LPS neurotoxicity was not observed in the absence of microglia, and was completely blocked by the NOS inhibitor diphenyleneiodoium chloride. Importantly, the neuronal death induced by microglia-derived NO was significantly attenuated in presence of agmatine. To further understand neuroprotective effects of agmatine Ahn et al. focused on the activity of iNOS in microglial models of hypoxia and LPS induced toxicity in vitro (Ahn et al., 2011, 2012) as well as in vivo. The observations made on BV2 immortalized murine microglia 20 h after exposure to hypoxic conditions (oxygen level less than 0.1% for 2 h) along with 100 μM of agmatine exhibited attenuation in nitrite production and apoptic cytotoxicity. Suppression of BV2 microglial cell death and nitrite production was observed when cells were threatened with LPS and treated with agmatine.

In order to investigate association of NO signaling with in vivo neuroprotective effect of agmatine, the number of microglia in ischemic penumbra was counted by expression of ionized calcium binding adaptor molecule 1 (Iba1), a microglial marker and iNOS immunochemically. Agmatine treatment following 24 h of MCAO hypoxic injury decreased the number of cells with Iba1 and iNOS in striatum and cortex in rats (Ahn et al., 2011). The observation was supported by the effect of agmatine on microinjected LPS induced neuroinflammation in the corpus callosum of mice (Ahn et al., 2012). Intracerebrally injected LPS damaged and activated microglial cells whereas agmatine treatment decreased the activation of microglia and attenuated the expression of iNOS, TNF-α and IL-1β. Although in earlier report by Abe et al. agmatine failed to alter the expression of iNOS in cultured microglia, this study demonstrated that agmatine could directly influence expression of iNOS in vivo (Abe et al., 2000) Differences between cellular (cells from two-day-old rat neonates) and animal (adult mice) experiments might have contributed for the reported discrepancy.

Edema is the consequence of brain ischemia and the channel proteins, aquaporins (AQPs), AQPs-1, -4, and -9 are implicated in water movement during the formation and resolution of cerebral edema after ischemia in rodent brain. In MCAO stroke rats, treatment of agmatine (100 mg/kg, i.p.) diminished the disruption of blood-brain barrier (BBB) and vasogenic edema after 22 h following 2 h MCAO. It reduced brain swelling volume and water content in brain tissue 24 h after ischemic injury (Kim et al., 2010). This effect of agmatine was correlated to decreased expression of AQP-1 in endothelial cells in cortex, striatum and choroid plexus after cerebral ischemia. The expression of AQP-4 was not significantly altered however, a reducing trend was observed in agmatine treated animals. The effect on AQP-4 was endorsed later with observation that AQP4 positive cells decreased in agmatine treated rats with transient cerebral ischemia produced by MCAO (Wang et al., 2010).

Following the surgical MCAO, treatment of agmatine (100 mg/kg, i.p.) at the beginning of reperfusion and continued again once daily for next 3 post-operative days accelerated the recovery of motor and proprioception deficits alongwith prevention of brain infarction (Wang et al., 2010). Agmatine decreased cerebral water contents, number of AQP-4 and GFAP- positive cells, neuronal apoptotic cells and iNOS expressing cells. The improved neurological outcomes were largely due to protection against apoptosis, gliosis, NO toxicity and cerebral edema. In the same animal model, agmatine treatment 5 min after beginning of reperfusion and again once daily for the next 3 post-operative days, attenuated the vasogenic (signal changes in T2-weighted images) and cytotoxic (intensities in diffusion weighted images) edema as well as cerebral ischemia and infarct (Huang et al., 2013). To further corroborate volume transfer constant and volume fraction of extravascular extracellular space were significantly lower in the agmatine-treated group than in the control group. The factor VIII, being an angiogenesis-related factor supporting formation of new microvessels, is a hallmark tissue response to ischemic injury and reduced expression of factor VIII-positive cells following agmatine treatment which may be a result of lowered need of angiogenesis with controlled BBB disruption in ischemia-reperfusion model (Ahn et al., 2015). In a transient ischemic cat model designed to simulate the clinical situation of hyperacute ischemic stroke, agmatine (100 mg/kg) administered intravenously decreased total number of TUNEL-positive cells and the area of severe ischemic neuronal damage as compared with the control ischemic cats that tolerated MCAO for one hour (Kim et al., 2006a, 2006b).

In all the discussed experiments, agmatine was employed exogenously in vitro or administered intraperitonially in animals and the role for endogenous agmatine is not accounted for protective effect. In an attempt to elevate the agmatine concentration endogenously, the study was undertaken to determine whether the expression of human ADC (hADC) and consequent increase of agmatine can prevent the cells from oxidative injury (Moon et al., 2010). RT-PCR and western blot analysis showed the specific and strong detection of hisADC genes in hisADC PT67 transfected cells as compared to normal control and pLXSN transfected PT67 cells. Immunocytochemical analysis demonstrated hisADC expression in the cytoplasm of *v*hisADC-NIH and high concentration of agmatine in the vhisADC-NIH was confirmed following H_2O_2 injury. The induced agmatine synthesis on the retroviral gene delivery prevented *v*hisADC-NIH from H_2O_2 injury suggested by decrease in LDH leakage into the medium and decreased number of propidium iodide positive cells during injury compared to control group. The expression of untagged hADC was evaluated in the cortical embryonic neural stem cell (NSC) infected with *v*hADC on being challenged with H_2O_2 injury (200 μM for 15 h) (Bokara et al., 2011). LDH leakage and intracellular reactive oxygen species formation were about 2-fold reduced whereas DNA fragmentation, chromatin condensation, and expression of apoptotic proteins such as p53, Bax, and caspase-3 cleavage were significantly decreased in ADC-NSCs when compared with control NSCs and NSCs infected with mock vector, suggesting the prevention of apoptotic cell death following H_2O_2 injury (Bokara et al., 2016). The hADC gene delivery into neural progenitor cells (mNPCs) triggered the expression of neural cell adhesion molecule (N-CAM) and microtubule-associated protein-2 (MAP-2), a neuronal marker (Bokara et al., 2016). Neurite outgrowth was significantly longer in hADC infected cells whereas the neurotrophic signal, brain-derived neurotrophic factor (BDNF) aided in the neuronal commitment, differentiation and maturation of hADC-mNPCs through phosphoinositide 3-kinase (PI3K) and extracellular signal regulated kinase I and II (ERKI/II) activation. The induction of neuron-like differentiation is believed to be regulated by the expression of glycogen synthase kinase3β (GSK-3β) and Wnt/β-catenin signaling pathways. Overall these findings suggest that hADC gene delivery favors cell fate commitment of mNPCs towards neuronal lineage and neuroprotection. Agmatine mediated enhancement of neurogenesis by increasing ERK1/2 expression, and suppresion of astrogenesis by decreasing bone morphogenic protein BMP-2, -4, and Sma and Mad 1,5,8 proteins' expression in subventricular zone neural stem cells have been reported (Song et al., 2011) (Sma and Mad i.e. SMAD proteins are the main signal transducers for receptors of TGFβ). The intracellular levels of agmatine, its precursor, arginine and byproduct putrescine, increased about 11-fold under the normal and oxidative stressed conditions in OGD model on primary cultured cortical astrocytes transduced with hADC-expressing retroviral vector. The hADC-overexpressing cells remained undamaged in OGD for 4 h and rescuing property was gradually potentiated with as restoration time proceeded for up to 10 h. The effect of endogenous agmatine seemed to be related to inhibited expression of iNOS and MMPs (Hong et al., 2014).

Ischemic preconditioning, still unclear at molecular level, is one of the most important endogenous mechanisms that protect the cells

against ischemia-reperfusion injury. It is suggested that agmatine may be a component of the ischemic tolerance response. The suggestion was based on studies in rats subjected to ischemic preconditioning by MCAO for 10 min three days before full ischemic offense by MCAO for 60 min in comparison to rats that suffered full insult of MCAO for 60 min without preconditioning or to normal rats (Kim et al., 2017a, 2017b). Thirty minutes after preconditioning, brain agmatine levels were increased and intensified gradually to about 3.5 folds at 3 days. The plasma agmatine levels increased synchronous to brain 30 min after preconditioning and increase was about ten-fold. On the other hand, the level of agmatine in liver 30 min after preconditioning reduced to about 20% compared with normal control. The upregulation of ADC was observed in brain as well as liver. The observations suggest that liver ADC actively metabolized L-arginine and secreted agmatine into the plasma after the early phase of preconditioning. This may be due to remote ischemic preconditioning where a brief ischemia of one organ confers protection to distant organs without direct stress to the organ. During full ischemic injury, agmatine level in the brain remained elevated in preconditioned rodents, attained the peak at 2 h of reperfusion and decreased gradually at 23 h that was still more than normal. However, in the non-preconditioned rodent group, the agmatine level increased with full injury, displayed peak at 1 h of reperfusion and increased gradually at 23 h of reperfusion. The time dependent variation hints at early and delayed protective response in preconditioned and non-preconditioned group. However, the neurological outcome as reduction in brain edema, infarct volume, iNOS and nNOS expression were superior in preconditioned animals than injury-only group (Table 3).

2.5. Spinal cord injury

The pathological sequelae associated with traumatic spinal cord injury (SCI) or brain injury where NMDA receptor and NOS play important roles in neuronal damage hint at the probable protection by agmatine. Hence the effects of agmatine have been studied in SCI models with contusion, compression, and transection injuries. Yu et al. employed contusion SCI produced at thoracic vertebra T10 by 10 g weight applied from a height of 12.5 mm in rodents. The first dose of agmatine (100 mg/kg) given 30 min after and later daily for 14 days significantly improved open-field locomotor function on Basso-Beattie-Breshnahan rating scale and provided greater tissue sparing from damage for upto 30 days following cessation of treatment (Yu et al., 2000). In clip compression model of SCI, the effect of agmatine (50 and 100 mg/kg/day for 10 days) was investigated in rats with laminectomy (T7-10) only and laminectomy with clip compression (Kotil et al., 2006). No statistically significant intergroup difference in motor function existed at any post injury interval between 50- and 100-mg/kg/day agmatine-treated rats. Administration of agmatine (100-mg/kg/day) reduced the NO levels as compared to animals with laminectomy only and laminectomy with clip compression. However, no significant intergroup difference in the reduction of NO levels was found between rats treated with 50- and 100-mg/kg/day doses of agmatine.

The co-localization of agmatine and imidazoline binding sites in several brain areas and interaction of agmatine with IBS to comprehend several pharmacological effects are explored abundantly. In view of this, very recently, the possibility of functional recovery from SCI by agmatine being attained through I-BS was tested (Dixit et al., 2018). In compression (5 g) of SCI following laminectomy at T10-12, injury resulted in hind-limb muscle paralysis in mice and effect of agmatine was measured by hind-limb motor function scoring system. Considerable recovery was noticed on 14th day with agmatine treatment for 14 days in dose dependant mannner. The effect of agmatine on SCI was significantly potentiated by I1-BS agonist, clonidine and I2-BS agonist, moxonidine. In contrast, it was completely blocked by pretreatment with I1-BS antagonist, efaroxan and I_2-BS antagonist, idazoxan. The results thus exhibited accomplishment of agmatine effects, at least partly, through I-BS. The disagreement of these results to Olmos et al. that I_2-BS were not involved in the neuroprotective effects of agmatine might be due to differences in experimental models (as in vivo and in vitro) and type of injury (Olmos et al., 1999). In complete transection SCI model at T9 in mice, agmatine treatment improved in locomotor function, especially surface righting reflex (Kim et al., 2011). The neuroregeneration was supported by reducing the collagen scar area (a physical barrier to axon regeneration) by decreasing the expression of transforming growth factor β-2 (TGFβ-2 that regulates glial/collagenous scarring) and increasing the expression of BMP-7 for 4 weeks after SCI. More detailed evaluations on inhibition of scar formation and role of BMPs in bringing about the neuroregeneration effects of agmatine were done in mice in which compression SCI was produced by a 15 g/mm^2 weight for 1 min at T9 segment (Park et al., 2013). Agmatine (100 mg/kg/day, i.p.) treatment within 1 h after SCI till 35 days resulted in improvement in locomotor recovery and bladder function. Immunohistochemical staining around the lesion sites, demonstrated reduction in scar area, inhibition in demyelination, reduction in neuronal loss. The results suggested that the total number of surviving neurons and oligodendrocytes were increased while the astrocytes population was decreased and the total number of surviving cells almost reached to normal in the agmatine treated mice. This corresponded with dramatic increase in BMP-2/7 expressions in neurons and oligodendrocytes. On the other hand, BMP-4 expressions were significantly decreased in astrocytes and oligodendrocytes around the lesion site. Since the glial scar formation consists predominately of reactive astrocytes, the reduction of gliosis and glial scar formation is thought to be controlled with the decreased expression of BMP-4 in astrocytes. The immunostaining results with 5-HT antibody (represents serotogenic fiber staining) also showed increase in the caudal 5-HT fiber density in the agmatine treated group and the morphology of the serotogenic fibers were almost similar compared to normal control mice. Agmatine treated mice showed increase of myelin and neurons stained cells, higher number of NeuN$^+$/NF$^+$ cells, less broken myelin sheaths and compact myelination around the lesion site. Therefore anticipated that agmatine treatment preserve the formation of dendrites and cell bodies of neurons around the lesion site in the injured spinal cord suggesting usefulness of agmatine for the attenuation of neuronal damage and support for neuronal survival.

BMP-2 is known to reduce M1 macrophage under inflammatory status, hence effect of agmatine on macrophage phenotypes, a key cellular component in neuroinflammation (type M1 induces a pro-inflammatory response and neurotoxic whereas type M2 inspires an anti-inflammatory response and promote axonal regeneration) was studied. The treatment of agmatine increased M2 macrophages (counted as CD206$^+$ & ED1$^+$ cells) on the caudal side to epicenter 1 week after SCI. Agmatine (100 mg/kg/day, i.p.) administered daily for 6 days (beginning the day after SCI), significantly increased only the BMP-2 expression in rats with SCI by contusion between T9 and T10 in rats (Kim et al., 2017a, 2017b). In addition, the expression of M2 macrophage markers, arginase-1 and CD206 mRNA as well as IL-10 mRNA was increased following agmatine treatment.

The local brain inflammatory response is exacerbated by the entry of peripheral immune cells into the brain and increased circulating proinflammatory cytokines. Under the compromised BBB activity, expression of adhesion molecules by vascular endothelial cells allows the entry of peripheral immune cells, including macrophages, neutrophils, leukocytes and T/B cells and increase in number of these cells represents an adaptive response of white pulp of the spleen to blood borne antigens. Agmatine do affect the immune response in the spleen after transient cerebral ischemia. Transient cerebral ischemia (23 h) demonstrate reduced white pulp area and increased number of CD11b$^+$ macrophages and CD4$^+$, CD25$^+$ regulatory T cells in the spleens of experimental animals. Agmatine treatment (100 mg/kg, i.p.) diminished the contraction of white pulp and decreased number of CD11b$^+$ macrophages as well as CD4$^+$,CD25$^+$ regulatory T cells (Uranchimeg

Table 3
Neuroprotective effects of agmatine against ischemic/hypoxic/OGD toxicity.

Injury type, By	In	Agmatine	Observed activity Funtional recovery measurement	Observation	Ref
Ischemic injury; MCAO (focal), BCAO (global)	Adult male mongolian gerbil, Male Wistar rats	10, 50, 100 mg/kg, i.p.	Infarct size, Motor function deficits, (neurological score)	Dose-dependent prevention of delayed neuronal cell death in hippocampus after forebrain ischemia	Gilad et al., 1996a
Hypoxic-ischemic; MCAO, 2.5 h of hypoxia 8% oxygen	7-day-old Sprague–Dawley rats pups	50, 100, 150 mg/kg i.p 5 min after reoxygenation & once daily thereafter for 3 day	Neuropathologic grading of brain, Hemispheric weight, Agmatine & nitrite/ nitrate metabolite levels	• Hypoxia elevated brain agmatine by 2-3 fold • Agmatine 100 mg/kg elevated brain agmatine by 50-fold at 3 h • blocked brain NO metabolites at 6 h	Feng et al., 2002
Hypoxic; OGD, O_2 < 0.2%, 1h	10–11 day primary mice cortical neuron culture	100 μM, 30 min before OGD, at the start of injury, & at reperfusion	Cell count, nNOS & iNOS expression & activity, NO production	• Reduction in cell death by agmatine presence in OGD insult • Protection associated with a reduction of NO & nNOS, but not iNOS.	Kim et al., 2004
Ischemic; MCAO, 2h	Male ICR mice	100 mg/kg i.p. 30 min before, at start of MCAO, at start of reperfusion, or 2 or 5 h after reperfusion	TTC staining (Infarct size), Cerebral blood flow (CBF), nNOS & iNOS expression & activity, NO production	• Reduction in infarct area in all treatments except when treatment was delayed 5 h. • Reduction in infarct size was unrelated to change in CBF • immunoreactivity for iNOS reduced with agmatine treatment before & at the start of MCAO	Kim et al., 2004
Hypoxic; OGD O_2 < 0.1%, 6h	Primary culture of murine cerebral endothelial cells	100 μM, 30 minutes before OGD at the start of injury and at the reperfusion	MMP-2, MMP-9 expression, NO production, eNOS expression	• Ischemia increased expression of MMP-2, -9 & decreased NO & expression of eNOS • Agmatine attenuated MMP-2 &-9 expression & increased the eNOS expression • Effect of agmatine on MMP-9 expression but not on MMP-2 were suppressed by NAME	Yang et al., 2007
Hypoxic ; OGD, O_2 < 0.1%, 4 h Restored for up to 20 h	Primary mice astrocyte culture	100 μM at start of injury	LDH assay, H & PI staining, Annexin V flow cytometric assay, NF-κB expression & phosphorylation	• Decreased viability of astrocytes following OGD & OGD-restoration • Agmatine increased cell viability & induced NF-κB translocation into nucleus.	Lee et al., 2009
Hypoxic; O_2 < 0.1%, 2 h	BV2 microglia	100 μM	LDH assay, H& PI staining, Nitrite determination	• Decrease in hypoxia-induced cytotoxicity & nitrite production with agmatine	Ahn et al., 2011
Hypoxic; OGD O_2 < 0.1%, 6h	bEnd.3 cell culture transfected with human hADC gene	100 μM at the reperfusion of 18 h.	LDH assay, Agmatine, NO, eNOS, MMP-2, MMP-9, ATF3 expression, Subcellular localization of ATF3	• Effects produced by endogenously elevated & exogenous agmatine • Decreased cell death, increased NO production • Exogenous agmatine & retrovirus induced endogenous agmatine inhibited MMP-2 & -9 expression regulated by eNOS, NO, ATF3 expression & decrease in eNOS expression	Jung et al., 2010
Ischemic; MCAO, 1.5 h	Male Sprague–Dawley rats	100 mg/kg i.p. at the start of MCAO	Cerebral blood flow, Iba1, iNOS expression	• Agmatine decreased activity of microglia & iNOS expression	Ahn et al., 2011
Ischemic; MCAO, 1 h	Cats	100, mg/kg i.v just after recanalization	MRI, TUNEL staining, H&E staining	• Agmatine treatment, prevented increase in number of TUNEL-positive cells in the areas of reperfusion hyperemia	Kim et al., 2006a
Ischemic; MCAO, 2 h	Mice	100 mg/kg i.p. at start of reperfusion	MMP-2 & MMP-9 expression Agmatine levels at 6 & 24 h	• Agmatine reduced expression of MMP-2,-9 in cortex, striatum. • MMP-2 & -9 expressions were markedly lower in blood vessels	Kim et al., 2008
Ischemic; MCAO, 2 h	Male ICR mice	100, mg/kg i.p. at start of reperfusion	TTC staining, H&E staining, Evans blue extravasation, AQPs expression, AQP-1, -4, & -9 positive cells	• Agmatine reduced brain edema, infarct volume, BBB disruption • Decreased expression of AQPs 22 h after ischemia	Kim et al., 2010
Ischemic; MCAO, 1.5 h	male Sprague-Dawley rats	100 mg/kg/day, for 4 days	Motor, proprioception functions, TTC staining, TUNEL assay, Water content, AQP-4 positive cells, GFAP-positive cells, iNOS expression	• Agmatine accelerated recovery of motor, proprioception deficits • Prevented brain infarction, edema, gliosis, apoptosis, iNOS expression	Wang et al., 2010

(*continued on next page*)

Table 3 (*continued*)

Injury type, By	In	Agmatine	Observed activity Funtional recovery measurement	Observation	Ref
Ischemic; MCAO, 1 h	Adult male Sprague-Dawley rats	100, mg/kg i.p.	TTC staining Expression of CD11b + macrophages, CD4 + CD25 + regulatory T cells, CD8+ cytotoxic T-cells	• After 23 h of ischemia, reduction in white pulp area in spleen & increase in number of CD11b + macrophages, $CD4^{+}CD25^{+}$ regulatory T cell, CD8+ cytotoxic T lymphocytes • Agmatine treatment prevented these effects except of CD8+ cells • Possible protective effect by minimizing neuroinflammation & prevention of depression of immune system	Uranchimeg et al., 2010
Global cerebral ischemic, 4-VO, 20 min	Adult male Sprague-Dawley rats	100 mg/kg i.p. with reperfusion	H&E staining, TUNEL staining, eNOS, iNOS, MMP-2, -9 expression, eNOS positive cells	• Ischemia increased MMP-9, iNOS & decreased eNOS espression • Agmatine reduced MMP-9, iNOS expressions in hippocampus, cortex & increased eNOS expression	Mun et al., 2010a
Global cerebral ischemic, 4-VO, 20 min	Rats	100 mg/kg, i.p. reperfusion	H&E staining, TUNEL assay, Expression of NOS, Grp78.	• Agmatine prevented delayed neuronal cell death in hippocampal neurons of CA1, cortical layers III–V • Induced Grp78 expression • suppressed expression of nNOS, iNOS& peroxynitrite formation • Protects ER-structure from ischemia	Mun et al., 2009, 2010
Ischemic; MCAO, 20 min	Rats	100 mg/kg, i.p. reperfusion	Body weight & mortality H&E staining, TUNEL assay	• Agmatine protects pyramidal neurons in cortical layers III–V of cerebral cortex	Mun et al., 2010a
Ischemic; MCAO, 0.5 h	Streptozotocine- induced diabetic Sprague-Dawley rats	100, mg/kg i.p at the beginning of reperfusion	Neurobehavioral assessment Regional CBF, TTC staining, microPET imaging NF-κB, IL-1β, TNFα positive cells Expression of HMGB1, RAGE, TLR-2, & -4, inflammatory cytokines	• No difference in regional CBF • Agmatine reduced infarct volume, preserved glucose metabolism • AGM ameliorates expression of NF-κB, IL-1β, TNF-α & microglial activation • Downregulated the protein levels of HMGB1, RAGE, TLR2, TLR4, NF-κB, IL-1β, & TNF-α. at 24 hours after reperfusion • Possible association of neuroprotective effect with suppression of neuroinflammation.	Kim et al., 2015
Ischemic; MCAO, 0.5 h	Streptozocine-induced diabetic rats	100 mg/kg i.p.the beginning of reperfusion	Motor function, Infarct & edema volume Caspase-3 activity TUNEL staining NOS & iNOS expression	• Agmatine decreased infarct & edema volume, caspase-3 & TUNEL-positive cells, NOS & iNOS expression • Possible association with. reduction in NOS expression & antiapoptosis	Cui et al., 2012
Ischemic; MCAO, 1.5 h	Adult male Sprague–Dawley rats	100, mg/kg i.p. 5 min after beginning of reperfusion & once daily thereafter for 3 days	MRI: T2WI, DWI, CE-T1WI, during 3 -72 h, Infarct volume, Evans blue extravasation assay, Nissl staining	• Agmatine-reduced infarct volume, Evans blue extravasation, attenuated decrease in Nissl-positive cells	Huang et al., 2013

et al., 2010). The brain infarction area was significantly reduced (5.51 ± 1.63% of the whole brain) in agmatine treated animals as compared to control (15.02 ± 4.28%). These finding signify that agmatine treatment may reduce brain infarction by reducing neuro-inflammation and lowers the risk of post-injury infection due to weakened immune system after stroke.

In rodent MCAO model, cellular release of high-mobility group box 1 protein (HMGB1) was observed early following ischemic reperfusion (Kim et al., 2006a, 2006b, Qiu et al., 2008). Importantly, stroke patients demonstrate high levels of serum HMGB1 compared with healthy control subjects (Muhammad et al., 2008). Its neutralization by anti-HMGB1 antibody and pretreatment with antagonist of HMGB1 at receptor for advanced glycation end products (RAGE) ameliorated ischemic brain damage in stroke patients. HMGB1 protein promotes pathogenesis of inflammatory actions once it gets into extracellular compartment. Therefore, early presence of HMGB1 into the extracellular compartment after ischemic injury may contribute to the initial inflammatory response via cascade of RAGE, toll-like receptor-2 (TLR2), and TLR4, transcription factor NFκ-B, tumor necrosis factor (TNF)-α, cyclooxygenase-2 (COX-2), iNOS, pro-inflammatory cytokines as IL-1β, IL-6 (Muhammad et al., 2008; Iadecola and Anrather, 2011; Broad et al., 2007; Marsh et al., 2009).

In connection, Kim et al. studied effects of agmatine on cytoplasmic translocation of HMGB1 and associated biochemical consequences. Agmatine (100 mg/kg, i.p.) treatment in normoglycemic and streptozotocin-induced diabetic rats subjected to MCAO followed by reperfusion improved the neurobehavioral activity and motor function in streptozotocin-induced diabetic rats at 24 and 72 h after reperfusion. The increase in baseline inflammatory cytokines before the ischemic event probably led to increased infarct size in diabetic brains compared with the normal brains after reperfusion. The infarct size was reduced in agmatine-treated diabetic rats compared with diabetic rats without agmatine treatment. Agmatine reduced the expression of HMGB1, RAGE, TLR2, and TLR4 while decreasing the level of TNF-α, NF-κB, and IL-1β anticipating the anti-inflammatory mechanism underlying the neuroprotection offered by agmatine (Kim et al., 2015). The reduced infarct size was associated with a decrease in apoptosis and NOS expression in similar animal model of neurotoxicity (Cui et al., 2012) (Table 4).

2.6. Traumatic brain injury

Along the lines of SCI models, a few studies are published for traumatic brain injury (TBI) model. Agmatine (50 mg/kg, i.p.) administered immediately after the onset of lateral fluid percussion injury in rat brain attenuated the TBI-induced increased hippocampal levels of lactate to pyruvate ratio, glycerol, intracranial hypertension, cerebral hypoperfusion, cerebral infarction as well as motor and proprioception deficits, reducing the excessive accumulation of both glutamate and nitric oxide (Kuo et al., 2007). Further, extension of this study suggested that agmatine therapy may attenuate TBI in rats via reducing neuronal and glial apoptosis, promoting angiogenesis, neurogenesis (Kuo et al., 2011). In diffuse brain injury created by Marmarou's impact-acceleration, agmatine (100 mg/kg, i.p.) displayed significant improvement in axonal, vascular, neuronal injury and beneficial effect in diffuse axonal damage (Sengul et al., 2008). Agmatine treatment after TBI produced by cold injury to the cerebral primary motor cortex of rats reduced edema by suppressing the expression of AQP-1, -4, -9 and reduced apoptotic cell death by suppressing the phosphorylation of MAPKs and increasing the nuclear translocation of NF-κB (Kim et al., 2015).

While in most of the studies the effect of agmatine in central neuronal injury was investigated; only a few studies have demonstrated the activity of agmatine on peripheral nerve injury. In an experimental model of peripheral nerve injury in rats for axonotmesis (i.e. second degree injury where axon is damaged but the surrounding connective tissue remains intact) and neurotmesis (i.e. third degree injury where axon and connective tissue are damaged) of sciatic nerve, agmatine (50 mg/kg/day for 10 days) had positive effects on recovery in distal part of traumatic nerve in both the models with decrease in axonolysis, axon degeneration and edema (Sezer et al., 2014).

The treatment of agmatine (100 mg/kg, i.p.) daily for 4 days after transection of mandibular branch of the facial nerve, generating a gap of 3 mm, either after unsutured or sutured vein graft reconstructions resulted in accelerated rate and degree of functional recovery by 65 days as observed by both vibrissae movement and electrophysiological recording (Berenholz et al., 2005). The maximal values of vibrissae motion grade were observed already by 25 days as compared with 65 days postoperative in the comparing groups. After facial nerve injury, agmatine was shown to accelerate functional recovery even at a dose 10 times lower (i.e., 10 mg/kg) than dose where robust functional recovery was seen.

Encouraged by the preclinical results for neuroprotection offered by agmatine, clinical trial of agmatine (ClinicalTrials.gov Identifier: NCT00405041) was carried out in patients of herniated lumbar disc-associated radiculopathy, commonly termed as sciatica that results from nerve damage caused by pressure on spinal lumbar nerve roots (Keynan et al., 2010). From open-label, dose-escalating, non-randomized study was performed to assess the safety, dose and side effects of agmatine sulfate. In randomized double-blind, placebo-controlled trial the sciatica patients were randomized to get 2.67 g agmatine per day for 14 days as an add-on to conventional treatments, or to a placebo add-on (In the treatment group participants assigned 51 and analyzed 31; In the placebo group participants assigned 48 and analyzed 30). Participants in agmatine treatment group experienced significantly greater pain relief and improved quality of life, as compared to the placebo-treated group and were without any adverse effects. With the proof-of-concept of agmatine's beneficial effects, open label study to evaluate the effectiveness of agmatine sulfate in people with painful small fiber neuropathy (ClinicalTrials.gov Identifier: NCT01524666) where agmatine (3.67 g per day for 2 months) as an add-on to conventional treatments, resulted in reduction in neuropathic pain and improved autonomic function (Tohidi et al., 2014). The safety of such high daily recommended dosage of agmatine has been evidenced even on long term consumption. The self-study, where a dose of 3 capsules of 445 mg of agmatine sulfate twice a day was (daily dose of 2.67 g) consumed by a couple of authors for at least 5 years demonstrated absence of any adverse effects on hematology, blood chemistry and urine analysis measures (Gilad and Gilad, 2014). The authors noted the absence of any record of adverse events related to high-dose agmatine sulfate regimen on postmarketing surveillance after its introduction as neutraceutical where 1015 individuals (46% women and 54% men) for periods ranging from 3 weeks to 3 years. Similarly, no reports of adverse events so far has been reported since the introduction of agmatine sulfate to commerce for body builders, based on unsubstantiated claims, in 2007 at recommended dosage up to 1.0 g/day and later, starting in 2012, at up to 2.0 g/day. However, the role of agmatine in altering feeding behavior in several pathological conditions like bacterial infections, stress, anorexia nervosa, obesity, cachexia and alcohol misuse, have also been demonstrated (Taksande et al., 2011, 2015a, 2015b, 2017; Benítez et al., 2018). The orexogenic behavior observed was not associated with weight gain and so differential physiological role of agmatine as mediator/regulator of feeding behavior and satiety is foreseen (Table 4).

2.7. Retinal ganglion cell protection

Agmatine can be presumed to have neuroprotective effects on retinal ganglion cells (RGCs) since it offers neuroprotection to various neuronal cells. With this presumption, Hong et al. examined effects of agmatine on apoptosis of undifferentiated immortalized rat RGCs (RGC-5) exposed to hypoxia (5% O_2) (Hong et al., 2007). The presumption

Table 4
Neuroprotective effects of agmatine in spinal cord injury and traumatic brain injury.

Injury type, By	In	Agmatine	Observed activity Funtional recovery measurement	Observation	Ref
Traumatic; Marmarou's impact-acceleration model	Adult male Sprague-Dawley rats	100 mg/kg/day i.p. for up to 4 days	Axonal, neuronal, vascular damage	• Agmatine treatment of 1 or 3 days & evaluation after 4 days did not present significant differences between treated & control groups, but evaluation after 8 days revealed a significant improvement in treatment group	Sengul et al., 2008
TBI, Traumatic; Elapse of 7-days	Adult male Sprague-Dawley rats	100 mg/kg i.p. 5 min after TBI & once daily thereafter for 3 days	Motor function deficit TTC staining, BrdU staining, NeuN-TUNEL assay, Caspase-3 assay Cells positive for VEGF, GFAP, Iba1, NeuN, n-NOS	• Agmatine attenuated TBI-induced motor function deficits, cerebral infarction • Reduced TBI-induced neuronal loss (NeuN-TUNEL double positive cells), glial loss (GFAP-TUNEL double positive cells), apoptosis (increased TUNEL-positive, caspase-3- positive cells), neuronal loss (NeuN-positive cells), gliosis (GFAP-positive cells; Iba1-positive cells), neurotoxicity (n-NOS-positive cells, 3-NT-positive cells), promoted angiogenesis (BrdU/endothelial cells; VEGF positive cells); neurogenesis (BrdU/NeuN positive cells)	Kuo et al., 2011
TBI, Traumatic; Cold-injury of the cerebral cortex Elapse of 1,2, 7-days	Adult male Sprague-Dawley	100 mg/kg i.p. 30 min after TBI and once daily until the end of the experiment	Evans Blue extravasation, TUNEL assay, Expression of NF-κB, MAPKs, AQPs, p-ERK p-JNK, p-P38, Motor functions	• Agmatine reduces brain edema after TBI • Suppressing expression of AQP-1, 4, 9. phosphorylation of MAPKs • Increases nuclear translocation of NF-κB	Kim et al., 2015
spinal cord ischemia model (balloon occlusion of abdominal aorta)	Wistar rats	100 mg/kg i.p. 5 min at start of reperfusion & once daily thereafter for 3 days	Motor performance Motoneuron cell counts	Agmatine accelerated recovery of motor deficits observed upto 17 days & prevented loss of motoneurons in the spinal cord	Gilad and Gilad, 2000a
SCI, Contusion	Female Sprague-Dawley rats	100 mg/kg/day, 30 min after SCI & for 14 days	Open-field locomotor function Tissue damage area	Improves locomotor function & reduces tissue damage for up to 44 days after SCI	Yu et al., 2000
SCI, Compression	Wistar albino rats	50 or 100 mg/kg/day i.p. 5 min after SCI & daily for 10 days	Iinclined-plane test, Motor function score, NO, MDA determination	• Agmatine improved functional recovery • Agmatine reduced NO & MDA levels	Kotil et al., 2006
Spinal Cord Injury, Complete transection	Male ICR mice	100 mg/kg/day i.p. 5 min after SCI & daily for 4 weeks	Open-field locomotor function Surface righting reflex test, Expression of BMP-7, TGFβ-2 positive cells, Collagen scar area	• Agmatine improved the surface-righting reflex. • Reduced collagen scar area • Reduced expression of TGFβ-2, increased expression of BMP-7	Kim et al., 2011
Spinal cord injury, Compression	Male ICR mice	100 mg/kg/day within 1 hour after SCI daily for 35 days	Behaviour, Bladder function, Luxol fast blue staining, Expression of MAP-2, BMP- 2/4/7 MAP-2, NeuN, NF NG2, Olg2 GFAP	• Agmatine improved locomotor recovery, bladder function • Inhibited demyelination events, gliosis, glial scar formation, loss of neurons, oligodendrocytes & formation of astrocytes • Increased BMP-2/7 expressions in neurons, oligodendrocytes & decreased in astrocytes • Decreased BMP-4 expression in astrocytes	Park et al., 2013
Axonotmesis or neurotmesis in sciatic nerve	Sprague-Dawley rats	50 mg/kg/day i.p. for 10 days	H & E staining for axonolysis, axon degeneration, edema, ranking	• 15 days after injury, histopathologic parameters showed recovery from injury in agmatine treated group in both models of peripheral nerve injury	Sezer et al., 2014

proved to be true when effects of agmatine 100 μM were compared to those of brain-derived neurotrophic factor (BDNF, 10 ng/ml), a well-known protective neurotrophin for RGCs as well as several molecular pathways associated with neuroprotective effects were observed. After 48 h of hypoxic culture, cell loss determined by LDH assay was 52.3%, which reduced to 25.6% and 30.1% in presence of agmatine and BDNF, respectively. The observed cell loss was primarily apoptotic and the total expression of mitogen-activated protein kinases (MAPKs; JNK, ERK p44/42 and p38) and NF- κB was not influenced by hypoxic injury. However, the phosphorylation of these proteins was increased. Agmatine reduced the phosphorylation of JNK and NF- κB, while BDNF suppressed phosphorylation of ERK and p38 implicating JNK and NF-κB signaling pathways in the neuroprotective effects of agmatine against hypoxia-induced retinal ganglion cell damage in RGC-5 cells. Next study was carried to elaborate the neuroprotective effects of agmatine on differentiated RGC-5 cells, before exposure to hypoxic damage (Iizuka et al., 2008). The pretreatment with agmatine (upto 100.0 μM) prior exposure to hydrogen peroxide as an oxidative stresser increased cell viability and attenuated apoptosis characterized by DNA fragmentation as determined by TUNEL assay. Agmatine also displayed the protection against TNF-α-induced apoptosis in RGC-5 cell line (Hong et al., 2009). The reversal of experiment to find effect of agmatine on TNF-α release by RGC-5 cells demonstrated significant reduction TNF-α level (Hong et al., 2008).

The presence of α_{2A}-adrenergic receptors have been identified on both undifferentiated and differentiated RGC-5 s (by succinyl concanavalin-A) (Wheeler and Woldemussie, 2001). The protective effects of agmatine (100 μM) pretreatment on RGC-5 s exposed to 1.0 mM H_2O_2 were completely abolished by 10 nM yohimbine but only partially decreased by 100 μM NMDA (Iizuka et al., 2010). Even the powerful NMDA antagonist, MK-801 did not reduce the RGC-5 cell death induced by H_2O_2. These results suggest that agmatine pretreatment may rescue RGCs from oxidative stress mainly through α_{2A} signaling rather than through the NMDA receptors. Data supporting the protection of RGCs by agmatine prompted the investigation of such effects in animal model of retinal ischemia–reperfusion-induced retinal toxicity (mimicking retinal ischemia and glaucoma associated with retinal ischemia) (Dastan et al., 2009). Ischemia induced by an elevated intraocular pressure (IOP) produces pathological features almost identical to those seen in patients after central retinal artery or ophthalmic artery occlusion. In guinea pigs transient acute ocular ischemia was achieved by cannulating the anterior chamber and IOP was increased to 150 mmHg for 90 min. Provided that agmatine reaches the brain following intraperitoneal administration, it may also reach the eye and exert its effect (Piletz et al., 2003). Hence, agmatine (50 mg/ml, i.p.) was administered before 45 min of ischemia (once or twice and infusion of agmatine was repeated at the same doses with a 12 or 24-h reperfusion period). The retinal thickness, thiobarbituric acid reactive substance (as indication of lipid peroxidation) and NO levels in the animals administered with agmatine during ischemia–reperfusion were significantly lower than control.

A method of agmatine use or a pharmaceutically allowable salt thereof and a pharmaceutical composition comprising the same are disclosed in United States patent (Seong et al., 2011). Additional evidence on the protection of RGC claims that agmatine prevents TNF-α induced apoptosis of RGCs by suppressing hypoxic induction activity of c-Jun N-terminal Kinase (JNK) and Nuclear Factor-kappa B (NF-κB). Further, it is suggested that it can effectively cure or prevent eye diseases, preferably including glaucoma, retinopathy and optic neuropathy. In the event of ocular application of agmatine (1 mM) solution in mice before occluding the ophthalmic artery by MCAO decreased proportion of apoptotic cells in the retinal sections determined after reperfusion (Hong et al., 2012).

Diabetic retinopathy is associated with microvasculature as well as to neural injury and neurodegenerative processes. Müller cells, the principal glial cells of retina, provide structural and metabolic support for retinal neurons and are activated by high glucose levels. Müller cells are essential for the removal of glutamate since these are the only cells in the retina that contain glutamine synthetase that convert glutamate into glutamine. In patients with diabetes mellitus, Müller cells are not able to transform glutamate to glutamine and, therefore, glutamate concentrations are elevated in the retinas of these individuals. During onset and progression of diabetic retinopathy, GFAP expression in Müller cells is unregulated; the nucleus is changed in addition to elevation in the activities of several growth factors, cytokines and inflammatory factors. The protective effects of agmatine were evaluated on high concentration glucose induced Müller cells (Han et al., 2015). LDH activity and TNF-α mRNA expression were significantly reduced in Müller cells exposed to a high glucose concentration following agmatine treatment. In addition, agmatine treatment inhibited glucose-induced Müller cell apoptosis, which was associated with the regulation of Bax and Bcl-2 expression. The glucose-induced phosphorylation of MAPK associated proteins as ERK, JNK, p38 was suppressed by agmatine treatment while the protective effects of agmatine on Müller cells were inhibited by NMDA. Therefore anti-inflammatory and anti-apoptotic effects of agmatine, as well as inhibition of the MAPK pathway via NMDA receptor suppression may be the underlying mechanisms related to the protective effects on Müller cells.

The real-time monitoring of light-induced changes in RGC-5 by electric cell-substrate impedance sensing (ECIS) (Bennet et al., 2013; Giaever and Keese, 1993) showed that initial light exposure (up to 4 h) promotes rapid production of ROS promote Ca^{2+} accumuation. Prolong light exposure of light radiations (after 4 h) increased NO and TNF-α levels and accumulation of these factors led to cell demise (Bennet and Kim, 2014). Agmatine controlled the elevation of free radicals, calcium gating, NO level, and TNF-α, thereby protecting the cells from photodamage. Loss of microvascular integrity is seen with major alterations in vascular permeability and disruption of BBB are the post ischemic events manifested through the degradation of the basal lamina that surrounds cerebral blood vessels. The vascular basal lamina is a constituent of extracellular matrix and its degradation is catalyzed by matrix metalloproteinases (MMPs). The MMPs are up-regulated by neuroinflammatory, ischemic injury and intensify disruption of matrix components, tight junctions between endothelial cells, and BBB, resulting in vasogenic edema following ischemia (Fujimura et al., 1999; Heo et al., 1999; Rosenberg et al., 1998).

In blood vessels, agmatine is stored in both endothelial cells (ECs) and vascular smooth muscle cells; however, ADC is only expressed in the endothelium (Regunathan et al., 1996). This implies that it would be possible for agmatine to affect vessels, especially ECs. Agmatine has been shown to decrease the expression of MMP-2 and -9 in cerebral ECs in normal and ischemic conditions (Kim et al., 2008). Earlier it has been shown that agmatine affects NO synthesis by activating eNOS (Morrissey and Klahr, 1997; Schwartz et al., 1997) and inhibiting inducible (iNOS) (Auguet et al., 1995; Galea et al., 1996) and neuronal NOS (nNOS) (Demady et al., 2001). The down-regulation of MMP-9 by agmatine was found parallel to the up-regulation of eNOS and maintenance of functional NO release in cerebral endothelial cells (Yang et al., 2007). The exogenous agmatine treatment attenuated the MMP-2 and MMP-9 protein and mRNA expression in primary cultured endothelial cells from murine brain. NO production which decreased in ECs after ischemic injury was augmented by agmatine treatment with concomitant elevation of eNOS expression. Administration of agmatine in presence of NOS inhibitor, Nω-nitro-L-arginine methyl ester (L-NAME), decreased the expression levels of MMP-2 mRNA and protein but did not affect that of MMP-9. The changes in regional distribution and the timing of eNOS and MMP expression by agmatine, especially during the early time (6 h) and 24 h postischemic periods after global cerebral ischemia/reperfusion, showed significant increase in eNOS levels observed at 6 h after 4-vessel occlusion with agmatine treatment both in rat hippocampus and parietal cortex (Mun et al., 2010a, 2010b). The number of eNOS positive microvessels were increased both 6 and

24 h after with agmatine treatment. During hypoxic-ischemic injury, agmatine treatment suppressed MMP-2 and -9 expressions in ADC genes transfected bEnd3 cells via the regulation of eNOS and NO through activating transcription factor 3 (ATF3) pathway (Jung et al., 2010). The observed alterations were combined effects of exogenous supplement and endogenously elevated concentration of agmatine.

The migration of endothelial cells is an important step in angiogenesis that promotes repair and regeneration of damaged brain tissue (Conway et al., 2001). There appears a connection between vascular and neuronal compartments with underlying common signals and substrates involved in angiogenesis. For example, neurons secrete pro-angiogenic factors such as vascular endothelial growth factor (VEGF) (Raab et al., 2004) and most neuronal mediators appear to participate in vascular responses following cerebral ischemia (Greenberg and Jin, 2005; Lazarovici et al., 2006). The association of agmatine and angiogenesis was explored in bEnd.3 cells under normal condition by migration assay to determine downstream signaling pathways (Jung et al., 2013). Agmatine treatment accelerated the migration of bEnd.3 cells in a concentration-dependent manner mediated via VEGF/VEGFR2 [VEGF receptor 2 (Flk-1/KDR or VEGFR2)], PI3K (phosphatidylinositol 3-kinase)/Akt (protein kinase B, also known as PKB, PI3K downstream effector protein/eNOS (endothelial nitric oxide synthase)/NO and ICAM-1 (intercellular adhesion molecule 1) pathway. This conclusion was based on the key findings that agmatine treatment induced VEGF, VEGFR2, PI3K, Akt/protein kinase B, eNOS, NO and ICAM-1 expressions during bEnd.3 cells migration. The expression of ICAM-1 and migration of bEnd.3 cells, induced by agmatine, were attenuated by treatment of wortmannin, a specific PI3K inhibitor (Table 5).

3. Mechanisms of neuroprotection

Primary brain injury occurs when cells are killed in a nonspecific manner at the moment of trauma (contusion, damage to blood vessels, axonal shearing, damage to BBB and meninges). The delayed effects of primary injury are manifested as secondary injury where neurons that were unharmed in the primary injury get damaged. The secondary injury arises due to events as ischemia, cerebral hypoxia, hypotension, cerebral edema, changes in the blood flow and supply to the brain, raised intracranial pressure, hypercapnia, acidosis, meningitis and brain abscess. The major event is lack of oxygen that fails the neuron's normal process of ATP generation. The cell switches to anaerobic metabolism, producing lactic acid. ATP-dependent ion transport pumps stop working, causing the cell to become depolarized, allowing ions, including Ca^{2+} to flow into the cell. In absence of working ion pumps, intracellular Ca^{2+} levels get too high. The presence of Ca^{2+} triggers massive efflux of the excitatory amino acid neurotransmitter, glutamate, into the extracellular space raising it to the toxic level. If the cell dies through necrosis, it releases glutamate and toxic chemicals into the surrounding affecting neighboring cells. Glutamate stimulates AMPA receptors and Ca^{2+}-permeable NMDA receptors, allowing entry of more Ca^{2+} into cells. The high Ca^{2+} levels, activate enzymes, affect structural or regulatory proteins, cleave the membrane lipids and increase generation of harmful chemicals like free radicals, reactive oxygen species (process called excitotoxicity). The calcium-dependent enzymes, phospholipases cleave membrane lipids to alter the permeability and fluidity of the membrane and compromise the functions of receptors, ion channels and other proteins. Mitochondrial break down releases toxins and apoptotic factors into the cellular matrix. The caspase-dependent apoptosis cascade is triggered and when reperfused, a number of factors lead to reperfusion injury. Structural damage during reperfusion is thought to be a consequence of excessive generation of oxygen free radicals. Peroxynitrite, formed by the reaction of O_2 with NO [produced by neuronal constitutive (nNOS) or inducible (iNOS)], is the implicated lipid peroxidation-initiating radical species during reperfusion alongwith inflammatory reaction following reperfusion injury. The recruitment of neutrophils to the area of injury along with

Table 5
Neuroprotective effects of agmatine in retinal cells.

Injury type, By	In	Agmatine	Observed activity, Funtional recovery measurement	Observation	Ref
Hypoxic; O_2 5%, 48 h	RGC-5	100, 500 μM	LDH, annexin V, caspase-3 assays. Expression & phosphorylation of MAPKs, JNK, ERK p44/42, p38, NF-κB	• Agmatine hypoxia induced apoptotic death • Effects associated with the activity of JNK & NF-κB pathways	Hong et al., 2007
Oxidative stress, H_2O_2, upto 2.5 mM, upto 48 h	RGC-5	Pretreatment 2 h, 100 μM	LDH, TUNEL assay	Agmatine pretreatment attenuated H_2O_2 induced apoptosis	Iizuka et al., 2008
Hypoxic; O_2 5%, 12 h	RGC-5	100 μM	TNF- α & its rereceptor-1 expression	Agmatine inhibits TNF- α production of RGCs in hypoxic condition	Hong et al., 2008
TNF-α, 50 ng/ml, 48 h	RGC-5	100 μM	LDH, annexin V assay, H& PI staining	Agmatine reduced TNF-α-induced apoptotic death	Hong et al., 2009
Oxidative stress, H_2O_2 1 mM, 16 h	RGC-5	Pretreatment 2 h, 100 μM agmatine or or NMDA or MK-801 or 500 nM yohimbine	LDH assay	• Agmatine pretreatment reduced H_2O_2 induced cell but MK-801 did not • Yohimbine but not NMDA inhibited agmatine effect • Effects of agmatine via α 2-AR signaling pathway	Iizuka et al., 2010
Phototoxicity	RGC-5	1-8 μM	MTT assay, ECIS impedance measurement, Determination of ROS, Ca2+, NO, TNF- α	Agmatine prevented phototoxic effects by controling elevation of ROS, Ca^{2+}, NO, TNF-α	Bennet et al., 2013; Bennet and Kim, 2014;
Transient ischemia–reperfusion	Retinal IR model	50 mg/kg i.p. once or twice	Retinal thickness, Lipid peroxidation, NO levels	Agmatine inhibited retinal thickening, lipid peroxidation, NO	Dastan et al., 2009
High concemtration glucose mediated Muller cell injury		100 or 200 μM		• Inhibited glucose-induced Müller cell apoptosis	Han et al., 2015

(continued on next page)

Table 5 (*continued*)

Injury type, By	In	Agmatine	Observed activity, Funtional recovery measurement	Observation	Ref
	Primary Müller cells culture from rat retina		LDH assay, Flow cytomety, Expression of GS, NMDAR, TNF-α protein & TNF-α mRNA, Bcl-2, Bax, caspase-3, Phosphorylation of ERK, JNK, p38	• Reduced LDH activity, TNF-α levels, glucose-induced phosphorylation MAPK protein • NMDA inhibited agmatine's effects	

multiple adhesion molecules, due to damage to the BBB and leakage of constituents of blood through the damaged BBB and osmosis produces vasogenic edema further accentuating the injury Recently, apoptosis has gained the attention as a mechanism for secondary neuronal death after transient ischemia. By demonstrating the nucleosomal ladders of DNA fragments in transient ischemia and reperfusion models, reports have shown that cell death during ischemia is mainly necrotic, whereas additional cell death due to the damage induced by reperfusion is principally through apoptosis.

With the established pathophysiology of brain injury and the preclinical studies of agmatine with respect to brain injury, the mechanisms of its neuroprotective effect can be assigned to its multimolecular biological effects. (1) Agmatine is an antagonist of NMDA subtype of glutamatergic receptors; accordingly, it impedes the intracellular accumulation of Ca^{2+}. (2) The α_2-adrenoceptor agonistic activity of agmatine may contribute to neuroprotecive action. It has been reported that agmatine and dexmedetomidine, another α_2-adrenoceptor agonist, have neuroprotective effects against cerebral injury (Li et al., 2006; Wei et al., 2002). (3) agmatine is a putative endogenous ligand for imidazoline binding sites and might contribute to neuroprotective action (Dixit et al., 2018). Available report imply that the activation of imidazoline receptors by increasing expression of glial fibrillary acidic protein (GFAP), reducing calcium overload exhibit neuroprotective actions. (4) Agmatine inhibits all isoforms of NOS, and reduces production of the neuromodulator NO (Auguet et al., 1995; Galea et al., 1996; Abe et al., 2000). Since NOS induction by activated NMDA receptors is considered as an important step in secondary injury (Iadecola, 1997; Wada et al., 1998; Sengul et al., 2008), inhibition of NOS by agmatine may be responsible for its protective effects during brain injuries. (5) Agmatine has ability to attenuate the oxidative/nitrosative stress and restore the antioxidant capacity (Dejanovic et al., 2018; Chai et al., 2016). (6) Agmatine suppresses harmful alterations in the immune system induced by the ischemia to curtail inflammatory events (Uranchimeg et al., 2010; Ahn et al., 2011), inhibits gliosis and edema (Wang et al., 2010). (7) Potential anti-apoptotic characteristics of agmatine reduce cell death (Zhu et al., 2006). (8) Agmatine interferes with intracellular-signaling pathways by inhibiting ADP ribosylation of proteins, a process implicated in neuronal injury following cerebral ischemia in rats (Takahashi et al., 1997; Moss et al., 1983; Laing et al., 2011). (9) Agmatine is a regulator of the polyamine pathway via formation of putrescine, a precursor for biosynthesis of polyamines, which are essentially involved in the response to cellular injury and in neuroprotection (Oble et al., 2004). (10) Agmatine can block ATP-sensitive potassium channels (Shepherd et al., 1996; Santhanam et al., 2007), and increase insulin release, leading to lowered levels of glucose and lactate in blood (Pfeiffer et al., 1981; Sener et al., 1989) helping to improve the outcome of ischemic injury (Béjot et al., 2012). (11) Agmatine can also block the voltage-gated calcium channels (Qiu and Zheng, 2006). (12) It can inhibit advanced glycation end-product formation, a process involved in damage to extracellular matrix proteins and implicated in the pathology of diabetes and neurodegenerative diseases (Vlassara et al., 1994; Marx et al., 1995).

3.1. Comments

Encouraged with positive findings about agmatine's neuroprotective action, most of the reports envisage that agmatine treatment may be a new therapy for mitigating cerebral damages and neurodegenerative diseases. However, it will be premature to proclaim agmatine as armamentarium to salvage neural injuries. The reasons are many fold. All the studies are executed with limited range of dose of agmatine such as in vitro studies with 100 μM and in vivo studies by intraperitoneal route with 100 mg/kg and sporadically with 50 mg/kg. Although it is known that the BBB is compromised in neural injury, the extent of agmatine transport into the brain has not been determined yet. The dose-response studies as well as the investigations on the detailed pharmacokinetic

profiling are also missing. The fact that agmatine is present endogenously in CNS is not counted in the preclinical findings and there is lack of data describing central agmatine levels before and after cerebral injuries. Moreover, the preclinical findings are not verified and supported by any clinical evidence so far. Nonetheless the neuroprotective effects produced by agmatine in animal models are undeniable and further pursuit of this molecule for its CNS related physiology, pharmacology, molecular mechanisms, analogue discovery and development may prove to be worthy of efforts. However, the beneficial effect of agmatine in the preclinical models of epilepsy and other neurodegenerative disorders including Alzheimer and Parkinsonian disease suggest that agmatine is not a mere mediator/marker of these neurotoxic events, but should be looked/investigated/explored further as target for pharmacotherapy/biomarkers for the clinically relevant neurotoxic pathologies.

Conflict of interest

Authors declares no conflict of interest

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