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Current Therapeutic Approaches from Imidazoline and Opioid Receptors Modulators in Neuroprotection

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Abstract

Due to brain plasticity, the nervous system is capable of manifesting behavioral variations, adapted to the influences from both external and internal environment. Multiple neurotransmitters are involved in the mediation of pathological processes at the molecular, cellular, regional, and interregional levels participating in cerebral plasticity, their intervention being responsible for various structural, functional, and behavioral disturbances. The current therapeutic strategies in neuroprotection aim at blocking on different levels, the molecular cascades of the pathophysiological mechanisms responsible for neuronal dysfunctions and ultimately for neuronal death. Different agents influencing these neurotransmitters have demonstrated beneficial effects in neurogenesis and neuroprotection, proved in experimental animal models of focal and global ischemic injuries. Serotonin, dopamine, glutamate, N-methyl-D-aspartate, and nitric oxide have been shown to play a significant role in modulating nervous system injuries. The imidazoline system is one of the important systems involved in human brain functioning. Experimental investigations have revealed the cytoprotective effects of imidazoline I2 receptor ligands against neuronal injury induced by hypoxia in experimental animals. The neuroprotective effects were also highlighted for kappa and delta receptors, whose agonists demonstrated the ability to reduce architectural lesions and to recover neuronal functions of animals with experimentally induced brain ischemia.

Keywords: neuroprotection, neurodegenerative diseases, ischemic stroke, imidazoline, opioids, nitric oxide

1. Introduction

Increase in life expectancy has led to aging of the population and consequently to an expansion of the prevalence of neurodegenerative diseases (NDDs) [1].

Clinical type	Disease/disorder
Signs of progressive dementia with no other neurological signs (absent/inconspicuous)	<ul style="list-style-type: none">• Alzheimer’s disease• Frontotemporal dementias• Some cases of Lewy-body disease• Posterior cortical atrophy (visuospatial dementia)
Signs of progressive dementia accompanied by other neurological abnormalities	<ul style="list-style-type: none">• Huntington’s disease (chorea)• Lewy-body disease (Parkinsonian features)• Some cases of Parkinson’s disease• Corticobasal ganglionic degeneration• Cortical-striatal-spinal degeneration (Jakob’s disease)• Dementia-Parkinson-amyotrophic lateral sclerosis complex• Cerebrocerebellar degeneration• Familial dementia with spastic paraparesis, amyotrophy, or myoclonus• Polyglucosan body disease• Frontotemporal dementia with parkinsonism or ALS
Signs of movement disorders or other posture abnormalities	<ul style="list-style-type: none">• Parkinson’s disease• Multiple system atrophy• Essential tremor• Progressive supranuclear palsy• Dystonia musculorum deformans• Huntington’s disease (chorea)• Acanthocytosis with chorea• Corticobasal ganglionic degeneration• Lewy-body disease• Restricted dystonia
Signs of progressive ataxia	<ul style="list-style-type: none">• Spinocerebellar ataxias• Cerebellar cortical ataxias• Complicated hereditary and sporadic cerebellar ataxias
Signs of slowly developing muscular weakness and atrophy	<ul style="list-style-type: none">• Motor disorders with amyotrophy• Spastic paraplegia without amyotrophy
Sensory and sensorimotor disorders	<ul style="list-style-type: none">• Hereditary sensorimotor neuropathies• Pure or predominantly sensory or motor neuropathic• Riley-Day autonomic degeneration
Signs of progressive blindness with or without other neurological disorders	<ul style="list-style-type: none">• Pigmentary degeneration of retina• Stargardt’s disease• Age-related macular degeneration
Signs characterized by degenerative neurosensory deafness	<ul style="list-style-type: none">• Pure neurosensory deafness• Hereditary hearing loss with retinal diseases• Hereditary hearing loss with system atrophies of the nervous system

**Adapted from Ropper A, Samuels M, Klein J. Adams and Victor’s Principles of Neurology. 10th ed. McGraw-Hill Education; 2014.*

Table 1.
Neurodegenerative diseases: main clinical types.

Neurodegeneration represents a loss of neurons and their structural components (dendrites, axons, and synapses) with a corresponding gradual atrophy in neuronal function [2].

NDDs (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, dementia with Lewy bodies) determine cognitive and memory deterioration or alteration of the ability to move, speak, and breathe. These chronic and progressive disorders are an important cause of reduced quality of life, morbidity, caregiver burden and also, of the increase in total healthcare expenditure [3–5]. **Table 1** lists the main clinical types of NDDs [6].

Neuroprotection can be defined as a “relative preservation of neuronal structure and/or function” or as an action that aims “to prevent neuronal damage over time (either acute or chronic)” [7]. Neuroprotective action is primary if it is exerted directly on the neuron, or secondary if it appears from an activity on an intermediary that endangers neuronal function [8].

The mechanisms by which most agents with efficiency in NDDs act are not fully elucidated, requiring multiple and in-depth experimental and clinical studies.

Several experimental investigations highlight the multiple and various inter-relations between adrenergic, serotonergic, dopaminergic, glutamatergic, opioid, imidazoline systems, and the nitric oxide pathways, which may elucidate the effects of different compounds involved in the mediation of pathogenic mechanisms responsible for numerous structural, functional, and behavioral disturbances.

This chapter presents a brief overview of the most studied mechanisms related to neuroprotection and details the possibilities to pharmacologically influence through the main known neurotransmitters the pathophysiological mechanisms linked to various NDDs.

2. The imidazoline system

Imidazoline receptors are located not only in the mammalian central nervous system (CNS) cells but also in the peripheral nervous system [9], being involved in the mediation of various physiological processes in the body. It is currently known that there are four types of imidazoline receptors: I1, I2, I3, I4 (non I1-non I2), from which the first three have been mostly studied [10].

It has been emphasized that these receptors play an essential role in cell proliferation, regulation of adipose tissue formation, body temperature maintenance, mediation of gastrointestinal motility, neuroprotection, inflammation, nociceptive sensitivity, and some neurological or psychiatric disorders (such as depression) [11]. Moreover, it is known that these imidazoline receptor subtypes exert control over the activity of the hypothalamic-pituitary-adrenal and noradrenergic axis [12, 13].

A number of different endogenous ligands have been characterized: agmatine, the best known and largely studied, harmaline and harmaline (derivatives of the beta-carboline group), and the newly discovered ribotide (acetic acid imidazole). Agmatine, the potent neurotransmitter of the imidazoline system, has an important role in the mediation of body's response to stress, analgesia, drug addiction, and abstinence syndrome, in modulation of seizures development, and in neuroprotection [14, 15].

Endogenous agmatine is produced in response to stress (in conditions of ischemia, prolonged exposure to cold) and/or to inflammation [16]. It is assumed that agmatine is also an effective neurotransmitter, due to its concentration in the brain similar to classical neurotransmitters [17, 18].

Literature data have revealed that agmatine stimulates the activity of endothelial nitric oxide synthase [16], this effect being also proved by its level in the rat brain after cerebral ischemia [19, 20].

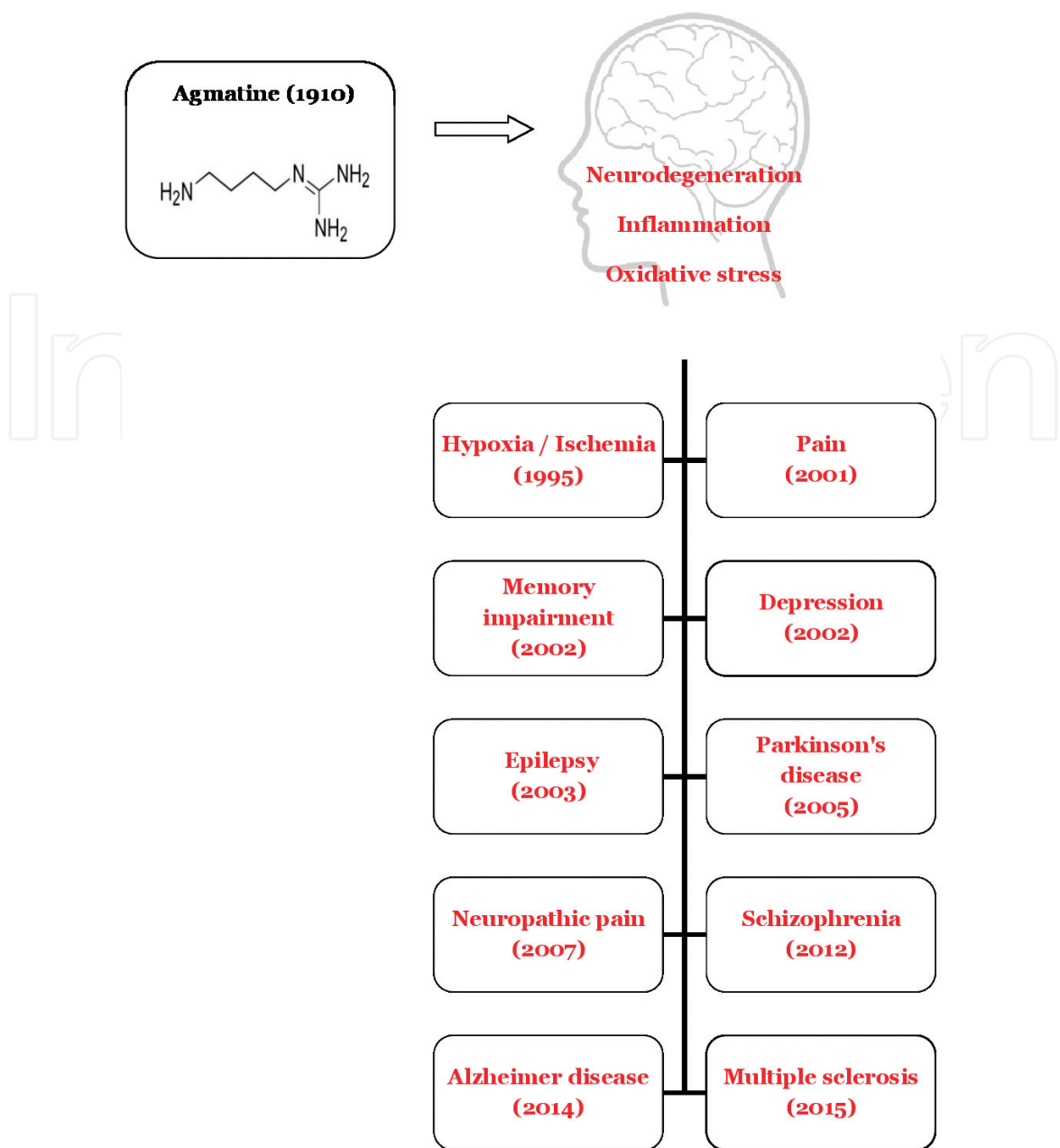


Figure 1.
Neuroprotective effects of agmatine and their first discovery.

Along with evidence of its neuromodulatory and neuroprotective properties, there are numerous preclinical studies demonstrating the beneficial effects of exogenous administration of agmatine in depression, anxiety, hypoxic ischemia, pain, morphine tolerance, memory impairment, Parkinson's disease, Alzheimer's disease, epilepsy, and other related conditions with traumatic brain injuries (**Figure 1**) [21–23]. All these are arguments in favor of the potential of agmatine as a new pharmacological agent for the treatment of various neurological diseases and NDDs [24].

3. The involvement of the imidazoline system in the mediation of cognitive functions

Electrophysiological studies involving various brain areas, performed on laboratory animals with experimentally induced cerebral alterations, have demonstrated the neurotropic effects of agmatine [25].

In vitro experimental researches have shown that activation of I2 receptors via the agmatine endogenous ligand exerts neuroprotective effects by increasing the

expression of glial fibrillary acidic protein in astrocyte cultures and by inhibiting MAO activity [26, 27]. Moreover, the beneficial effects of agmatine have been observed on ischemic-hypoxic lesions, on glutamate-induced neurotoxicity by activating the imidazoline receptors [28, 29]. It was also demonstrated that agmatine administration improves learning activity and memory of rats in experimental models of Alzheimer's disease and streptozotocin-induced type two diabetes mellitus [22, 30].

Other experimental investigations highlight the neuroprotective effect of intranasal administration of agmatine in elderly female rats, with a significant improvement of neurological status and increase of survival rate [28, 29].

The neuroprotective effects of agmatine on the morphological changes determined by repeated induced stress on medial prefrontal cortex and hippocampus of the rat were also investigated [31]. It was emphasized that under constant stress conditions, morphological alterations of the brain are associated with the reduction of endogenous agmatine levels [measured by high-performance liquid chromatography (HPLC)] and with an increase of arginine-decarboxylase level in the prefrontal cortex, hippocampus, striatum, and hypothalamus [32].

The exogenous administration of agmatine lowers brain morphological impairment, suggesting thus its neuroprotective effects against structural changes in the rat brain, under recurrent stress circumstances [32]. Moreover, elevated levels of agmatine have been evidenced in the blood, cortex, hippocampus, and hypothalamus, immediately after brain hypoxic ischemia. Other studies emphasize the neuroprotective influences of agmatine, highlighted by the increase of its brain levels, in rats subjected to prolonged cold-exposure stress conditions [33].

The neuroprotective potential of agmatine was also highlighted in the experimental model of 1-methyl-4-phenyltetrahydropyridine (MPTP)-induced Parkinson's disease in mice [29]. The use of agmatine attenuates the loss of cellular dopamine from the black substance and repeated treatment improves short-term memory impairment induced by MPTP in elderly mice. The behavioral benefits of agmatine are associated with the decrease in MPTP-induced glutamate capture in the hippocampal area, suggesting thus its involvement in modulation of glutamate recapture, the possible mechanisms responsible for lowering glutamate extracellular levels, thereby alleviating its neurotoxicity [29].

It is known that alteration of spatial memory in Parkinson's disease and schizophrenia is attributed to several factors, including hypofunction of glutamate and reduction of hippocampal volume [34]. Literature data report that the administration of the N-methyl-D-aspartate (NMDA) receptor antagonists (phencyclidine, also coded MK801) frequently impairs the late alternation performance in a standardized behavioral model of cognitive functions alteration similar to schizophrenia in laboratory animals [34]. The use of the glutamate/NMDA receptor antagonist phencyclidine induces a spectrum of behavioral, neurochemical, and anatomical changes, manifested by locomotor hyperactivity, motor-negative deficits, and cognition alterations (with memory impairment and visual attention) in laboratory animals. This substance was used to induce the experimental schizophrenia in laboratory animals [35, 36]. Agmatine attenuates cognitive and behavioral deficiency in rats with experimental phencyclidine-induced schizophrenic manifestations [37].

The effects of agmatine on memory alterations similar to those found in Alzheimer's disease have been evaluated in rats; in the pathogenesis of this degenerative disorder (which causes cognitive deficits in rodents), the fragment beta amyloid A β 25–35 plays an essential role. Studies have shown that agmatine significantly reduces the alterations in memory and spatial learning induced by the beta amyloid A β 25–35 fragment (the neurotoxic component of beta amyloid A β 1–42) in various behavioral experimental models, such as: the swimming test, the radial arm maze test, and the object recognition test [38].

It has been revealed that agmatine diminishes the activation of hippocampal caspase-3 (the early indicator of neuronal apoptosis) and prevents the alteration of spatial memory induced by lipopolysaccharides, in the swimming test in rat [39], suggesting its neuroprotective effects.

The neurotropic activity of agmatine has been also evidenced in the structural and cognitive alterations after the administration of NMDA (N-methyl-D-aspartate) in rats [40]. The use of high performance liquid chromatography (HPLC) and electrochemical detection allowed highlighting that the treatment with NMDA is associated with low concentrations of monoamines (epinephrine, norepinephrine, dopamine, and serotonin) in rat PC12 cells [29, 40]. In this experimental model (swimming test), agmatine protects against NMDA-induced PC12 cell lesions, augmenting the levels of epinephrine, norepinephrine, and dopamine, but not influencing serotonin values, together with lowering intracellular Ca^{2+} overload. These results indicate that the neuroprotective action of agmatine may be related to NMDA-receptor modulation and/or to controlling the decrease in monoamine content and NMDA-induced intracellular Ca^{2+} overload [40].

Immunohistochemical studies and electrophysiological investigations performed on the brain have validated the neuroprotective actions of both imidazoline receptor antagonists idazoxan and efaroan in rats with cerebral damages caused by the use of quinolinic acid [41], and also in mice with experimentally induced autoimmune encephalomyelitis, confirming the improvement of brain structural alterations and blood brain barrier lesions curtail [42].

A new (+)-2-(ethyl-2,3-dihydrobenzofuranyl)-2-imidazoline derivative—dexefaroxan—the (+) enantiomer of efaroan has been characterized. It has a potent and selective α_2 antagonist activity, with facultative effects on cognitive functions in the passive avoidance test in rats with memory-deficiency induced by scopolamine, diazepam, or by the 2-adrenergic agonist UK 14,304.

Dexefaroxan improves the cognitive performances in the passive avoidance test, facilitates spatial memory in the Morris swimming test in rats, and increases the object recognition ability in the specific behavioral test in mice [43, 44]. It has also proved to ameliorate the animal's memory deficits in these tests, particularly by attenuation of spontaneous memory loss, and to improve their spatial recognition ability, rather than through the acquisition skills or various other non-cognitive effects.

After subcutaneous administration of dexefaroxan, its pharmacodynamic effects persist for about 21–25 days, indicating that tolerance does not occur during prolonged treatment. Moreover, it was emphasized that dexefaroxan exerts protective effects on the spatial memory deficit caused by cortical devascularization in the Morris swimming test in rats [43].

Dexefaroxan has also been shown to exhibit neuroprotective effects on the devascularization-induced neurodegeneration, to ameliorate the structural changes in the hippocampus, and to remove the cognitive deficits induced by cerebral ischemia in rats [45, 46]. Its neuroprotective effects were present also in the excitotoxic lesions produced at the region of the basal magnocellular nucleus, increasing the olfactory discriminative capacity of rats, suggesting thus the possibility of its use in the treatment of memory disturbances in Alzheimer's disease [43].

Studies performed on genetically modified animals revealed that dexefaroxan improves cognitive performance in knockout mice with Alzheimer's disease [47].

Literature data regarding the neuroprotective action of imidazoline agonist and antagonist agents in human studies are only few, and the mechanisms involved in these effects are not completely deciphered.

Some investigations suggested that agmatine manifests protective activity against brain cell injury in different *in vivo* models of Parkinson's disease, as well as

in vitro studies, performed on human-derived dopaminergic neuroblastoma cell lines. It was postulated that the neuromodulatory properties of agmatine are related to the protective effects on the dopaminergic neurons, to NMDA receptor blocking, and to the decrease in oxidative stress, due to the inhibition of nitric oxide synthase (NOS) activity [48, 23].

Other clinical trials highlighted that the treatment with agmatine was associated with cytoprotective actions, in patients with spinal cord injury, proved by lessening the glial scar construction, decreasing the collagen scar zone, relieving the neuronal alterations, and recovering remyelination [49]. Moreover, the beneficial effects of agmatine have been demonstrated in various CNS lesions such as: cerebrovascular accident, brain trauma, neuropathic pain, lumbar degenerative disc disease, and different other types of neuropathy [50–52].

The administration of dexmedetomidine has also shown neuroprotective effects in humans with acute cerebral lesions [53].

In patients with dementia due to brain frontal lesions, idazoxan alleviates attentional and executive dysfunctions evoked by classical cognitive function tests [54].

4. The interrelation between the adrenergic and the imidazoline systems in the mediation of cognitive functions

Clonidine, both a non-specific α_2 adrenergic and imidazoline receptor agonist, decreases the cognitive function alterations induced by phencyclidine and MK801, facilitating spatial memory in the radial arm maze test in rats [35], but does not influence the behavioral and cognitive deficits in the experimental NMDA-induced excitotoxic dorsal hippocampal lesions [35]. Such findings indicate that clonidine improves memory alterations caused by glutamate hypofunction, but not by hippocampal injury, implying that multiple and distinct mechanisms are involved in the development of memory disorders.

The administration of the α_2 adrenergic receptor agonist clonidine or guanfacine prevents some of the behavioral effects of NMDA antagonists, proving that the monoaminergic system mediates a number of aspects of the cognitive deficit. Clonidine and guanfacine improve the lack of visual attention and spatial memory induced by phencyclidine in rats [34, 36]. It was demonstrated that low doses of clonidine recover the animal's ability to accurately choose the object and prevent the performance deficit induced by phencyclidine. At high doses, clonidine decreases the response time and induces a lack of the choice accuracy. These results indicate that clonidine treatment can alleviate phencyclidine-induced deficit of attention and of working memory, probably by preventing some of the neurochemical and anatomical effects of this psychotomimetic drug [34].

On the other hand, the use of only the selective α_2 adrenergic receptor antagonist does not impair the animal's spatial memory, but dramatically aggravates the phencyclidine-induced memory deficit [36]. These data demonstrate that α_2 adrenergic receptors mediate the inhibition of spatial memory disturbances, suggesting their important role in cognitive deficits associated to NMDA receptor hypofunction [34, 36].

The role of moxonidine (an α_2 adrenergic imidazoline I1 receptor agonist) on cognitive function in rats with Huntington's disease experimentally induced with 3-nitropropionic acid (3-NPA) was investigated in the Morris swimming test and in the elevated plus maze test. The administration of 3-NPA induces degenerative brain damage, progressive motor dysfunction, loss of grip force, emotional disturbances, weight loss, anxiety, and impairment of learning activity and memory. An increase in cerebral acetylcholinesterase level, enhancement of oxidative stress, and

impairment of the activity of mitochondrial enzyme complexes I, II, and IV were also noted [28]. The treatment with moxonidine resulted in the alleviation of disturbances caused on animal weight, motor activity, gripping ability, anxiety, impairment of learning ability and memory, and biochemical disturbances, thus indicating that substances modifying the activity of I1 receptors may be potential pharmacological agents for the treatment of degenerative brain disorders [55].

The effects of clonidine have also been evaluated in mice with subacute brain ischemia obtained after permanent ligation of common carotid arteries. The subsequent brain damages consisted of expansion of cerebral infarction areas, assessed by computed tomography scans. This experimentally induced chronic cerebral hypoperfusion was associated with a significant impairment of animal's learning ability and memory in the Morris swimming test [25, 28]. Subacute treatment with clonidine for 7 days increases the expression of neuronal nuclei, glutamic acid-decarboxylase-67, and gamma-aminobutyric acid (GABA) B receptor (GABA_B1) in hippocampal subregion cornu amonis (CA₁) but does not influence the level of these elements in the hippocampal area CA₃, nor in the dentate gyrus. These data support the idea that clonidine exerts neuroprotective effects on chronic cerebral ischemic lesions, by regulating GABA_B1 receptors and the activity of glutamic acid-decarboxylase-67 [25].

Additionally, the decrease in superoxide dismutase (SOD), catalase (CAT), and glutathione levels as well as the increase of both malondialdehyde (MDA) level and cerebral acetylcholinesterase activity were noted in animals with brain ischemic lesions [28].

Both moxonidine and clonidine have shown a decrease in histopathological changes, oxidative stress, central cholinesterase activity, as well as a reduction in memory disturbances and learning deficits in mice with vascular dementia induced by subacute ischemia after permanent bilateral cerebral artery ligation [28, 40].

In vitro cell culture studies from the rat frontal cortex with glutamate-induced neurotoxicity revealed the partial neuroprotective effects of moxonidine, with a significant decrease in the number of dead cells [26]. Moxonidine has shown beneficial effects on cerebral spasm in an experimental rabbit model of subarachnoid hemorrhage [40, 56].

5. The interrelations between the imidazoline system and the oxidative stress in the mediation of cognitive functions

Different pathological conditions of the body, as well as the physiological process of aging, can cause cognitive impairment and free oxygen radicals production, being responsible for abnormal functioning and cell death. Subsequently, a new idea has emerged claiming that nitric oxide (NO), along with the free radicals, plays a key role in the aging process, due to neurotoxic effects on the brain exerted by its excessive levels [57]. Nitric oxide is generated from L-arginine under the action of nitric oxide synthase (NOS). The three isoforms of NOS have different roles in the body: neuronal NOS (nNOS) is responsible for synaptic plasticity, learning and memory processes; endothelial NOS (eNOS) provides stabilization and regulation of vascular micro-environment and contributes to neuroplasticity [58]; and inducible NOS (iNOS) is involved in various pathophysiological conditions [57].

Numerous experimental researches reveal that NOS activity is significantly elevated in the brain of elderly rats, being associated with existing cognitive alterations [57, 59]. Mediated by the competitive inhibition of nNOS and iNOS, and correlated with the stimulation of NOS, agmatine contributes to the improvement of cognitive functions [19, 60, 61], while exhibiting neuroprotective effects [38, 39].

Literature data have shown that agmatine eliminates neuroinflammation and lipopolysaccharide-induced memory impairment (which is known to stimulate iNOS activity and, implicitly, the NO production) in laboratory animals. It prevents cognitive alterations, probably as a result of inhibition of iNOS activity [39]. Other researchers have disproved these results by showing that agmatine can cause cognitive impairment due to the inhibition of NMDA receptors and of NO, important elements in the modulation of learning and memory processes [62, 63].

On the other hand, it is known that the central cholinergic system plays a crucial role in the mediation of cognitive functions. Cognitive deficits have been induced in laboratory animals by using an anticholinergic agent, scopolamine, its administration producing a significant reduction in NOS activity, and an increase in arginase activity, of L-ornithine and putrescine levels in the hippocampus [51]. It has been observed that agmatine eliminates the scopolamine-induced alterations of memory and learning capacity [64, 65].

Although glutamatergic activity is required for cognitive processes, it is assumed that the increase of glutamate levels or of NMDA activity would also be responsible for the scopolamine-induced cognitive disturbances [66].

Knowing that agmatine blocks NMDA receptors and also interferes with the pathways of NO, NOS, and L-arginine, it was assumed that the removal of scopolamine-induced cognitive deficits can be attributed to its modulating effect on NO/NOS activity, on L-arginine, and also to the antagonization of NMDA receptors, with subsequent suppression of excessive glutamatergic activity [61, 65].

Abnormal release and disturbances of neuromodulatory activities due to variation in cerebral agmatine levels may be correlated to different CNS diseases (such as schizophrenia). Interactions of agmatine with other central neurotransmitter systems (such as glutamatergic and nitrenergic) appear to be particularly important in the pathophysiological mechanisms of CNS disorders associated with brain damage and cognitive functions deficit.

6. The opioid system

Neurodegeneration can be caused by chronic disease progression or by acute injury (cerebral ischemia—stroke or trauma) [67]. Ischemic stroke represents a vascular ailment with neurological consequences produced by the obstruction of the arteries in a part of the brain, therefore by blood supply privation [68]. Stroke can determine long-term neurological and psychiatric impairments, its therapy being focused on confining secondary injury processes [67].

In a recent review article, Chamorro presented that ischemic stroke is “the first cause of permanent disability in adult people, the second single most frequent cause of death for people older than 60 years, the second most common cause of dementia, representing approximately 3% to 7% of the total health-care expenditure in high income countries” [69].

A superpose of pathologies in different neurological disorders was proposed since NDDs and ischemic stroke are frequently concomitant, hence the neuroprotective therapy could be similar [70].

Opioids are substances with morphine-like action binding to specific opioid receptors (ORs). In the early 1990s, three important opioid receptor families [μ (MOR), κ (KOR), and δ (DOR)] were identified, and in 1994 another opioid receptor was discovered [nociceptin, orphanin FQ receptor (NOP), or the opioid receptor-like orphan receptor (ORL)]. ORs are found in the nervous system, lungs, heart, liver, and gastrointestinal and reproductive tracts. They have been intensively studied and it was emphasized that they not only are related to

Opioid receptor	Agonist
δ	DADLE [D-Ala2, D-Leu5]-enkephalin
	DPDPE (D-Pen2,D-Pen5)-enkephalin
	SNC80
	Tan-67
	Remifentanil
κ	BRL 52537
	CI-977
	GR89696
	Salvinorin A
	U-50,488H
	Dynorphin
μ	DAMGO [D-Ala2,N-MePhe4,Gly-ol]-enkephalin
	Endorphin 1 and 2 (EM 1/2),
	Morphine

Table 2.
Opioid receptors and their agonists tested for neuroprotective action.

antinociceptive action, but also have a role in cell proliferation, ionic homeostasis, emotional response, immune function, epileptic seizures, feeding, obesity, respiratory and cardiovascular control, hibernation, and neuroprotection [71, 72].

In the last decades, researches have pointed out that the opioid system can be promising to get neuroprotective treatment in the event of stroke, through OR agonists at lower doses, to avoid tolerance and/or physical dependency. DOR agonists followed by KOR agonists have revealed the most intense neuroprotective efficacy [64]. Major OR agonists tested for neuroprotection are listed in **Table 2** [71, 72].

DOR activation is beneficial against ischemic, hypoxic, and excitotoxic injuries [73]; recent studies promote DOR and especially DADLE (an analog of endogenous delta-opioid enkephalin) as promising targets for treating NDDs like stroke and PD [74–76].

DADLE alleviates apoptotic pathways, supports not only cell survival of peripheral organs (such as lung, heart, kidney, and liver) but also neuronal survival, and protects neurons and glial cells from ischemia-induced cell death [76–78].

In a cellular model of PD, DADLE administration augmented cell survivability with concurrent downregulation of the unfolded protein response stress sensors and protein aggregates [79].

Findings from a rat middle cerebral artery occlusion (MCAO) stroke model proposed that neuroprotection of DADLE treatment was based on the activation of PI3K-Akt pathway by reducing nerve cell apoptosis [80].

Non-selective opioid receptor agonists were also tested: LYS739 (fluorinated enkephalin-fentanyl derivative) and the most promising compound—biphalin—which proved to be effective both *in vitro* and *in vivo* stroke models [72].

The latter is a dimeric enkephalin analog (Tyr-D-Ala-Gly-Phe-NH-)2 with high potency and affinity for MOR and DOR and low affinity for KOR. Biphalin crosses blood-brain barrier reaching spinal and supraspinal sites expressing OR and produces less physical dependence and tolerance compared to morphine [72, 81–84].

Different studies using a mouse MCAO stroke model reported that biphalin reduced brain edema and infarction, ROS production, and NMDA-induced excitotoxicity. It also increased locomotor activities and neurological score after stroke resembled to saline-treated animals [72, 81, 85, 86]. Biphalin notably diminished penumbral expression of Na^+ , K^+ , 2Cl^- cotransporter (NKCC), and the translocation of the conventional isoforms of protein kinase C [81].

It has been hypothesized that biphalin's neuroprotective effects are more intense compared to subtype-selective agonists due to concomitant activation of the three types of OR [72, 85].

7. Conclusion

Various pharmacological substances influencing the pathways of the main neurotransmitters have confirmed valuable effects in neurogenesis and neuroprotection, being validated in different *in vitro* researches and *in vivo* experimental animal models of limited or extensive ischemic brain lesions.

Deciphering the roles of the neurotransmitters in central nervous system activity other than the signaling function will represent a starting point to deepen the knowledge about the complex mechanisms of the brain functions and to obtain new agents useful for protection of ischemic neurons and for preventing their irreversible damage.

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