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REVIEW

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Molecular and cellular mechanisms of neuroprotection by oligopeptides from snake venoms

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Abstract: Venom snake-derived peptides have multiple biochemical, pharmacological, and toxicological profiles, allowing for the discovery of new medicinal products and therapeutic applications. This review specifically examines the fundamental elements of neuroprotection offered by different oligopeptides derived from snake venom. It also includes a brief evaluation of short peptides that are being considered as potential therapeutic agents. Proline-rich peptides and tryptophyllin family peptides isolated from the crude venom of Viperidae family snakes, specifically *Bothrops atrox*, *Bothrops jararaca*, and *Bothrops moojeni*, have been shown to have pro-survival properties, the ability to reduce oxidative stress, and the ability to promote cell viability and mitochondrial functions. Three significant mechanisms are related to the neuroprotection mediated by snake venom oligopeptides: (1) Activation of the L-arginine metabolite pathway, such as polyamines from ornithine metabolism, which reduces N-methyl-D-aspartate (NMDA)-type glutamate receptor activity; (2) Enhancement of cell viability by activating the nerve growth factor-signaling pathway; and (3) Activation of the Muscarinic acetylcholine receptor subtype M1 (mAChR-M1). These small peptides show promise as neuroprotective agents against a variety of neurodegenerative disorders.

Introduction

Snake venom is secreted from a specific gland positioned above the upper jaw bone [1]. It is released by a compressor muscle surrounding the gland and transported through a duct to the fangs [1]. When injected into an individual's tissues, usually under accidental circumstances, snakebite envenoming causes considerable annual mortality, and surviving victims suffer ranging from mental and physical issues [2]. It is considering a severe public health issue that the World Health Organization (WHO) classified as a neglected disease [2]. The extensive complexity of venom composition [3] is responsible for prominent local tissue damage and systemic disturbances [2]. Toxins can cause cognitive abnormalities, bleeding, renal modifications, cardiovascular shock, lung and muscle inflammation, and reproductive problems, depending on the location they act [2,4,5].

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Composition of snake venom

The specific composition of venom can vary based on factors such as the species of snake, environmental conditions, age, sex, and the sort of prey that is accessible [6]. Snake venom is primarily composed of a variety of components, with bioactive peptides and proteins being the most abundant [7]. Aside from these, lipids, amino acids, carbohydrates, metal ions, nucleosides, and amines can constitute up 5%–10% of snake venom crude [8].

Researchers have developed venomics techniques to characterize venom toxins qualitatively and quantitatively in various areas of venom biology [8]. Most snake toxins are in the mass range of 4 to 100 kDa, while a number of multimeric toxins may exceed a total mass of 250 kDa [8]. Studies have demonstrated the categorization of snake venom protein families into different hierarchical groups based on their occurrence and abundance [7]. Based on a comprehensive analysis of the proteome composition of venom from 179 species (including 68 elapids and 111 vipers), 42 different protein families have been found [7].

They represent a combination of occurrences across many species, together with the proportional abundance of these protein families expressed as a percentage of the overall venom composition [7]. Among them, 4 protein

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families were classified as dominant: Phospholipase A2 (PLA2) is responsible for acute skeletal, muscle necrosis, flaccid paralysis, oedema, leukocyte influx into tissues, and pain [7]; Snake venom metalloprotease (SVMP) has hemorrhagic activity, as well as the ability to degrade fibrinogen and fibrin by proteolysis, induce apoptosis, and prevent platelet aggregation [7]; Three-finger toxins (3FTx) are non-enzymatic toxins that cause neurotoxicity effects, paralysis, muscle fasciculations, and cardiac failure [7]; And snake venom serine protease (SVSP) affects he coagulation cascade in the fibrinolytic and kallikrein-kinin pathways [7].

Various pharmacologically active peptides derived from the low molecular mass fraction (LMMF) of venom have also been reported, such as disintegrins [9] and proline-rich oligopeptides (PROs), commonly known as bradykinin (Bk) potentiating peptides (BPPs) [10].

PROs served as the basis for the discovery of antihypertensive medications like Captopril and its derivatives [13].

PROs have 5 to 14 amino acids and are structurally classified as oligopeptides. They have a high proline content, a pyro-glutamic acid residue in the amine-terminal position (<E), a PXP module, and at least one proline residue in the carboxy-terminal [14]. Over 85 snake proteins have been discovered, and analysis of their sequence features has revealed common components like N-terminal pyroglutamic acid and C-terminal QIPP, as well as varying components like AP or WAQ found in some of them [14]. These peptides include multiple proline residues in their interior regions [14–17] resulting in them being resistant to hydrolysis by aminopeptidases, carboxypeptidases, and endopeptidases [18,19] (Table 1).

TABLE 1

Structure-activity properties of snake proline-rich oligopeptides (PROs). Researchers identify peptides based on the number of amino acids in their primary structure and the order of their discovery. Differential effects of PROs are presented on the Bk potentiation, ACE inhibition, human Ass activity, and stability when submitted to the action of the endogenous animal proteolytic enzymes. PRO; proline-rich oligopeptide, Bk; Bradykinin, ACE; Angiotensin-converting enzyme, AsS; Argininosuccinate synthetase, *Peptide sequence in Clustal format; nd: untested; Red arrows represent the cleavage points

Peptide	Sequence*	Snake species	MW (Da)	Bk potentiation (nmol)	ACE-Ki (nM)	Human AsS activity (% in relation to basal activity)	Stability of PROs peptides found in the urine	Ref.
PRO-5a	<EKWAP	*B. jararaca*	611.7	0.36 ± 0.03	1280	59	<EKW↓AP	[18,20–22]
PRO-7a	<EDGPIPP	*B. jararaca*	705.8	10.77 ± 0.60	40.000	42	<EDGPIPP	[17,19–23]
PRO-9a	<EWPRPQIPP	*B. jararaca*	1101.3	0.22 ± 0.02	1	117	<EWPRPQIPP <EWPRPQIP↓P <EWPRP↓QIPP	[18,22,24]
PRO-10c	<ENWPHPQIPP	*B. jararaca*	1196.3	0.48 ± 0.02	0.5	405	<ENWPHPQIPP <ENWPHPQIP↓P	[24–27]
PRO-11e	<EARPPHPPIPP	*B. jararaca*	1189,4	2,68 ± 0,20	2000	281	nd	[16,17,19,20]
PRO-12b	<EWGRPPGPPIPP	*B. jararaca*	1281.5	0.83 ± 0.30	150	250	No fragments (unstable)	[17,18,20,23]
PRO-AP	<EARPPHPPIPPAP	*B. jararacussu*	1374.6	nd	350	nd	nd	[28]

Venom-derived peptides

Snake venom disintegrins are cysteine-rich non-enzymatic proteins derived from snake venom metalloproteinase, grouped based on sequence size and disulfide bond number [11]. Disintegrins possess a particular arrangement that enables them to recognize integrins within their structure [9]. This recognition facilitates their interaction with transmembrane adhesion receptors, thereby inhibiting the binding of these receptors to proteins found in the extracellular matrix and other cells [9]. Disintegrins possess diverse biological activities, such as the ability to prevent platelet aggregation and exhibit anticancer activity [9]. PROs were the first naturally found inhibitors of Angiotensin I-Converting Enzyme (ACE) described from snake venom studies [12]. The structures and functions of

For many years, it was believed that the antihypertensive effects of various BPPs were due to the suppression of ACE and the enhancement of Bk [15]. However, despite their structural similarity, the different biological function profiles suggest that they change their targets through various mechanisms [23]. These mechanisms include functioning as agonists of the M1 muscarinic acetylcholine receptor (mAChR-M1) [29,30] and activating argininosuccinate synthetase (AsS) [23,25].

Neuroprotective potential in the LMMF components of snake venom

Research has shown that compounds found in snake venom have the potential to be used as treatments for neurological diseases [31,32]. Martins and collaborators found that the LMMF of *Bothrops atrox* (*B. atrox*) venom (*Ba*-LMMF),

consisting exclusively of components with a molecular weight less than 14 kDa, is neuroprotective by reducing mitochondrial permeability transition (MPT) [33]. MPT is characterized by the enlargement of mitochondria due to an elevation in reactive oxygen species (ROS) and calcium ion (Ca^{+2}) levels [34]. Although MPT occurs before the initiation of apoptosis, it is believed that *Ba*-LMMF venom inhibits neuronal apoptosis [33].

Similarly, the LMMF obtained from *Bothrops jararaca* (*B. jararaca*) snake venom (*Bj*-LMMF), containing many bioactive peptides <10 kDa, showed neuroprotective efficacy against H_2O_2-induced toxicity in primary cultured hippocampus cells (Figs. 1A–1E) [35]. The *Bj*-LMMF lowered superoxide dismutase (SOD), caspases 3, and 8 levels, indicating that may boost hippocampus cell survival by reducing oxidative stress [35]. On the other hand, *Bj*-LMMF did not show a protective capacity against H_2O_2-induced damage in neuronal cell line SH-SY5Y (Fig. 1C) [36]. Querobino and collaborators proposed that *Bj*-LMMF treatment would elicit distinct responses in the primary culture of hippocampus cells, which comprises various cell types such as neurons, glia cells, and astrocytes, compared to cell lines [36]. *Bj*-LMMF-mediated neuroprotection was tested against oxidative stress in two different cell types, the PC12 and C6 cell lines (dopaminergic neuronal and astrocyte-like, respectively) [37]. It is interesting to note that *Bj*-LMMF protected neuronal PC12 cells from oxidative stress caused by H_2O_2 but not C6 cells (Fig. 1B).

Researchers commonly employ the C6 cell line to investigate various aspects of astrocytic function. It demonstrates rapid responsiveness to external stimuli, including H_2O_2, which can induce oxidative-nitrosative stress [37] indicating that C6 cells are more vulnerable to the effects of *Bj*-LMMF compared to PC12 or SH-SY5Y cells (Figs. 1B–1D). The neuroprotective effects of *Bj*-LMMF in PC12 cells were demonstrated by enhancing cell survival and metabolism (Fig. 1D) in response to H_2O_2-induced neurotoxicity. This was accomplished by reducing the generation of ROS, the production of NO, and the activity of arginase through the process of urea formation [37]. Studies reveal that the neuroprotection mechanism of this fraction relies on the synthesis route of L-arginine metabolites [37].

Argininosuccinate synthetase (AsS) and nitric oxide synthase (NOS) play crucial roles in the metabolic route of L-arginine, as shown in Fig. 2 [38]. AsS enzyme produces argininosuccinate which is metabolized by argininosuccinate lyase (AsL) to L-arginine [39]. L-arginine enhances several cellular processes and promotes the production of nitric oxide, agmatine, polyamines, and ornithine [38,39]. The neuroprotective properties of *Bj*-LMMF were diminished when AsS and NOS were suppressed. The mechanism of action is affected by the production pathway of L-arginine metabolites such as specifically NO and polyamines derived from ornithine hydrolysis [37]. The polyamines have well-known for their substantial contribution to the neuroprotection process, as evidenced in studies [40].

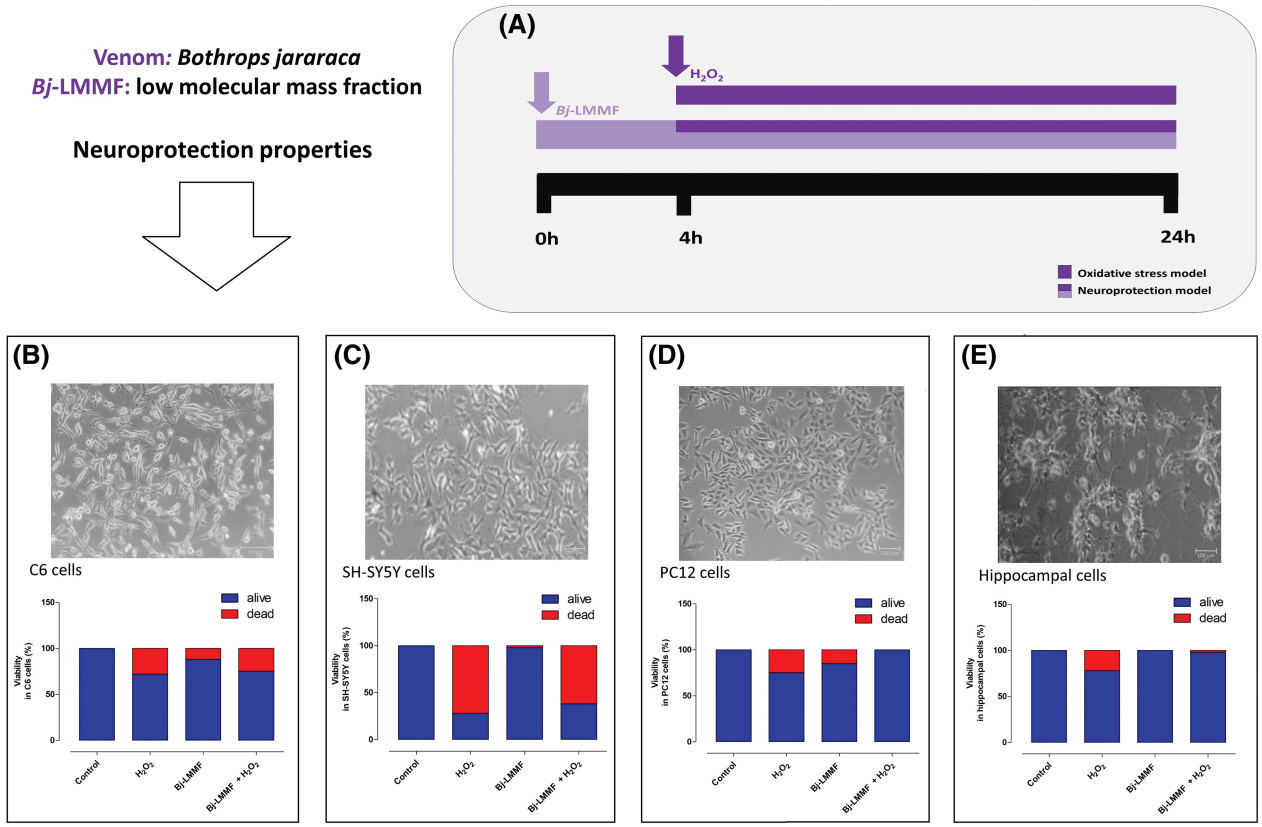

FIGURE 1. *Bj*-LMMF-mediated neuroprotection against oxidative stress in different cell lines reported in the literature [35,37]. (A) Studies have investigated the effects of oxidative stress on cells, specifically focusing on neuroprotective models. The cells received a 4 h pre-treatment with *Bj*-LMMF. Subsequently, the medium was substituted with *Bj*-LMMF, a novel medium, and/or H_2O_2 solutions, and left to incubate for an additional 20 h. Neuroprotective effects of *Bj*-LMMF against H_2O_2-induced toxicity in C6 (B), SH-SY5Y (C), PC12 (D) and hippocampal (E) cells.

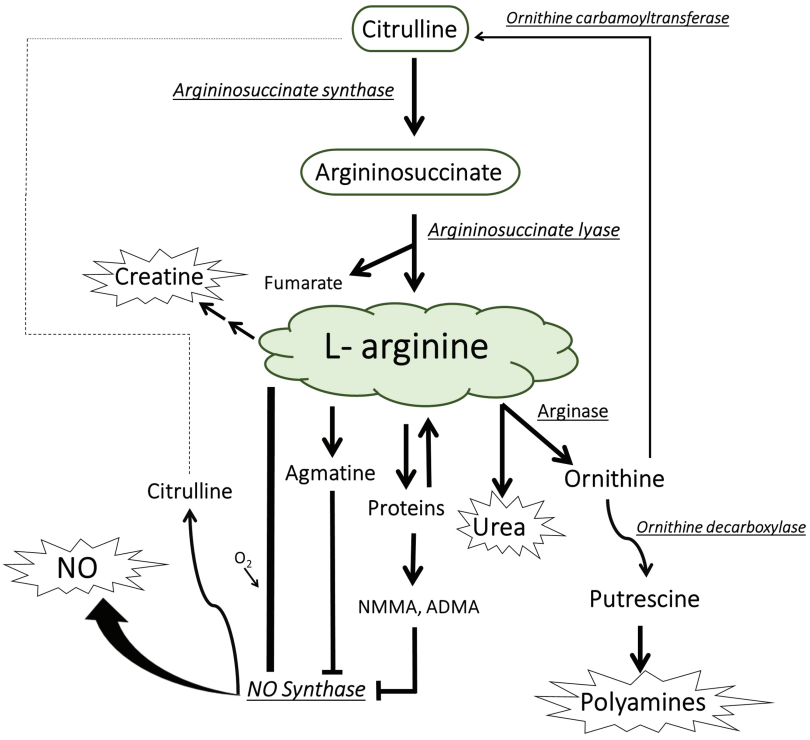

FIGURE 2. Metabolism of L-arginine in mammalian cells, including its involvement with the generation of nitric oxide (NO) and the citrulline-NO cycle [41]. NMMA; N^G-monomethyl-L-arginine, ADMA; asymmetric N^G, N^G-dimethyl-L-arginine.

Neuroprotection mediated by venom-derived peptides

Natural component-derived peptides are being considered as possible therapeutic potential for neuroprotection [42]. Pharmaceutical industries are particularly interested in peptides that possess advantageous characteristics such as low molecular mass, high activity and specificity, effortless absorption, and a good safety profile with minimal toxicity [43]. Venom-derived peptides and venom peptide-inspired molecules contributed to the discovery of new drugs approved by regulatory institutions or undergoing preclinical and clinical studies [44]. Captopril (antihypertensive), Tirofiban (antiplatelet), Eptifibatide (another antiplatelet), Batroxobin (Defibrase, for the care of acute brain injury and stroke), and α-Cobrotoxin (analgesic for moderate to severe pain) are exemplary instances of authorized pharmaceuticals that are generated from peptides found in snake venoms [8]. Some oligopeptides generated from snake venom have been demonstrated to provide neuroprotection using different models. Proline-rich peptides and tryptophyllin family peptides derived from the snake venoms (*B. atrox*, *B. jararaca*, and *B. moojeni* species) have demonstrated pro-survival features, the capacity to reduce oxidative stress, and enhance cell viability and mitochondrial functions.

The neuroprotective properties of several PROs were investigated for H_2O_2-induced toxicity in different types of neurons in culture, as seen in Table 2 [45,46]. However, the p-BTX-I, PRO-5a, PRO-7a, and PRO-10c peptides showed better neuroprotective effects improving cell viability and decreasing oxidative stress biomarkers against ROS-induced changes [45,46]. Curiously, although they have a similar structure, the biological mechanisms responsible for their neuroprotective activities are distinctive.

PRO-10c-mediated neuroprotection in SH-SY5Y cell cultures can be seen by a reduction of ROS, lipid peroxidation, inducible NOS, NO levels, and total glutathione [36]. PRO-10c was reported as an AsS activator, which improves the enzyme's ability to bind to ATP and citrulline, while also raising the levels of L-arginine [25]. Elevated levels of L-arginine can enhance the synthesis of agmatine, which is known for its neuroprotective properties [47]. Agmatine blocks the N-methyl-D-aspartate (NMDA) receptor channel in hippocampuss [48]. The neuroprotective impact of PRO-10c is attributed to enhanced L-arginine production, which is accomplished via upregulating AsS (Fig. 3A), producing agmatine, and maintaining mitochondrial membrane integrity [36]. Interestingly, it was observed that the neuroprotective efficacy of PRO-10c against H_2O_2-induced toxicity in SH-SY5Y cells was attenuated by AsS inhibition using α-Methyl-DL-aspartic acid (MDLA) [36], demonstrating that its neuroprotective effects are dependent on L-arginine metabolism.

PROs 5a and 7a exhibit neuroprotective effects in SH-SY5Y cells, but distinct molecular mechanisms seem to underlie their neuroprotection [45]. MDLA did not change the improvement of cell viability or decrease oxidative stress biomarkers in PRO-5a and PRO-7a-mediated neuroprotection [45] in contrast to PRO-10c [36]. PRO-7a exhibited a heightened response to increasing NO and prevented the decline in mitochondrial membrane potential against oxidative stress, unlike PRO-5a [45].

A peptide p-BTX-I was obtained from *B. atrox* venom and presents neuroprotective effects against dopaminergic neurotoxicity induced by 1-methyl-4-phenylpyridinium (MPP+) in neuronal PC12 cells, which reproduces Parkinson's disease [46]. This peptide enhances cellular

differentiation and vitality in cells against MPP+, suggesting that the protective effect operates through the activation of the Nerve Growth Factor (NGF)-signaling pathway [31,49]. An examination of the biotransformation of PRO-5a (also known as EKWAP) has shown that a metabolite (also known as EKW) is found in urine when PRO-5a is injected intraperitoneally. This suggests that PRO-5a is easily broken down by proteolytic enzymes naturally present in animals [18]. PRO-5a is hydrolyzed by ACE, specifically cleaving between WA amino acids [50] (Table 2). It indicates that the metabolized form of PRO-5a could possess similar neuroprotective properties to the <EVW peptide (Table 2).

TABLE 2

Neuroprotection mediated by venom-derived peptides in different cell lines and oxidative stress models. The quantification of the effect of peptides on oxidative stress was done using a neuroprotection index. This index assesses the correlation between the viability of cells that were not treated and the viability of cells that were treated with MPP+ or H_2O_2 in combination with the peptides. ID; Peptide identification, PRO; Proline-rich oligopeptide, MPP+; 1-Methyl-4-phenylpyridinium, H_2O_2; Hydrogen peroxide

ID	Snake venom	Cell line	Oxidative stress model	Cell density (0.33 mm^3)	Concentration tested	Neuroprotection index	Ref.
p-BTX-I	*B. atrox*	PC12[a]	MPP+	2×10^4	350 $\mu g.mL^{-1}$	0.89	[46]
PRO-5a	*B. jararaca*	SH-SY5Y [b]	H_2O_2	1×10^5	1 μM	0.85	[45]
PRO-7a	*B. jararaca*	SH-SY5Y [b]	H_2O_2	1×10^5	1 μM	0.80	[45]
	B. jararaca	PC12[c]	H_2O_2	5×10^3	1.56 μM	1.10	[51]
	B. jararaca	C6[c]	H_2O_2	5×10^3	1.56 μM	0.69	[51]
PRO-10c	*B. jararaca*	SH-SY5Y[d]	H_2O_2	1×10^5	1 μM	0.80	[36]
PRO-11e	*B. jararaca*	SH-SY5Y[b]	H_2O_2	1×10^5	1 μM	0.45	[45]
PRO-12b	*B. jararaca*	SH-SY5Y [d]	H_2O_2	1×10^5	1 μM	0.45	[36]
PRO-AP	*B. jararacussu*	SH-SY5Y[b]	H_2O_2	1×10^5	1 μM	0.47	[45]

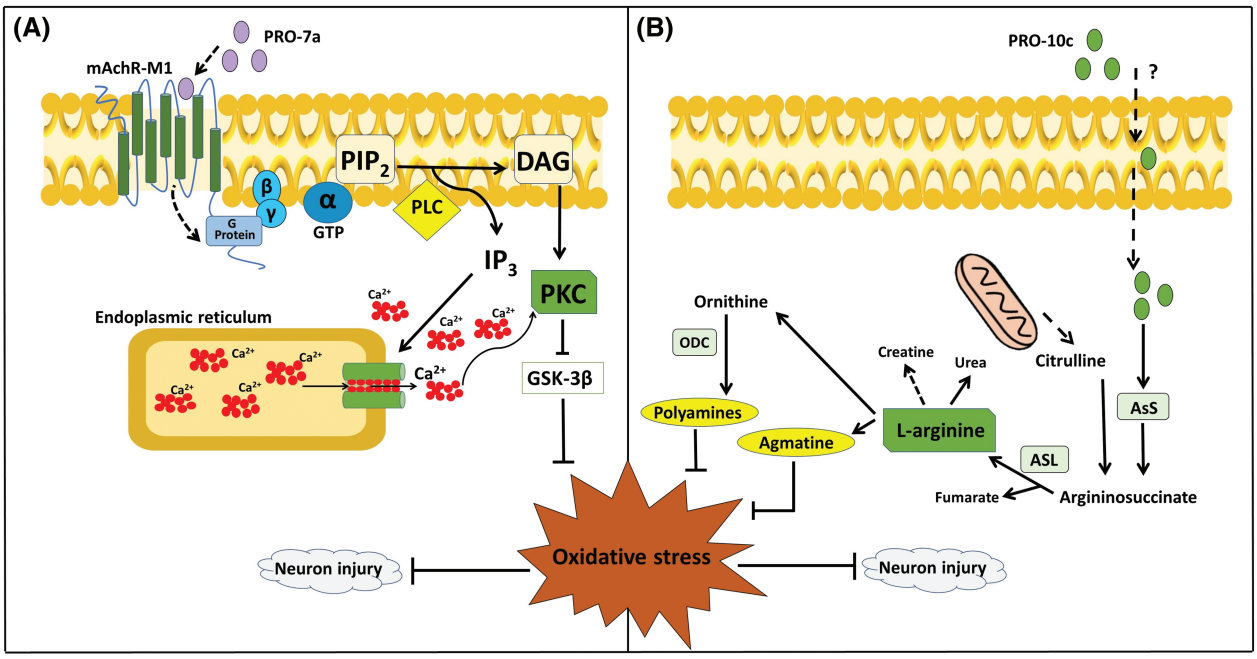

FIGURE 3. Proposals for PROs-mediated mechanisms of neuroprotection. (A) PRO-7a activates mAChR-M1, leading to various consequences through the signaling of PKC and MAPK. These effects occur independently of the rise in L-arginine caused by AsS activation, and they trigger a biological response to the effects of oxidative stress [51]. (B) PRO-10c is taken up into cells by an unknown process, increasing the production of L-arginine by upregulating the expression and activity of AsS. Due to the increased levels of L-arginine and its metabolism (polyamines and agmatine). These compounds have neuroprotective properties and have a role in the response to oxidative stress in neurological disorders [36]. AsL; Argininosuccinate lyase, AsS; Argininosuccinate synthetase, DAG; Diacylglycerol, GSK3β; Glycogen synthase kinase 3β, GTP; Guanosine triphosphate, IP3; Inositol triphosphate, mAChR-M1; M1 muscarinic acetylcholine receptor, MAPK; mitogen-activated protein kinase, PIP2; Phosphatidylinositol 4,5-bisphosphate, PKC; Protein kinase C, PLC; Phospholipase C.

PROs 5a and 7a are agonists of mAchR-M1 [29,30]. The mAchR-M1 stimulated have neuroprotective effects in the central nervous system (CNS) [52,53], and it is considered when the mechanisms that explain PROs 5a and 7a-mediated neuroprotection are discussed. Indeed, the neuroprotection induced by PRO-7a against oxidative stress in PC12 cells is dependent on mAchR-M1 activation [51].

mAChR-M1 is conventionally associated with the G-protein family to trigger the stimulation of phospholipase C (PLC) and protein kinase C (PKC), which in turn suppress the activity of glycogen synthase kinase 3 beta (GSK 3 β). This suppression leads to oxidative stress reduction [54]. Stimulating the activation of mAchR-M1 receptors enhances brain function and modifies the start or progression of Alzheimer's disease [55]. Additionally, it has been recognized as a crucial therapeutic focus for neurodegenerative condition [56].

PRO-7a-induced neuroprotection appear to be achieved via activating the mAchR-M1, leading to a decrease in stress markers and neuronal toxicity [51]. PRO-7a also does not affect AsS activity or L-arginine availability, as reported by PRO-10c [51]. Then, it has been proposed that the neuroprotection by PRO-7a may be mediated by unique mAChR-M1 through G-protein/PLC/PKC signaling, in contrast to PRO-10c. This mechanism involves the inhibition of GSK3β, resulting in a reduction of oxidative stress and neuron damage (Fig. 3B).

Antioxidants neutralize free radicals, consequently preventing a chain reaction, and reducing the ROS production [57,58]. Antioxidant peptides have the capacity to decrease the generation of oxidative compounds by activating cellular antioxidant enzymes, such as SOD and catalase, which are accountable for sustaining the antioxidant system within cells [42]. Some PROs of *B. jararaca* were examined for their potential as antioxidants, but the PROs 5a, 7a, and 10c were incapable of capturing free radicals [36,45]. However, the BmT-2 peptide (FPWIIS-NH_2) was identified in *B. moojeni* and belongs to the tryptophyllin family and showed antioxidant functions [59]. Tryptophyllins are a class of peptides found in the skin secretions of amphibians [59]. The inclusion of aromatic residues in the main structure of tryptophyllins, such as BmT-2, significantly contributes to their antioxidant effects [59,60].

Conclusion

The latest discoveries described in this review demonstrate that various oligopeptides are found in snake venom, each with distinct sequences and functions, have the potential to be a compelling subject of investigation. They may function as promising candidates for novel neuroprotective therapy and function as prototypes for the development of neuroprotective drugs.

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Availability of Data and Materials: All data generated or analyzed during this study are included in this published article, and original data are available from the corresponding author upon reasonable request.

Ethics Approval: Not applicable.

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