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Antinociceptive properties of the mastoparan peptide Agelaia-MPI isolated from social wasps

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ABSTRACT

Analgesic therapy is based on the sequential treatment of pain, in which opioids are drugs of last resource and known to be highly effective, but are also responsible for undesirable side effects, tolerance and addiction. There is a need for new drugs with alternative targets in order to minimize side effects and improve treatment efficacy. Mastoparans are an abundant class of peptides in wasp venom and have shown great potential as new drugs, as well as being excellent tools for the study of G-protein-coupled receptors. The objective of this study was to investigate the antinociceptive activity of the mastoparan Agelaia-MP I and the mechanisms involved. Agelaia-MP I (MW 1565 Da) showed dose-dependent antinociceptive activity in mice submitted to i.c.v. injection in two different models. The highest dose produced a maximum effect for up to 4 h, and nociception remained low three days after injection. Further experiments showed that Agelaia-MPI induced partial and reversible blockade of the amplitude of action potential, probably interacting with voltage-gated sodium channels. These results revealed the significant potential impact of compounds isolated from wasp venom on the central nervous system (CNS). In addition, the antinociceptive effect described here is a novel activity for mastoparans.

1. Introduction

Pain pharmacotherapy is based on the prescription of analgesics, which are the second most prescribed class of drugs. The main classes of analgesics are non-steroidal anti-inflammatory drugs (NSAIDs) and opioid antagonists. In some cases antidepressants and anticonvulsants can be used not only to control chronic pain, but to treat pain-related morbidities, such as depression (Kroenke et al., 2009).

Analgesic opioid drugs are the most widely used medication for moderate to severe chronic pain. They are highly effective, but induce systemic side effects such as nausea, constipation, dizziness, somnolence, vomiting, immunosuppression, tolerance, physical dependence and respiratory depression, resulting in negative medical, social and economic consequences for patients and society (Benyamin et al., 2008; Provenzano and Viscusi, 2014). Morphine derivatives are the most commonly used analgesics for chronic pain, acting on opioid receptors by stimulating the endogenous system to suppress pain.

Problems related to opioid analgesic use in patients prompted the search for alternative drugs, with new mechanisms of action. For instance, Ziconotide, a peptide from the venom of the sea snail *Conus* sp. and a neuronal calcium channel blocker, was approved in 2004 as a chronic and neuropathic painkiller (Prialt®). Ziconotide exhibits stronger analgesic effects than morphine and is a better alternative in that it does not cause tolerance or dependence (Fedosov et al., 2012).

Venomous arthropods are known to use their toxins mainly as a defense against predators, and/or to paralyze prey (Mebs, 2001). When inoculated in humans, these toxins may cause allergic

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reactions, pain, tissue damage and CNS alterations, such as sleepiness, paralysis and seizures. Due to their high affinity and selectivity to CNS receptors, these toxins are considered promising drugs for the treatment of several neurological diseases, including epilepsy, Parkinson's and Alzheimer's, as well as the development of more efficient analgesics (Gazerani and Cairns, 2014; Monge-Fuentes et al., 2015; Silva et al., 2015).

Wasps are arthropods from the *Hymenoptera* order with potent venom capable of paralyzing prey. Solitary and social wasp venom contains a complex mixture of compounds, including biogenic amines (<1 kDa), peptides (1–10 kDa) and proteins (>10 kDa), which are responsible for a range of biological activities, such as allergic reactions, antimicrobial, anxiolytic, antinociceptive, anticonvulsant, and hemolytic effects, among others (Baptista-Saidemberg et al., 2012; Mendes et al., 2004; Nakajima et al., 1986).

Peptides are responsible for most of the activities described in wasp venom. In addition to being an antimicrobial and exhibiting neurological properties, the small size of these molecules allows them to interact with different cell types and tissues, capable of causing hypertension, muscle contraction, cell lysis, and polymorphonuclear cell chemotaxis (Baptista-Saidemberg et al., 2012). Given their distinct functions and significant presence in wasp venom, peptides are divided into classes according to their most characteristic biological activity, as follows: kinins, mastoparans, chemotaxic peptides, antimicrobials and neuroactive peptides.

Mastoparans exhibit similar functional and structural characteristics to those of melittin, a peptide isolated from bee venom. These peptides were first isolated from hornet wasp venom, but are widely found in solitary and social Eumeninae wasps (Konno et al., 2016). Mastoparan is an α-helical amphiphilic peptide that contains 10 to 14 amino acids, no cysteine in its primary sequence, and a strong presence of lysine (Nakajima et al., 1986). Lysine facilitates the release of histamine from mast cells and is positively charged, thus increasing affinity with negatively charged biological membranes (De Souza et al., 2011; Higashima et al., 1990; Konno et al., 2000). Mastoparan is a component of social and solitary wasp venom involved in inflammation, cell membrane lysis, mast cell degranulation, histamine release, as well as neutrophil and T-helper chemotaxis (Hancock and Diamond, 2000). Mastoparans also activate phospholipase A2 (Argiolas and Pisano, 1984), and G-protein-coupled receptors (Higashijima and Ross, 1991). However, studies on the effects of the interaction of mastoparans with central nervous system targets are scarce, with research focusing more on their ability to cross the blood brain barrier and carry compounds to the brain (Chen and Liu, 2012). This class of peptides has recently been studied for the development of an antidote for Botulinum toxin (Zhang et al., 2013).

The present study identified and analyzed the antinociceptive properties of the mastoparan peptide Agelaia-MP I from the social wasp *Parachartergus fraternus* (Hymenoptera, Vespidae). Moreover, the action of the peptide was evaluated in isolated action potentials of the frog sciatic nerve in order to determine the therapeutic target.

2. Material and methods

2.1. Purification of the peptide

Two *Parachartergus fraternus* nests were collected in Brasilia, Federal District (IBAMA license number 21723-1). Access to and remittance of genetic patrimony for the purposes of scientific research was granted by the National Council of Technological and Scientific Development (CNPq nº 010476/2013-0). The specimens collected were immediately frozen using dry ice and stored at −75 °C until use. Venom sacs were dissected immediately after thawing, extracted in Milli-Q water and centrifuged at 10000 g (3 min, 4 °C). The supernatant was freeze dried, diluted with 1:1 acetonitrile–water, filtered through a Microcon (Millipore, USA) membrane with a 3000 Da cutoff filter, and lyophilized. The venom extracted was submitted to reversed-phase HPLC (Shimadzu Prominence, Japan) using C18 ODS columns, 15 μm, 250 × 10 mm (Phenomenex, USA), with a linear gradient from 0 to 65% CH_3CN/H2O/0.1% TFA over 65 min at a flow rate of 1.5 ml/min to produce Agelaia-MP I, eluted at 62 min.

2.2. Mass spectrometry

All mass spectra were acquired on a MALDI-TOF/TOF UltraFlex III mass spectrometer (Bruker Daltonics, Germany). An a-cyano-4-hydroxycinnamic acid matrix (Sigma-Aldrich, USA) was prepared at a concentration of 10 mg/ml in 1:1 CH3CN/0.1%TFA. The sample (0.5 ml) was dropped onto the MALDI sample plate, added to the matrix (0.5 ml) and left to dry at room temperature. The equipment was used in positive reflective mode to determine mass from the peaks, and for peptide sequencing the MS/MS spectra were obtained using the LIFT method. Spectra analysis and *de novo* sequencing were performed manually using FlexAnalysis 3.0 software (Bruker Daltonics, Germany). In order to address the distinction between Leu/Ile and Lys/Gln, we identified d and w-type fragment ions using MALDI TOF/TOF. Similarity searches were performed online using the BLASTP and Fasta programs. The Clustal W2 tool (also available online) was used for multiple sequencing alignment, which allows more than three DNA or protein sequences to be compared simultaneously (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

2.3. Peptide synthesis

After preliminary tests, the fraction that exhibited antinociceptive activity was synthesized by AminoTech P&D, Brazil. The homogeneity and the sequence of the peptide were confirmed by MALDI-TOF MS analysis.

2.4. Bioassays

Experiments were performed according to the Ethical Principles on Animal Experimentation stipulated by the Brazilian National Council for the Control of Animal Experimentation (BRASIL, 2008), the National Institute of Health's Guide for the Care and Use of Laboratory Animals (NIH Publication No. 8023, revised in 1978) and Brazil's Arouca Law (No. 11.794/2008). This study was approved by the Animal Research Ethics Committee of the University of Brasília (nº 63878/2011).

2.4.1. Neurosurgery

Male Swiss Webster mice (16–20 g) were submitted to surgery to implant a stainless steel cannula (10 mm long and 0.7 mm in external diameter, fixed with dental acrylate) in the lateral ventricle. The animals were previously anesthetized with ketamin (75 mg/kg) and xylasin (15 mg/kg) and then fixed in a stereotaxic device (Insight Equipamentos, Pesquisa e Ensino, Brazil). After 4–6 days of recovery, the bioassays were performed. The peptide (6.4, 4.8 and 3.2 nmol; 1 μL) was injected through the cannula using an infusion pump (Harvard Apparatus, UK).

2.4.2. Hot plate test

Experiments were carried out based on the protocol described by Bannon and Malmberg (2007). The mice were placed on an aluminum hot plate (AVS Projetos®, Brazil) at a temperature of 55.5 ± 0.5 °C. The latency until animals jumped or licked their hind

paws was recorded. Before the test the mice were evaluated and a 15-s cut-off time was established. Those who did not respond to the thermal stimulus within this time range were not used in the experiment. Prior to peptide administration, basal responses (mean of three latencies at 5-min intervals) were obtained from the mice (Mortari et al., 2007). Escape latencies were recorded after treatment, at the following time intervals: 20, 40, 60, 90, 120, 150, 180, 210, 240 min, 24 h and 3 days. Independent groups of animals (n = 6) were treated with saline (vehicle), morphine (12 nmol), or the peptide, all administered i.c.v.

Motor response latencies (AL – antinociception latencies) were normalized by the analgesia nociception index (ANI) using the equation:

$$AI = \frac{AL - BML}{TL - BML}$$

where BML is the basal mean latency and TL is the time limit of the experiment (50 s).

The results were expressed as AI averages ± SEM and area under the curve.

2.4.3. Tail flick test

The assays were performed according to the instruction manual for the equipment and in line with the protocol described by Bannon and Malmberg (2007), with modifications. The mice were placed on the digital analgesimeter (Insight®, Brazil) and their tails positioned on top of a Ni-Chrome filament, which heats at a rate of 9 °C/s, reaching a maximum temperature of 75 °C (temperature limit for the equipment). Basal measurements (three times, at 5-min intervals) were performed prior to treatment. Escape latencies were recorded after treatment at the following intervals: 20, 40, 60, 90, 120, 150, 180, 210, 240 min, 24 h and 3 days. Independent groups of animals (n = 6) were treated with saline (vehicle), morphine (12 nmol), or the peptide, all administered i.c.v.

Escape temperatures (ET) were normalized by the analgesia nociception index (AI), using the equation:

$$AI = \frac{ET - BMT}{MT - BMT}$$

where BMT is the basal mean temperature and MT is the temperature limit for the experiment (75 °C).

The results were expressed as AI averages ± SEM and area under the curve.

2.4.4. Motor incoordination

The Rotarod test (Insight®, Brazil) was performed to assess changes in the motor coordination of animals after treatment with Agelaia-MP I. Three days before injection, the animals were trained on the equipment, using five cycles of 5-min sessions with a 5-min rest between each cycle. Animals were selected on the day of the test prior to injection; those who did not remain on the device for 5 min were excluded.

The effect of mastoparan in terms of latency to fall was evaluated after injection at the following intervals: 20, 60, 90, 150, 210 and 1440 min. The parameter assessed in this model was the time (latency) it took for the animals to lose their balance and fall off the rod.

2.4.5. Histological analysis

After the experiments, the mice were euthanized with CO_2 and 10 μL of methylene blue was administrated through the cannula to check whether it was correctly positioned. The brain was then removed and fixed in a 4% formaldehyde solution. A cut was made in the ventricle region and blue staining in both lateral ventricles indicated correct cannula placement.

2.5. Sucrose gap assay

The mechanism of action of Agelaia-MP I was also evaluated in the frog peripheral nervous system. The sciatic nerve was removed from euthanized *Lithobates catesbeianus* bullfrogs, and connective tissue was removed prior to the experiments. Compound action potentials were recorded using the sucrose gap technique (modified from Strong et al., 1973) using a five chamber arrangement. The chambers were isolated from each other using petroleum jelly. Ringer's solution (composition: 111 mM NaCl, 1.9 mM KCl, 1.1 mM $CaCl_2$, 2.4 $NaHCO_3$) was used in all chambers except the fourth, which received 216 mM of sucrose solution. The first two chambers were used for supramaximal stimulation consisting of 6–7 V voltage pulses 25 μs in duration, emitted by an S8 Stimulator (Grass Instruments, USA). The central chamber, with a volume of 350 μL, received the peptide suspended in deionized water. Potential differences between the test chamber and fifth chamber were measured by Ag-Cl electrodes, using a DC differential amplifier with high impedance and a voltage gain of 50, coupled to a TDS 360 digital oscilloscope (Tektronix, USA).

2.6. Statistical analysis

Results with normal distribution were submitted to two-way repeated measures analysis of variance (two-way ANOVA) and the Bonferroni correction. The area under the curves was analyzed by one-way ANOVA, with $p < 0.05$, followed by Tukey's test. ED_{50} was calculated using non-linear regression. GraphPadPrism 6 software for Mac (GraphPad Software, Inc., USA) was used for all analyses.

3. Results

Agelaia-MP I (MW 1565 Da) was isolated from an active fraction of *Parachartergus fraternus* social wasp venom (Fig. 1). Its sequence was determined by MALDI-TOF/TOF analysis (Fig. 2) as Ile/Leu- Asn-Trip-Ile/Leu-Lys/Gln-Ile/Leu-Gly-Lys/Gln-Ala-Ile/Leu-Ile/Leu-Asp-Ala-Ile/Leu – NH_2 and identified as a mastoparan identical to that previously described in another wasp species, *Agelaia pallipes pallipes* (Fig. 3) (Mendes et al., 2004). The peptide was synthesized and tested in bioassays, after checking the purity of the compound.

Agelaia-MP I displayed dose-dependent antinociceptive activity in mice submitted to i.c.v. injection at concentrations of 6.4, 4.8 and 3.2 nmol, in the hot plate model (Fig. 4A and B). The value of ED_{50} was calculated as 4.798 nmol (CI 95% = 4.41 to 5.21) using the data obtained 2 h after injection, when the peptide's maximum effect was obtained (Figs. 4A and 5).

The i.c.v. injection of the peptide was chosen because Agelaia MP I is a highly hemolytic peptide (Mendes et al., 2004) and a different administration route, such as i.v., could cause severe systemic effects in mice. In cases of chronic cancer or acute pain, an Ommaya reservoir can be implanted in the right ventricle to inject morphine directly into the CNS (Cramond and Stuart, 1993; Lobato et al., 1987).

In this test, the highest dose produced a maximum antinociceptive effect for up to 4 h and nociception remained low for three days. Results showed significant differences for treatment effect [$F_{(4,41)} = 12.34$; $p < 0.0001$], time [$F_{(10,410)} = 16.16$; $p < 0.0001$] and treatment-time interaction [$F_{(40,410)} = 3.624$; $p < 0.0001$]. In the first 24 h, Bonferroni's post-test revealed significant differences for the highest peptide concentration and morphine compared to saline and 3.2 mM ($p < 0.001$) in all the time

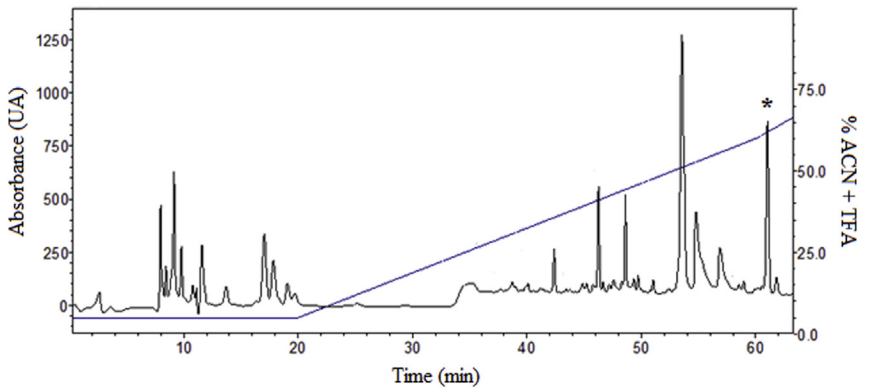

Fig. 1. HPLC profile of low molecular weight compounds (<3000Da) of venom from the social wasp *Parachartergus fraternus*. A reversed phase column was used (C18 ODS, 15 μm, 250 × 10 mm), under the following conditions: 5% isocratic flow of CH_3CN/H2O/0.1% TFA in the first 20 min, followed by a 5–60% linear gradient of CH_3CN/H2O/0.1% TFA for 40 min, at a flow rate of 1.5 ml/min. A total of 4 mg of venom was injected into each chromatography column, and the eluted fractions were monitored at 216 nm.

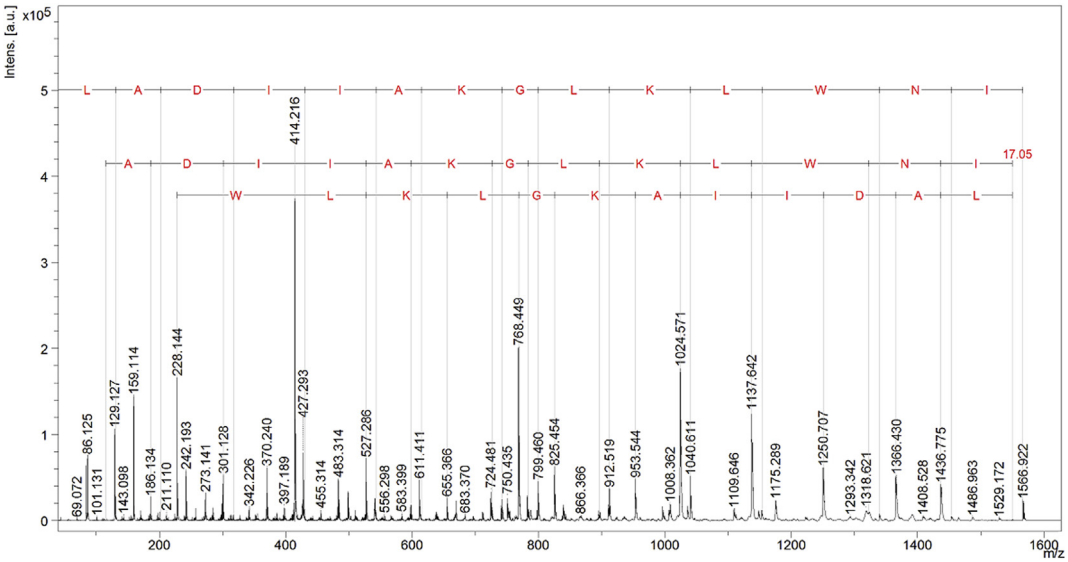

Fig. 2. *De novo* sequencing of the major peptide of the HPLC fraction PfTx 10 by MALDI-TOF/TOF mass spectrometry, using the LIFT method.

periods analyzed. Moreover, three days after peptide administration, concentrations of 6.4 and 4.8 nmol induced an antinociceptive effect when compared to saline, morphine and lower concentrations (Fig. 4).

Analysis of the area under the antinociception index curve in the hot plate test showed a dose-dependent increase in activity (Fig. 4B). One-way ANOVA showed a significant difference between the highest dose and all other treatments, demonstrating the potent antinociceptive effect at this dose. There was also a significant difference between 6.4 and 4.8 nmol doses and the lowest dose of peptide ($p < 0.05$ and $p < 0.001$ respectively).

```
                                10          20
                          ....|....|....|....|.
Parachatergus fraternus   INWLKLGKAIIDAL
Agelaia p. pallipes       INWLKLGKAIIDAL--100%
Protopolybia exigua       INWLKLGKAIIDAL--92.9%
Polybia paulista          INWLKLGKMVIDAL--85.7%
P. paulista               IDWLKLGKMVMDVL--64.3%
```

Fig. 3. Mastoparan sequences from social wasps aligned using the Clustal W multiple sequencing alignment tool and Bioedit software v.7.0.4.1.

The animals tested in the tail flick assay showed no significant antinociceptive effect in relation to peptide, but significant differences were observed for morphine (Fig. 6A and B). Two-way ANOVA revealed significant treatment [$F_{(4,25)} = 44.759$; $P < 0.0001$], time [$F_{(10,25)} = 8.089$; $p < 0.0001$] and treatment-time interaction [$F_{(40,250)} = 5.329$; $p < 0.001$]. Morphine differed significantly from all other treatments at 4 h; however, the effect was completely reversed on the second and third days after injection (Fig. 6A). Similarly, significance was not observed for peptide action in the area under the curve. Statistical analysis showed a significant difference between morphine, the peptide doses and saline [$F_{(4, 27)} = 8.419$, $p < 0.001$] (Fig. 6B).

With respect to the peptide's effect on motor coordination, significant differences were observed [$F(5,15) = 6.403$; $p < 0.05$] (Fig. 7). At the maximum dose at which analgesia tests were performed (6.4 nmol), there was no change in time spent in the testing equipment compared to saline ($p > 0.05$ for all time periods). However, at doses 5 and 10 times higher (32 and 64 nmol), analysis showed a change after 3 h experiment (180 min), which remained until the last evaluation period (24 h after administration).

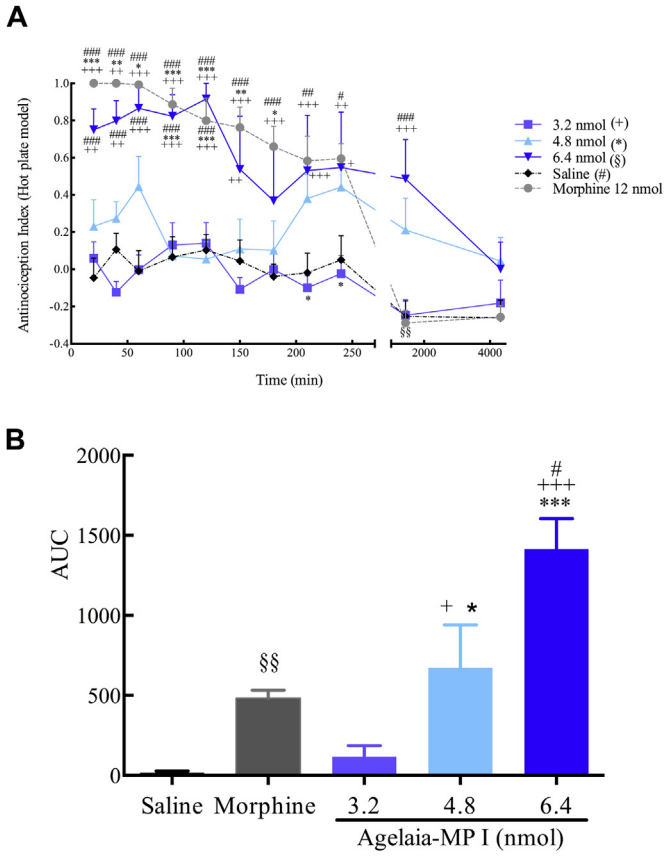

Fig. 4. Hot plate escape latencies after i.c.v. administration of Agelaia MP I in mice. **A)** Antinociception index (AI). Data were analyzed by two-way ANOVA, followed by the Bonferroni post hoc test. **B)** Area under the AI curve. Data were analyzed by ANOVA, followed by Tukey's post hoc test. * significant differences compared to saline, *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$. # significant differences compared to morphine; + significant differences compared to the 3.2 mM dose. § significant differences compared to the 4.8 mM dose.

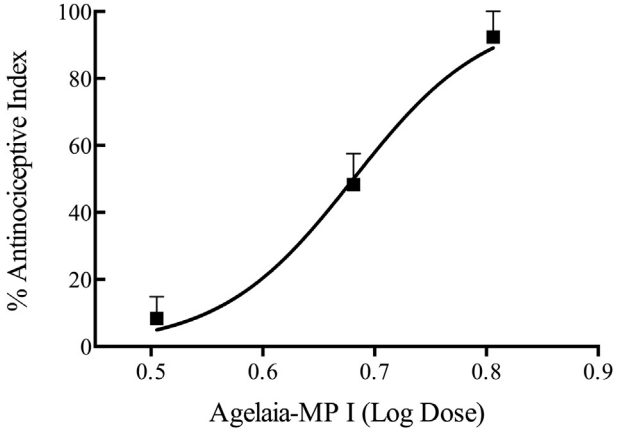

Fig. 5. Dose-response curve of the antinociception index induced by Agelaia-MPI.

Additional experiments on isolated frog sciatic nerve using the sucrose gap technique showed that Agelaia-MP I induced partial (67%) blockade of the amplitude of action potential at 1 mM. The maximum effect of the peptide was achieved after 15–16 min of incubation. Removal of the peptide by continuous washing with physiological solution for 5 min enabled full recovery of the initial action potential amplitude (Table 1).

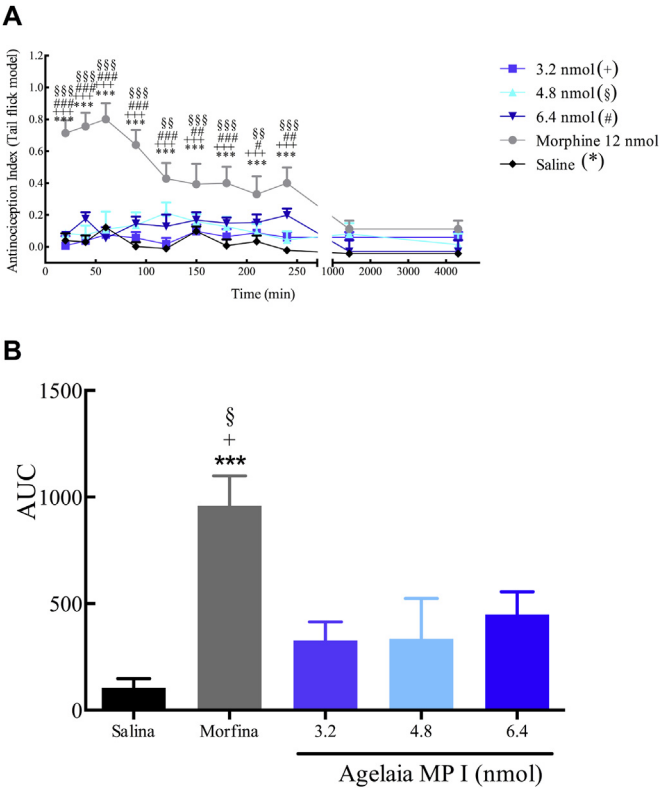

Fig. 6. Tail flick times after i.c.v. administration of Agelaia-MPI in mice. **A)** Antinociception index (AI). Data were analyzed by two-way ANOVA, followed by the Bonferroni post hoc test. * significant differences compared to saline, *** = $p < 0.001$; ** = $p < 0.01$; * = $p < 0.05$. # significant differences compared to the 6.4 mM dose; + significant differences compared to the 3.2 mM dose. § significant differences compared to the 4.8 mM dose. **B)** Area under the AI curve. Data were analyzed by ANOVA, followed by Tukey's post hoc test. * significant differences compared to saline; # significant differences compared to morphine.

4. Discussion

The present study describes for the first time the antinociceptive effect of a mastoparan peptide. CNS activity of mastoparans was first described by Blazquez and Garzon in 1994. The authors demonstrated that mastoparan isolated from *Vespula lewisii* could interact with G-protein-coupled opioid receptors, and that this effect lasted up to nine days after administration, interfering with the analgesic effect of opioid agonists (Sánchez-Blázquez and Garzón, 1994). In addition, a number of discoveries have been made regarding the functions of this molecule in vivo and *in vitro*

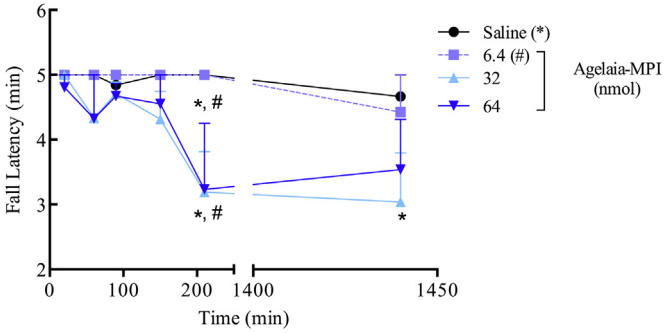

Fig. 7. Effect of the peptide Agelaia-MPI on motor incoordination evaluated using the rotarod performance test.

Table 1
Effects of the Agelaia-MP I on the peak amplitude of the compound action potentials of frog sciatic nerve. For control experiments, a deionized water was used to dilute the peptide (30 μL of vehicle). Percentage recovery is the % of initial amplitude recovered after washing the peptide with deionized water. Unpaired T test; *p < 0.05.

Group	N	% Reduction (Mean ± SEM)	% Recovery
Control	3	11.7 ± 11.73	100 ± 0
Agelaia-MPI	4	68.07 ± 11.87*	100 ± 0

and it is known to act as an antimicrobial, antitumor, cytolitic and insulinotropic agent (Baptista-Saidemberg et al., 2012; de Azevedo et al., 2015; dos Santos Cabrera et al., 2008; Leite et al., 2015). Mastoparans are the most abundant peptide components in wasp venom (Nakajima et al., 1985), and can be found not only in the venom of social and solitary wasps, but also in the hemolymph of *Sphodromantis viridis* (Zare-zardini et al., 2015). It is structurally and functionally similar to melittin, a peptide found in bee venom, and bombinin, found on the skin of the *Bombina variegata* toad (Argiolas and Pisano, 1984; Csordas and Michl, 1969).

Structurally, mastoparan is an amphiphilic peptide with an α-helix structure, no cysteine in its primary sequence, and typically contains 10–14 amino acid residues (Nakajima et al., 1986). The large amount of lysine residue in its chains likely facilitates the release of histamine from mast cells and positively charges the molecule by increasing its affinity with the negatively charged biological membranes (dos Santos Cabrera et al., 2008; Higashima et al., 1990; Konno et al., 2000).

The data in the present study show that mastoparan has an antinociceptive effect when injected directly into the CNS of mice in the hot plate test. In addition, its effect was dose-dependent, and the largest dose showed a prolonged antinociceptive effect up to three days after injection.

To date, only two antinociceptive compounds from wasp venoms have been identified and evaluated. Thr^6-BK (RPPGFTPFR), isolated from *Polybia occidentalis*, exerted a strong effect in i.c.v. administration in rats. Pallipin-III, a 22 residue amidated sequence (SIKKHKCIALLKRRGGSKLPFC-NH2) isolated from the social wasp *Agelaia pallipes pallipes*, exhibited antinociceptive and anti-inflammatory effects when injected peripherally into mice (Baptista-Saidemberg, 2011).

Differences were observed between both antinociceptive assays performed with mice, and the peptide Agelaia MP I was more active in the hot plate test. This may be due to i.c.v. administration, since the tail flick response is modulated by receptors in the spinal cord. Thus, the tail flick test has often been used to evaluate drugs with opioid-like activity, although largely via systemic administration (Le Bars et al., 2001). The antinociceptive effect of the peptide in the hot plate model was about 2.5 times greater than in the tail flick test. According to Le Bars et al. (2001), this result suggests that Agelaia MP I may act on non-opioid receptors.

Direct i.c.v. administration of drugs has long been used in a number of CNS conditions, such as refractory pain, brain infection, brain tumor, and intracerebral hemorrhage (Cook et al., 2009). For example, in cases of cancer-related pain, an Ommaya reservoir containing morphine can be implanted directly into the right ventricle of the CNS (Cramond and Stuart, 1993; Lobato et al., 1987). In addition, icv and intrathecal delivery are the most commonly used drug administration routes for in vivo research in rodents, and are ideal to investigate both desired and undesired in vivo brain effects. (Kuo and Smith, 2014). Moreover, i.c.v. administration of novel compounds from drug discovery programs makes it possible to determine their intrinsic efficacy and adverse effects, which are mediated predominantly by supraspinal mechanisms. Another advantage is that the brain parenchyma has a low capacity to metabolize exogenous compounds, allowing a high rate of interaction.

It is important to note that, according to ataxia tests for the antinociceptive effective dose (6.4 nmol), Agelaia-MP I caused no change in animals, indicating that the peptide was well tolerated at this dose. However, motor disturbances were observed when the peptide concentrations used were 5–10 times higher than that capable of inducing analgesia.

The sucrose gap test revealed possible action on Na_v channels, blocking the compound action potential evoked from the frog nerve. The effect of the peptide was reversible after continuous washing, therefore excluding the hypothesis of membrane lesions in the axons of the nerve. In this respect, the peptide could block direct transmission of the action potential to the CNS by Na_v channels blockade and cause pre-synaptic inhibition, thus preventing neurotransmitter release (Mizoguchi et al., 2012).

As shown in the chromatographic profile of *Parachartergus fraternus* venom, there are still a number of low molecular weight compounds to be studied in this venom, which has significant pharmacological and biotechnological potential. With respect to mastoparan, the present study reports on an additional function for this multifunctional molecule: antinociceptive activity. This property requires further study in order to better understand its mechanisms of action and enable the future development of a new treatment alternative for pain.

5. Conclusions

The results of this investigation demonstrated the significant potential of compounds that act on the central nervous system isolated from wasp venom. In addition, the antinociceptive effect of the peptide Agelaia MP I described here is a novel activity for the mastoparan class of peptides.

Acknowledgements

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Transparency document

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