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Agelaia MP-I: A peptide isolated from the venom of the social wasp, *Agelaia pallipes pallipes*, enhances insulin secretion in mice pancreatic islets

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ABSTRACT

Peptides isolated from animal venoms have shown the ability to regulate pancreatic beta cell function. Characterization of wasp venoms is important, since some components of these venoms present large molecular variability, and potential interactions with different signal transduction pathways. For example, the well studied mastoparan peptides interact with a diversity of cell types and cellular components and stimulate insulin secretion via the inhibition of ATP dependent K^+ (K_{ATP}) channels, increasing intracellular Ca^{2+} concentration. In this study, the insulin secretion of isolated pancreatic islets from adult *Swiss* mice was evaluated in the presence of synthetic Agelaia MP-I (AMP-I) peptide, and some mechanisms of action of this peptide on endocrine pancreatic function were characterized. AMP-I was manually synthesized using the Fmoc strategy, purified by RP-HPLC and analyzed using ESI-IT-TOF mass spectrometry. Isolated islets were incubated at increasing glucose concentrations (2.8, 11.1 and 22.2 mM) without (Control group: CTL) or with 10 μM AMP-I (AMP-I group). AMP-I increased insulin release at all tested glucose concentrations, when compared with CTL ($P < 0.05$). Since molecular analysis showed a potential role of the peptide interaction with ionic channels, insulin secretion was also analyzed in the presence of 250 μM diazoxide, a K_{ATP} channel opener and 10 μM nifedipine, a Ca^{2+} channel blocker. These drugs abolished insulin secretion in the CTL group in the presence of 2.8 and 11.1 mM glucose, whereas AMP-I also enhanced insulin secretory capacity, under these glucose conditions, when incubated with diazoxide and nifedipine. In conclusion, AMP-I increased beta cell secretion without interfering in K_{ATP} and L-type Ca^{2+} channel function, suggesting a different mechanism for this peptide, possibly by G protein interaction, due to the structural similarity of this peptide with Mastoparan-X, as obtained by modeling.

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1. Introduction

Peptides occur in the whole animal kingdom and are involved in most, if not all, physiological processes in animals. The knowledge of the amino acid sequence of peptide hormones or neurotransmitters is important for the synthesis of large quantities of peptides, in order to perform further functional analysis (Baggerman et al., 2004). Wasp venoms are a rich source of peptides involved in pain and local tissue damage, among other effects. Some of these peptides can interact with G-protein

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coupled receptors (GPCR), and are involved in the activation of different types of basophiles, chemotaxis of polymorphonucleated leukocytes (PMNL), smooth muscle contraction and neurotoxicity (Ishay et al., 1975; Nakajima, 1984; Oliveira et al., 2005; Rocha et al., 2008).

The most abundant classes of peptides, isolated from wasp venoms, are the mastoparans, followed by antibiotic and chemotactic peptides (Nakajima et al., 1986). Classically, peptides from the mastoparan group are reported to be 10–14 amino acid residues long and to have an α helix conformation (Nakajima et al., 1986; Mendes et al., 2005). These peptides are also rich in lysine residues, which are thought to perform a key role in the stimulation of histamine release from mast cells (Higashijima et al., 1990), serotonin from platelets and prolactin from the anterior pituitary gland (Hirai et al., 1979a; Kuroda et al., 1980). In addition, recent studies proposed the classification of peptides based on their physicochemical properties, instead of primary sequence similarities (Saidemberg et al., 2011).

Mastoparan, the first peptide of this class, was reported to be capable of stimulating the release of granules from mast cells (Hirai et al., 1979a). However, different studies have shown that this peptide can stimulate the degranulation of other cell types, such as: MIN6 cells (Ohara-Imaizumi et al., 2001), INS-1 cells (Amin et al., 2003) and beta pancreatic cells (Gil et al., 1991; Komatsu et al., 1992, 1993; Hillaire-Buys et al., 1992; Eddlestone et al., 1995; Konrad et al., 1995; Kowluru et al., 1995; Straub et al., 1998; Kowluru, 2002; Amin et al., 2003; Chen et al., 2004; Omata et al., 2005). Mastoparan can alter some of the biochemical mechanisms involved in the secretory response of these cells, enhancing, for example, the activity of phospholipase A_2 (PLA_2) (Argiolas and Pisano, 1983; Gil et al., 1991; Joyce-Brady et al., 1991; Komatsu et al., 1992) and phospholipase C (PLC) (Okano et al., 1985; Mousli et al., 1989; Perianin and Snyderman, 1989; Wallace and Carter, 1989; Gusovsky et al., 1991; Choi et al., 1992). This peptide can also reduce phosphoinositide separation via the suppression of PLC, or by the direct interaction of the peptide with phosphoinositides (Nakahata et al., 1989; Wojcikiewicz and Nahorski, 1989; Eddlestone et al., 1995).

The Mastoparan peptide is reported to be capable of stimulating (Wheeler-Jones et al., 1992) or suppressing (Nakahata et al., 1989; Joyce-Brady et al., 1991) adenylate cyclase activity, since this peptide can bind to calmodulin in a stochiometric proportion of 1:1 (Barnette et al., 1983; Malencik and Anderson, 1983). Other activities of this peptide include the augmentation of DNA synthesis due to the improvement of the GTP/GDP exchange of heterodimeric G proteins; mastoparan also stimulates arachidonic acid release via a pertussis toxin-sensitive G protein in Swiss 3T3 cells. Arachidonic acid, like mastoparan, stimulates DNA synthesis in the presence of insulin (Higashijima et al., 1988; Mousli et al., 1989; Gil et al., 1991; Higashijima and Ross, 1991; Eddlestone et al., 1995). In addition, Mastoparan may also be capable of lysing eukaryotic cells (Hirai et al., 1979a, 1979b; Kurihara et al., 1986; Katsu et al., 1990; Tanimura et al., 1991).

To date, Mastoparan, is the only peptide toxin to be isolated from wasp venom that is reported to stimulate the release of insulin (Daniel et al., 2002). This stimulation occurs by enhancing intracellular Ca^{2+} concentration, via inhibition of the K_{ATP} channels (Eddlestone et al., 1995). Considering the importance of the discovery of anti-diabetes drugs and the reported action of Mastoparan on pancreatic beta cells, the study of similar molecules is fundamental, since this kind of study also increases knowledge regarding envenomation due to wasp sting accidents.

Agelaia MP-I (AMP-I) is a mastoparan peptide (INWLKLGKAIIDAL–NH_2), isolated from the venom of the social wasp venom *Agelaia pallipes pallipes*, that has 14 amino acid residues and exhibits significant hemolytic, mast cell degranulation, and chemotactic activities (Mendes et al., 2004; Baptista-Saidemberg et al., 2011). Due to the characteristics reported for these peptides, we have investigated the ability of the AMP-I peptide to modulate the secretion of insulin from langerhans islets isolated from mice, both in the presence of low and high concentrations of glucose. The mechanism involved in this modulation is independent of the K_{ATP} and L-type Ca^{2+} channels.

2. Materials and methods

2.1. Peptide synthesis, purification and molecular structure studies

2.1.1. Peptide synthesis

The peptide (INWLKLGKAIIDAL–NH_2) was prepared by step-wise manual solid-phase synthesis using *N*-9-fluorophenylmethoxy-carbonyl (Fmoc) chemistry with Novasyn TGS resin (NOVABIOCHEM). Side-chain protective groups included *t*-butyl for serine and *t*-butoxycarbonyl for lysine. Cleavage of the peptide–resin complexes was performed by treatment with trifluoroacetic acid/1,2-ethanedithiol/anisole/phenol/water (82.5:2.5:5:5:5 by volume), using 10 mL/g of complex at room temperature for 2 h. After filtering to remove the resin, anhydrous diethyl ether (SIGMA) was added at 4 °C to the soluble material causing precipitation of the crude peptide, which was collected as a pellet by centrifugation at 1000 × *g* for 15 min at room temperature. The crude peptide was solubilized in water and chromatographed under RP-HPLC using a semi-preparative column (SHISEIDO C18, 250 mm × 10 mm, 5 μm), under isocratic elution with 60% (v/v) acetonitrile in water [containing 0.1% (v/v) trifluoroacetic] at a flow rate of 2 mL/min. The elution was monitored at 214 nm with a UV-DAD detector (SHIMADZU, mod. SPD-M10A), and each fraction eluted was manually collected into 1.5 mL glass vials. The homogeneity and correct sequence of the synthetic peptides were assessed using a gas-phase sequencer PPSQ-21A (SHIMADZU) based on automated Edman degradation chemistry and ESI-MS analysis.

2.1.2. Peptide purification

The synthetic peptide was purified by using a CAPCELL PACK C-18 UG120 column (10 mm × 250 mm, 5 μm, SHISHEIDO) under isocratic elution with 38% (v/v) MeCN [containing 0.1% (v/v) TFA]. The elution was monitored at 214 nm, and fractions were manually collected into 5 mL glass vials.

2.1.3. Mass spectrometric analysis

MS analyses were conducted on an ion trap/time-of-flight mass spectrometer (IT-TOF/MS) (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization source. The setting conditions for optimized operations were: positive mode, electrospray voltage 4.5 kV, CDL temperature 200 °C, block heater temperature 200 °C, nebulizer gas (N_2) flow of 1.5 L/min, trap cooling gas (Ar) flow of 95 mL/min, ion trap pressure 1.7×10^{-2} Pa, TOF region pressure 1.5×10^{-4} Pa, ion accumulation time 50 ms. The auto-tuning was performed with a Na-TFA solution and showed the following parameters: for the positive mode, error 3.1 ppm and resolution 11,000; and for the negative mode, error 2.3 ppm and resolution 13,000.

2.1.4. Molecular modeling

The search for templates for the AMP-I target sequence was performed with Blastp (Altschul et al., 1997) and the alignment (Table 1) was formatted and input into the program. The structure of the homologous peptide (Mastoparan-X) was selected from the Protein Data Bank (PDB) (Berman et al., 2000), which was solved experimentally by RMN (PDB ID: 1A13) (Kusunoki et al., 1998). The AMP-I model was built with restrained-based modeling implemented in MODELLER9v8 (Sali and Blundell, 1993), with the standard protocol of the comparative protein structure modeling methodology, by satisfaction of spatial restraints (Sali and Overington, 1994; Marti-Renom et al., 2000). A total of 1000 models were created and the best models were selected according to MODELLER objective function (Shen and Sali, 2006) and stereochemical analysis with PROCHECK (Laskowsky et al., 1993). The primary sequence similarity between the peptide with the template was 65% (identity 58%). The final models were selected with 100% residues in favored regions of the Ramachandran plot (Fig. 1), with the best values of the overall *G*-factor and the lower values of energy minimization (Table 2). For visualization of the model of AMP-I, the PyMOL program was used (DeLano, 2002).

2.1.5. Analysis of models

The overall stereochemical quality of the final models for Agelaia MP-I was assessed by the PROCHECK program (Koradi et al., 1996). The root mean square deviation (rmsd) between Cα–Cα atom's distance was superposed using the program LSQKAB from CCP4 (Konno et al., 2007). The cutoff for hydrogen bonds and salt bridges was 3.3 Å. The contact area for the complexes was calculated using AREAIMOL and RESAREA (Konno et al., 2007). The root mean square deviation (rmsd) differences from ideal geometries for bond lengths and bond angles were calculated with X-PLOR (Krishnakumari and Nagaraj, 1997). The *G*-factor value is essentially just log-odds score based on the observed distributions of the stereochemical parameters. This is computed for the following properties: torsion angles (the analysis provided the observed distributions of $\varphi - \psi$, $\chi1 - \chi2$, $\chi - 1$, $\chi - 3$, $\chi - 4$, and ω values for each of the 20 amino acid types) and covalent geometry (for the main-chain bond lengths and bond angles). The average of these values was calculated using PROCHECK (Koradi et al., 1996). The Verify-3D measures the compatibility of a protein model with its sequence, using a 3D profile (Laskowsky et al., 1993; Kusunoki et al., 1998; Lee et al., 1999).

Table 1
Amino acid sequences for Agelaia MP-I described by Mendes et al. (2004) and revisited by Baptista-Saidemberg et al. (2011), Mastoparan described by Hirai et al. (1979a), and Mastoparan-X described by Hirai et al. (1979b) aligned by multialign software (http://multalin.toulouse.inra.fr/multalin/cgi-bin/multalin.pl, accessed in November 24th, 2011). The red color indicates that the amino acid residue is present in all peptides. The blue color indicates an amino acid match for at least two peptides. The black color indicates a difference between the amino acids in the sequences. (For interpretation of the references to colour in this table legend, the reader is referred to the web version of this article.)

Peptide	Peptide sequence	C-Terminal
Mastoparan	INLKALAALAKKIL	NH_2
Mastoparan-X	INWKGIAAMAKKLL	NH_2
Agelaia MP-I	INWLKLGKAIIDAL	NH_2
Consensus	INWK.LAA.AKK.L	NH_2

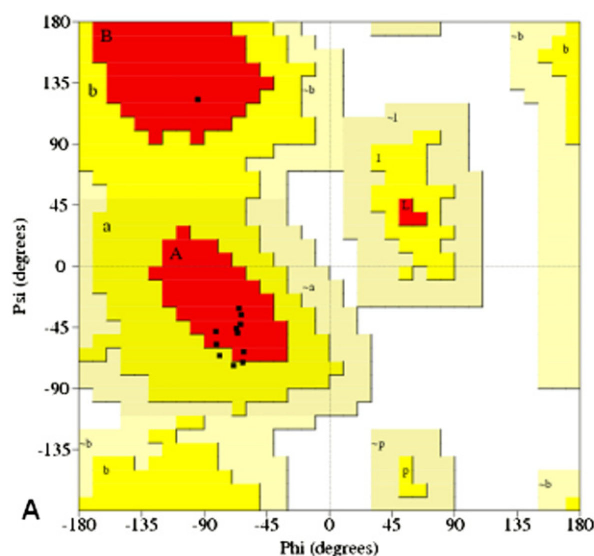

Fig. 1. Ramachandran plot from Procheck for Agelaia MP-I model.

2.2. AMP-I and insulin secretion

2.2.1. Animals

All experiments were approved by the ethics committee at the Universidade Estadual de Campinas – UNICAMP (protocol number 2585-1). The studies were carried out on 90-days-old male *Swiss* mice obtained from the breeding colony at UNICAMP and maintained at 22 ± 1 °C, on a 12-h light–dark cycle, with free access to food and water.

Table 2
Access PDB code for Agelaia MP-I model presenting the identity and similarity values between both peptides and the model resolution method.

Peptide	Template	Access	Identity (%)	Similarity (%)	Sequence resolution
Agelaia MP-I	Mastoparan-X (MP-X)	1A13	58	65	MNR

Table 3
Analysis of the stereochemical quality for Agelaia MP-I.

Peptide	Ramachandran diagram area				G-factor		
	More favorable (%)	Additionally allowed (%)	Favorably allowed (%)	Not allowed (%)	Torsion angle	Covalent geometry	Total average
Agelaia MP-I	100	0	0	0	0.52	−0.09	0.26

2.2.2. Langerhans islet isolation and static insulin secretion

Islets were isolated by collagenase digestion of the pancreas. For static incubations, four islets were first incubated for 30 min at 37 °C in Krebs–bicarbonate (KRB) buffer with the following composition in mM: 115 NaCl, 5 KCl, 2.56 CaCl2, 1 MgCl2, 10 NaHCO3, 15 HEPES, supplemented with 5.6 mM glucose, 3 g/L of bovine serum albumin (BSA) and equilibrated with a mixture of 95% O_2/ 5% CO_2 to give pH 7.4. This medium was then replaced with fresh buffer, and the islets were incubated for 1 h with 2.8, 11.1 or 22.2 mM glucose without (control group: CTL) or with AMP-I peptide (AMP-I group). For analysis of whether the AMP-I peptide interacts with KATP or L-type Ca2+ channels, the islets were incubated with 2.8 or 11.1 mM glucose plus 250 μM diazoxide or 10 μM nifedipine. At the end of the incubation period, the insulin content of the medium was measured by radioimmunoassay (Ribeiro et al., 2010).

2.2.3. Statistical analysis

Results are presented as means ± S.E.M. for the number of determinations (n) indicated. The statistical analyses were carried out using ANOVA Bonferroni, $P \leq 0.05$ were performed using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results and discussion

After AMP-I synthesis, fractionation and purification, the ESI-MS analysis of the synthetic peptide presented a compound with m/z 1566.5 as $[M + H]^+$ and 784.1 as $[M + 2H]^{2+}$. The sequencing and homogeneity of AMP-I was confirmed by mass spectrometry and Edman degradation chemistry (not shown data, for reference see Baptista-Saidemberg et al., 2011).

AMP-I sequence differs from the original Mastoparan peptide (from *Vespula lewisii*), as shown in Table 1. However, considering the characteristics of the data obtained to develop the molecular modeling of AMP-I, the results of biological assays of hemolysis ($ED_{50} = 6 \times 10^{-6}$ M) and mast cell degranulation ($ED_{50} = 4 \times 10^{-5}$ M)obtained by Baptista-Saidemberg et al. (2011), besides *in silico* classification using physicochemical properties by PCA (Saidemberg et al., 2011) it is possible to confirm that AMP-I is also a mastoparan class peptide.

Agelaia MP-I was modeled using Mastoparan-X as a template model (Table 3) and the Ramachandran plot (Fig. 1) shows that the structure obtained for AMP-I was correct. The 3D structure of AMP-I was shown in Figs. 2 and 3. Fig. 2 demonstrates the amino acid sequence, while Fig. 3 shows the charge distribution along the sequence. These characteristics of a high percentage of alpha helices, net charge, and hydrophobicity are in accordance with the PCA grouping of this peptide, as described recently by Saidemberg et al. (2011). The molecular modeling of this peptide is fundamental for understanding its activity in relation to structure.

Fig. 4 shows insulin secretion in isolated islets incubated with AMP-I peptide. AMP-I increased glucose-induced insulin secretion in a dose-dependent manner. Isolated islets incubated with AMP-I showed enhanced insulin release at all glucose concentration tested, when compared with the CTL islets ($P < 0.05$).

The effects of AMP-I upon pancreatic islets were not due to lysis, since islets from the AMP-I group at the end of the experiments were re-incubated under the same conditions of glucose concentrations, without AMP-I, and showed a similar secretory function to that observed for CTL islets (data not shown).

To verify a possible action of AMP-I upon K_{ATP} and L-type Ca^{2+} channels in pancreatic beta cells function, we used diazoxide (DZX) and nifedipine (NIF) (Fig. 5). The DZX drug is a selective ATP-sensitive K^+ channel activator in both vascular smooth muscle and pancreatic β-cells, and is anti-hypertensive (Grimmsmann and Rustenbeck, 1998); while NIF is a L-type Ca^{2+} channel blocker that induces apoptosis in human glioblastoma cells (Mayer and Thiel, 2009).

Enhanced insulin release was also observed in the AMP-I group when incubated with DZX or NIF ($P < 0{,}05$). On other hand, in the CTL group, DZX and NIF completely inhibited glucose-induced secretion. In contrast to the results of AMP-I, the Mastoparan peptide has been shown to increase the intracellular free calcium concentration by inhibition of ATP-sensitive potassium channels (Eddlestone et al., 1995), suggesting that different mastoparan peptides can act by different mechanisms. Mastoparan and its

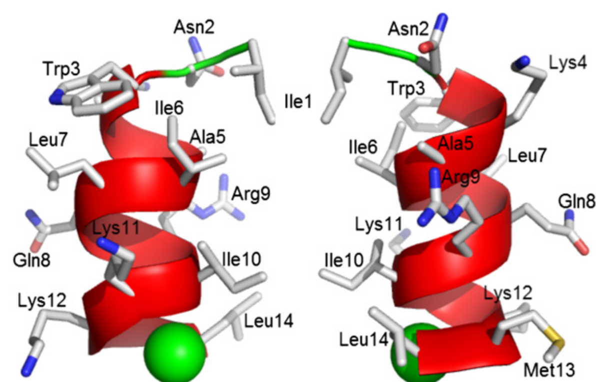

Fig. 2. Images generated by MolMol program for the secondary structure (ribbon) of the Agelaia MP-I peptide. The coil region is the N-terminal, the green circle represents the amidated C-terminal ($COONH_2$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

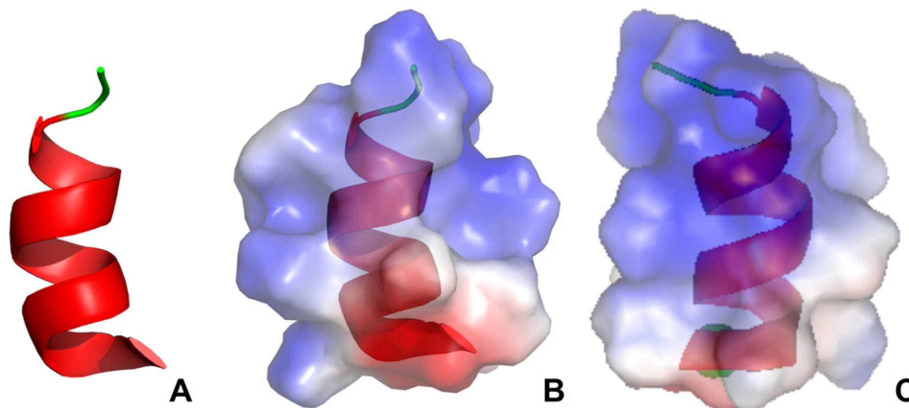

Fig. 3. Images generated by the PyMol program showing the ribbon structural conformation of the Agelaia MP-I peptide.

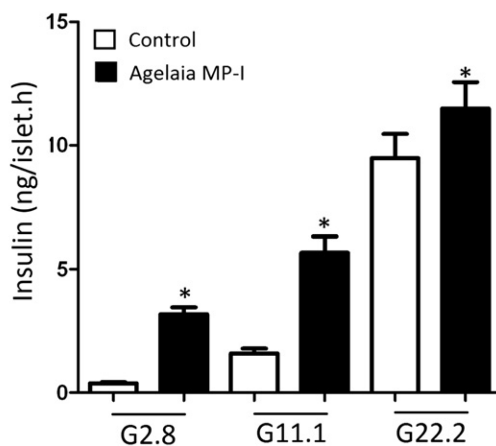

Fig. 4. Insulin secretion in response to increasing glucose concentrations, in combination or not with 10 μM Agelaia MP-I. Data represents mean ± SEM, $n = 8$. $^*P < 0.05$ vs CTL.

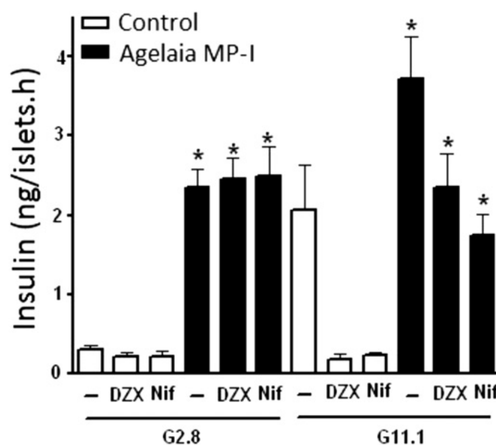

Fig. 5. Insulin secretion in response to 2.8 (G2.8) and 11.1 (G11.1) mM glucose with or without 10 μM Agelaia MP-I. Data represents mean ± SEM, $n = 8$. $^*P < 0.05$ vs CTL.

analogue are also reported to interact with G proteins (Weingarten et al., 1990; Wakamatsu et al., 1992), therefore due to the similarity of AMP-I with Mastoparan-X, a very well described G protein interacting peptide (Sukumar and Higashijima, 1992; Wakamatsu et al., 1992), this is a very good clue about the mechanism of action of AMP-I, since several important sites regulating stimulus-secretion coupling and release of insulin from pancreatic beta-cells are modulated by G proteins (Robertson et al., 1991).

The principal component analysis (PCA) classification, described by Saidemberg et al. (2011), of the Mastoparans also indicates that some edge peptides from this large class, in addition to having similar general physico-chemical properties, can show some superposition with other peptide groups. Therefore, besides having the same general activities, different mastoparans can have different mechanisms of action and properties. This feature is closely related to its structure and physico-chemical properties, which can lead to the opening of new structure–function relationship studies of peptides for pharmacological applications.

4. Conclusion

Agelaia MP-I, like the Mastoparan peptide, is a peptide capable of interacting with different components of cells (phospholipids, receptors, ionic channels) and promoting the degranulation of different granulocytes. As such, AMP-I showed a positive and non-lytic effect upon pancreatic beta cell function. In contrast to Mastoparan, AMP-I did not affect K_{ATP} nor L-type Ca^{2+} channel activity in pancreatic beta cells, suggesting a different mechanism for this peptide, possibly by a G protein interaction due to the structural and physicochemical similarity of this peptide with Mastoparan-X, as obtained by modeling. This study may open interesting new structure–activity relationship perspectives for peptides with pharmacological interest for future studies related to metabolic endocrine disease.

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Institute of UNESP – Rio Claro/SP, while the biological assays were assayed at the Endocrine Pancreas Laboratory – Biology Institute of UNICAMP – Campinas/SP. This research was supported by FAPESP (2011/51684-1), and CAPES grants. MSP and EMC are researchers of CNPq.

Conflict of interest

No competing interests.

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