Two new bradykinin-related peptides from the venom of the social wasp Protopolybia exigua (Saussure)

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1. Introduction

In 1954 Jacques and Schachter [28] reported the presence of bradykinin-related peptides in the venom of social wasp Vespa vulgaris. These peptides were named wasp kinins due to pharmacological properties similar to the mammalian pain producing nonapeptide bradykinin (BK) [27].

Kinins are polypeptides (9–18 amino acid residues) containing a bradykinin-like sequence at the C-terminal [25]. In some cases the whole nonapeptide sequence (BK) is present within the kinin sequence. The primary sequences of most of the kinin-related peptides from animal venom are longer, their pharmacological actions more potent and effects longer lasting compared to BK [17,25]. A series of different bradykinin-related peptides have been identified in the venoms from different species of the social and solitary wasps [2,7,10,20,21,32–34]. Wasp kinins cause hypertension in rats, dogs, rabbits and cats, hypotension in chickens, brochoconstriction in guinea pigs, contraction of isolated smooth muscle preparations (rat uterus, rabbit jejunum, guinea pig ileum, rabbit colon and frog stomach fundus) and relaxation of rat ileum muscle and had no algesic effects. However, Protopolybiakinin-I caused less potent constriction of the isolated rat ileum muscles than bradykinin (BK). In addition, it caused degranulation of mast cells which was seven times more potent than BK. This peptide causes algesic effects due to the direct activation of B₂-receptors. Protopolybiakinin-II is not an agonist of rat ileum muscle and had no algesic effects. However, Protopolybiakinin-II was found to be 10 times more potent as a mast cell degranulator than BK. The amino acid sequence of Protopolybiakinin-I is the longest among the known wasp kinins.

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wasp Colpa interrupta [15]. Glycosylated wasp kinins have been described in the venom of Paravespula maculifrons [7,25]. These wasp kinins block the excitatory nicotinic transmission with a concurrent activation of inhibitory GABA-ergic system and delayed reversible block of synaptic transmission [24,25].

The function of kinin-related peptides in wasp venom is not fully clear. Kinin-related peptides can cause constriction/relaxation of muscles [17], activation of leukocytes followed by a release of cytokines, prostaglandines, leukotrienes, reactive oxygen species and the blockage of the cholinergic transmission in the insect central nervous system (CNS) [25]. These observations suggest that the kinin-related peptides are used as venom toxins for defense or predation, depending on the type of animal venom under consideration.

Kinin-related peptides have also been isolated from the venoms of ants, bees, snakes, frogs and spiders [6,15,16,24,26,29]. Protopolybia exigua is an aggressive wasp that causes frequent stinging accidents in southeast Brazil, where this insect is endemic. Therefore it is important to investigate the biochemical and pharmacological characterization of the kinin-related peptides present in P. exigua venom to understand its composition so that it can help in envenomation mechanisms. In the present work, identification and biochemical/pharmacological characterization of two novel wasp kinin peptides isolated from the venom of neotropical wasp P. exigua is described. These peptides were extracted from the wasp venom with 50% (v/v) acetonitrile, followed by RP-HPLC fractionation. Their amino acid sequences were determined by automated Edman degradation chemistry. These observations suggest that the kinin-related peptides are used as venom toxins for defense or predation, depending on the type of animal venom under consideration.

2. Materials and methods

2.1. Biological material and extract preparation

Colonies of P. exigua (Saussure) were collected in Rio Claro, SP, southeast Brazil, immediately frozen and stored at −20 °C. The venom reservoirs were removed by micro-dissection using surgical micro-scissors from 400 workers and the venom was extracted with 1:1 acetonitrile/ultra pure water. The extract was lyophilized and kept at −20 °C.

2.2. Materials and Instruments

Acetonitrile (HPLC grade) was obtained from ALDRICH, and trifluoroacetic acid (TFA) analytical-reagent grade, was from CARLO ERBA. For preparation of the eluents, high-purity water (Nanopure Barnstead) was used. The purification was carried out in a HPLC system (SHIMADZU, model CBM-10A), equipped anisole/phenol/water (82.5:2.5:5:5:5 by volume), using ethanedithiol, anisole, phenol and ethyl ether were purchased from ALDRICH.

2.3. Sample preparation and peptide purification

The lyophilized venom extract (1800 μg crude venom) was solubilized in 5% (v/v) MeCN in a concentration of 100 μg/ml and chromatographed by RP-HPLC on a SHISEIDO Nucleosil C-18 (ODS) column (250 mm × 4.6 mm; 5 μm), at a flow rate of 2 ml/min using a 5–60% MeCN (containing 0.1% TFA) gradient (v/v), at 30 °C for 45 min. The elution was monitored at 215 nm with a UV-DAD detector (SHIMADZU, mod. SPD-M10A) and the fractions for each peak were manually collected into 2 ml plastic vials. Peaks of interest were resubmitted to RP-HPLC using a SHISEIDO Nucleosil C-18 (ODS) column (250 mm × 4.6 mm; 5 μm), under isocratic elution with 40% (v/v) MeCN (containing 0.1% TFA) at a flow rate of 700 μl/min for 30 min at 30 °C. The elution was monitored and fractions were collected as before. The homogeneity of each peak was checked by ESI-MS analysis.

2.4. Mass spectrometry

Mass spectra were acquired on a triple quadrupole (Quatro II) mass spectrometer instrument (Micromass, UK), equipped with a standard electrospray probe, adjusted to ca. 5 μl min⁻¹. During all the experiments source temperature was maintained at 80 °C and the needle voltage at 3.6 kV by applying a drying gas flow (nitrogen) of 200 l h⁻¹ and a nebulizer gas flow (nitrogen) of 20 l h⁻¹. Mass spectrometer was calibrated with intact horse heart myoglobin and its typical cone-voltage induced fragments. The cone sample to skimmer lens voltage, controlling the ion transfer to the mass analyzer, was maintained at 30 V. About 50 pmol of each sample was injected into electrospray transport solvent. The ESI mass spectra were obtained in the continuous acquisition mode, scanning from m/z 100 to 2000 with a scan time of 7 s.

2.5. Peptide sequencing

The amino acid sequence was determined by a gas-phase sequencer PPSQ-21 (Shimadzu) based on automated Edman degradation chemistry.

2.6. Peptide synthesis

The peptides were synthesized by step-wise manual solid-phase synthesis using N-9-fluorophenylmethoxy-carbonyl (Fmoc) chemistry with Novasyn TGS resin (Novabiochem) as previously described [17,32]. Side-chain protective groups included t-buty1 for serine and t-butoxycarbonyl for lysine. Cleavages of the peptides–resin complexes were 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. Samples were filtered to remove the resin, ethyl ether was added at 4 °C for the precipitation of the crude peptides and centrifuged at 1000 × g for 15 min at room temperature and the pellet collected. The crude peptide pellets were solubilized in water and chromatographed by RP-HPLC using a semi-preparative column (SHISEIDO C18, 250 mm × 10 mm, 5 μm), under isocratic elution with 40% (v/v) acetonitrile in water [containing 0.1% (v/v) trifluoroacetic acid] at a flow rate of 2 ml/min. The
elution was monitored at 215 nm with a UV-DAD detector (Shimadzu, model SPD-M10A) and each fraction eluted was manually collected in 2 ml plastic vials. The homogeneity and correct sequence of the synthetic peptides were evaluated by comparing their retention times during RP-HPLC under isocratic conditions with 40% (v/v) MeCN containing 0.1% (v/v) TFA against the natural peptides. ESI-MS analysis was also used to check the purity of peptides (considering as criteria the presence of a single molecular ion, equivalent the expected molecular mass for the amino sequence of each peptide). The sequence of synthetic peptides was confirmed by automated sequencing based on Edman degradation chemistry.

2.7. Biological activities

2.7.1. Mast cell degranulation

Mast cell degranulation was determined by measuring the release of β-D-glucosaminidase (co-localizes with histamine), as proposed by Hide et al. [9]. Mast cells were obtained by peritoneal washing of adult Wistar rats with a solution containing 0.877 g NaCl, 0.028 g KCl, 0.043 g NaH2PO4, 0.048 g KH2PO4, 0.10 g glucose, 0.10 g BSA, 90 μl 2 M of CaCl2, 50 μl Liquemine (heparin, ROCHE) in 100 ml water. Mast cells were incubated in the presence of peptides for 15 min at 37 °C. After centrifugation, the supernatants were sampled for the determination of the percentage of total β-D-glucosaminidase, which was determined from lysed mast cells in the presence of 0.1% (v/v) Triton X-100.

2.7.1.2. Nociceptive behavioral responses.

Rats were injected with HOE-140 (300 nmol/kg) or with saline (1 ml/kg) subcutaneously and placed in observation boxes (30 cm × 30 cm) with nontransparent walls and their behavior was observed from below using a mirror [8]. After 10 min to habituate to the new environment, rats under brief nitrous oxide analgesia received a subplantar injection of bradykinin (50 nmol) or Protopolybiakinin-I and Protopolybiakinin-II (50 nmol) in 50 μl phosphate-buffered saline and returned to observation box. Their behavior was monitored for 60 min by a person unaware of pretreatment of rats. The behavioral responses were rated with scores: 0 = no reaction; 1 = favoring the non-injected contra lateral paw; 2 = elevating the injected paw from the ground; 3 = slicking or biting the injected paw [14]. Score were recorded for 1 min and mean scores was calculated. The behavioral responses were recorded both in control and rats pretreated with HOE-140 (300 nmol/kg; s.c.). All experiments were carried out in accordance with the guidelines of the Institutional Committee for Research and Animal Care of the São Paulo State University and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.8. Statistics

Data was analyzed by non-parametric tests, Mann–Whitney U-test or Kruskal–Wallis H-test, followed by a Tukey–Kramer post-test as needed [27]. A value of P < 0.05 was considered statistically significant. The results are presented as means ± standard error (S.E.M.).

3. Results

3.1. Peptide purification

The venom extracts of P. exigua were isolated by RP-HPLC fractionation, and 20 fractions were collected and used for biological assays (Fig. 1). Fractions 1 and 2 contained complex mixture of endogenous biogenic amines and neurotransmitters, and fractions 3, 4, 9, 11, 12, 16 and 18 contained only small amounts of biological material and were not identified. Fraction 5 was identified as serotonin, while fractions 6, 7, 8, 14 and 20 contained unidentified peptide components. Fractions 15, 17 and 19 were previously identified and characterized as mastoparan peptides [17]. Bioassay of fractions 10 and 13 (assigned with asterisks in Fig. 1) revealed pronounced mast cell degranulation activity and characterized further.

![Fig. 1 – RP-HPLC profile of the fractionation of P. exigua venom (800 μg) on a Nucleosil C-18 (ODS) SHISEIDO column (250 mm × 10 mm, 5 μm) using linear gradient, 5–60% (v/v) MeCN (containing 0.1% TFA), at a flow rate of 2.0 ml/min for 45 min at 30 °C, by monitoring at UV 215 nm.](image-url)
The ESI-MS spectra revealed that fractions 10 and 13 contained peptide components with molecular masses of 2422.8 and 2202.7 Da, respectively (data not shown). The mass spectra also show that these peptides were pure enough for amino acid sequencing by automated Edman degradation chemistry.

### 3.2 Structural analysis

The primary sequences of both peptides as determined by Edman degradation chemistry are:

- Fr-10: DKNKPKRVGGRPPGFTPFR-OH (2422.8 Da)
- Fr-13: DKNKPKWAGFPFPTFIR-OH (2202.7 Da)

These sequences provided above molecular masses which are identical to experimental values if the C-terminal residues are in the free acidic form. The sequence of both of the above peptides show similarity to BK and other bradykinin-related peptides (Table 1). Based on these similarities peptides present in fractions 10 and 13, were named Protopolybiakinin-I and Protopolybiakinin-II, respectively (Table 1).

Undesired protease-induced cleavage of natural peptides is a key concern when dealing with peptides/polypeptides from venom sources. In order to verify if the peptides corresponding to the primary structures of Protopolybiakin-I and Protopolybiakin-II exist naturally, the venom the social wasp *P. exigua* to the primary structures of Protopolybiakin-I and Protopolybiakin-II were naturally, the venom the social wasp *P. exigua* was also fractionated in presence of protease inhibitors (phenylmethylsulfonyl chloride and pepstatin A) and analyzed by mass spectrometry. The chromatographic profile obtained was exactly the same as obtained in the absence of protease inhibitors (Fig. 1). Molecular masses (2422.7 and 2202.8 Da) of peptides with and without protease inhibitors were identical (data not shown). These results suggest that the synthethic peptides are present in the venom of *P. exigua* as natural compounds and they are not the products of protease degradation.

Since the yield of Protopolybiakin-I (30 μg) and Protopolybiakin-II (75 μg) from the wasp venom was not enough for bioassay characterization, they were manually synthesized on-solid phase. These synthetic peptides were purified to homogeneity on C18 columns by RP-HPLC. Chromatographic profiles for both peptides, after cleavage with TFA (crude peptides) and purification (assayed peptides) are shown in Fig. 2. Protopolybiakin-I after TFA cleavage revealed four peaks of by-products, while the main-product eluted with a retention time of 15 min, by isocratic elution (20% (v/v) MeCN containing 0.1% TFA) at 30 °C (Fig. 2A). Re-fractionation of the main-product resulted in a sharp and homogeneous peak (Fig. 2B) with a molecular mass of 2422.8 Da as analyzed by ESI-MS (data not shown).

The chromatographic profile of crude products obtained from the synthesis of Protopolybiakin-II after TFA cleavage, revealed six peaks of by-products, while the main-product eluted with a retention time of 19 min (Fig. 2C), and its re-fractionation resulted in a sharp and homogeneous peak (Fig. 2D), with a molecular mass of 2202.7 Da. The sequences of synthetic peptides were confirmed by automated sequencing by Edman degradation chemistry. The results show that the synthetic peptides are homogeneous ensuring the authenticity of pharmacological characterization obtained with synthetic peptides.

#### Table 1 – Amino acid sequence alignments of bradykinin, Protopolybiakinins and some wasp kinins

<table>
<thead>
<tr>
<th>Peptides</th>
<th>Amino acid sequencesa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td>RPPGFSPFR</td>
</tr>
<tr>
<td>Protopolybiakinin-I</td>
<td>DKNKPKRVGGRPPGFTPFR</td>
</tr>
<tr>
<td>Protopolybiakinin-II</td>
<td>DKNKPKWAGFPFPTFIR</td>
</tr>
<tr>
<td>[Thr6] Bradykinin</td>
<td>RPPGFPTFR</td>
</tr>
<tr>
<td>Vesponinin-M</td>
<td>GRPGFSPFRID</td>
</tr>
<tr>
<td>Vesponinin-X</td>
<td>ARPGFSPFRIV</td>
</tr>
<tr>
<td>Vesponinin-A</td>
<td>GRPGFSPFRIV</td>
</tr>
<tr>
<td>Vesponinin-T</td>
<td>GRPGFSPFRIV</td>
</tr>
<tr>
<td>Polisteskinin-3</td>
<td>pETNKKKLKGRPPGFSPFR</td>
</tr>
<tr>
<td>Polisteskinin-R</td>
<td>ARRPGFPTFR</td>
</tr>
<tr>
<td>Polisteskinin-J</td>
<td>RRPPGFSPFR</td>
</tr>
<tr>
<td>Polisteskinin-C</td>
<td>SKRPPGFSPFR</td>
</tr>
</tbody>
</table>

Conserved residues are shown in bold.

a Nakajima et al. (1986) [18].

**Fig. 2** – (A) RP-HPLC profile of the crude products obtained from the synthesis of Protopolybiakin-I after TFA cleavage. Method details same as for Fig. 1. (B) Re-fractionation of the main-product after its purification (assigned with an arrow) under the same conditions described above. (C) Chromatographic profile of the crude product obtained in the synthesis of Protopolybiakin-II after TFA cleavage, under reversed-phase HPLC, with a Nucleosil C-18 (ODS) SHISEIDO column (250 mm × 10 mm, 0.5 μm) using isocratic elution with 30% (v/v) MeCN (containing 0.1% TFA), at 30 °C and a flow rate of 2.0 ml/min for 25 min at 30 °C by monitoring at UV 215 nm. (D) Re-fractionation of the main-product after its purification (assigned with an arrow in (C)) under the same conditions described above.
3.3. Biological activities

The function of synthesized peptides were characterized by its effects on muscle contraction (isolated rat ileum muscle), nociceptive responses and rat mast cells degranulation. The dose–response curves of these assays are shown in Figs. 3–5, respectively.

3.3.1. Muscle constriction assays

Protopolybiakinin-I produced some constriction of the rat ileum, while Protopolybiakinin-II had no effect compared to BK (Fig. 3). The results are expressed as percentage of the maximal responses to 10 μM BK. The log-dose curves obtained with established doses (10⁻⁹ to 10⁻⁵ M/assay) were used to calculate the ED₅₀ value for both peptides, i.e., at the concentration of 0.3 and 3.8 μM for BK and Protopolybiakinin-I, respectively.

To characterize the receptor-type mediating the contractile effects of Parapolybiakinin-I, specific antagonists of B₂ (HOE-140) and B₁ ([Des-Arg₁₀] HOE-140) receptors were tested. HOE-140 inhibited the muscle constriction induced by 100 μM BK or Protopolybiakinin-I. While the specific B₂-receptor antagonist (100 μM) had no effect (data not shown) indicating that the B₂-receptor is responsible for the contractile effects of Protopolybiakinin-I.

3.3.2. Nociceptive behavior

Subplantar injections of BK (50 nmol) and Protopolybiakinin-I (50 nmol) consistently produced nociceptive behavioral responses in unanesthetized rats. The nociceptive responses became apparent after the first 2 min and the most frequently observed behavioral response was elevating and licking of the injected paw and lasted for 20–30 min (Fig. 4). No behavior indicative of nociception response was observed 30 min after the injection. Pretreatment of rats with HOE-140 (300 nmol/kg; s.c.) 20 min prior to the subplantar injections prevented behavioral responses produced by the peptides. Rats showed normal behavior (sitting, grooming or exploring the observation chamber) throughout the 60 min observation period (only

Fig. 3 – Dose–response curves of bradykinin and Protopolybiakinin-I and Protopolybiakinin-II causing constrictions of isolated rat ileum muscle strips. Values are mean ± S.D. (n = 5). *P < 0.05.

Fig. 4 – Nociceptive behavioral responses following subplantar injections of: (A) bradykinin (50 nmol), (B) Protopolybiakinin-I (50 nmol) and (C) Protopolybiakinin-II (50 nmol) in unanesthetized rats. The subplantar injections were given under brief nitrous oxide analgesia. HOE-140 (300 nmol/kg) was injected s.c. 20 min prior to peptide injections. Control rats received NaCl (154 mM) 1 ml/kg shown as “vehicle”. The behavior scores were calculated for 1-min each as described in Section 2. Values are mean ± S.D. (n = 5). *P < 0.05.
3.3.3. Mast cell degranulation

Protopolybiakinin-I and Protopolybiakinin-II and BK caused degranulation of 35, 52 and 4% of the mast cells, respectively (Fig. 5).

4. Discussion

Two novel peptides with BK-related amino acid sequences were found in fractions 10 and 13 during the purification of wasp venom. A comparison of the sequences of Protopolybiakinin-I and Protopolybiakinin-II, with BK and other wasp kinins (Table 1) revealed that eight amino acid residues of Protopolybiakinin-I and Protopolybiakinin-II are identical to BK. Protopolybiakinin-II shares the common positions of seven amino acids with BK. A more detailed comparison with the sequences of other kinins reveal that Protopolybiakinin-I consists of a [Thr6] Bradykinin molecule elongated at the N-terminus with 12 amino acid residues. Additionally the sequence data show that the Protopolybiakinins are among the largest wasp kinins and share sequence similarity to Polisteskinin-3 also.

The in vivo activity of the kinins may be enhanced by structural modifications designed to prevent attack by proteases, so as to increase their half lives due to: (i) by the elongation of the BK sequence, incorporating amino acid residues at one or both terminus; (ii) by introduction of one or more D-isomers in the amino acid sequence; (iii) by modification of the terminal amino or carboxyl moieties to the corresponding acyl derivatives, alcohols or amides [10]. The high activity of the wasp kinins may be due to a elongated amino terminal (Table 1).

A comparison between the ED50 data for ileum muscle contraction (0.3 and 3.8 μM for BK and Protopolybiakinin-I, respectively), reveals that the Protopolybiakinin-I is about 10 times less potent muscle agonist than BK and Protopolybiakinin-II is not a smooth muscle agonist (Fig. 3). This is in contrast to the observations suggesting that most of wasp kinins are less active as smooth muscle agonists than BK [10].

The general pharmacological effects of kinins have been attributed to the stimulation of specific B1- or B2-receptors [2,12,13]. Our observation that HOE-140 suppresses muscle contraction caused by BK and Protopolybiakinin-I, while [Des-Arg10] HOE-140 is inactive, confirm that Protopolybiakinin-I is a B2-type agonist. It is however, likely that the interaction of Protopolybiakinin-I with other receptor sub-types may also contribute to its effect on muscle contraction. The incomplete S-shape of the dose–response curve observed in Fig. 3, suggests that Protopolybiakinin-I at high concentrations may have caused some ileum muscle contraction, probably due to the interactions of Protopolybiakinin-I with still unidentified receptor sub-types. Thus, there is a distinct possibility that the Protopolybiakinin-I is a partial B2-receptor agonist rather than full agonist like BK.

Bradykinin is a B2-type receptor ligand belonging to the seven transmembrane helix class of G-protein-coupled receptors [4,31]. The bioactive conformation of peptides which act as receptor ligands is of great pharmaceutical and theoretical interest. Evidence suggests that BK molecule is flexible and is in rapid equilibrium between multiple conformations in aqueous solution [19]. Experimental evidence suggests that the last four carboxy-terminal residues of BK show a β-turn conformation (Ser6-Arg9) in hydrophobic medium that is important for its binding to its natural receptor [12]. NMR analysis of the kinetics of interaction between BK and B2-receptor suggested that the entire nonapeptide (BK) in trans conformation at all three Xaa-Pro amide bonds, is involved [11,12,13,19]. By using a combination of homology modeling, molecular dynamics and docking simulations it was proposed that the BK molecule adopts a twisted “S” shape, wherein the C-terminal β-turn is buried in the receptor, while the N-termius interacts with some negatively charged residues of the loop 3 (extracellular) of B2-receptor [13]. Thus only the short BK-related peptides could fit properly into B2-receptor binding site for optimal interaction and to cause potent smooth muscle contractions. Since the Protopolybiakinins are larger molecules with long primary structure and thus may not properly bind to B2-receptor to promote potent smooth muscle contractions. These considerations could explain the potency differences between BK and Protopolybiakinins in causing smooth muscle contraction.

A comparison of primary structure reveals that the basic domain RVGGRR in the middle of the structure of Protopolybiakinin-I is replaced by a relatively hydrophobic sequence WMAGF in Protopolybiakinin-II (Table 1). In addition, the phenylalanine residue at the C-terminus of Protopolybiakinin-II (important for the activity of BK-related peptides) is replaced by an Isoleucine residue. The activation of B2-receptor by BK-related peptides requires a series of hydrophilic interactions between the negatively charged residues of the loop 3 at level of extracellular domain of B2-receptor and the most of the primary structure of the kinin peptides, extending from the N-terminus into the basic domain mentioned above [12,13]. Thus, it seems that the structural differences between the Protopolybiakinins could explain the poor interaction of Protopolybiakinin-II with other receptor sub-types, extending from the N-terminus into the basic domain mentioned above [12,13]. Thus, it seems that the structural differences between the Protopolybiakinins could explain the poor interaction of Protopolybiakinin-II with B2-receptor and its inactivity concerning to the constriction of ileum muscle preparation. The antagonist HOE-140 completely abolished the nociceptive behavioral effects of BK and
Protopolybiakinin-I, suggesting that pain-related effects of these peptides may be due to direct B2-receptor activation. Protopolybiakinin-II did not cause any algesic effects.

In addition to the agonist effect on B1/B2-receptors of smooth muscle preparations, another mechanism has been proposed for the BK-related peptides in rat peritoneal mast cells. The secretary activity of mast cells has been attributed to the direct activation of G-proteins similar to a mechanism proposed for the mastoparan peptides from wasp venom [17,31]. BK has been shown to interact first with sialic acid residues on the cell surface and then with G-protein, activating phospholipase C and intracellular calcium mobilization, in a cascade of effects resulting in mast cell degranulation [4].

Protopolybiakinins are polycationic peptides containing 18–21 amino residues, which potentially may either interact directly with plasma membrane phospholipids or G-protein-like receptors, causing histamine release. Our results show that Protopolybiakinins are also mast cell degranulators, with a ED50 of 100 and 10 μM, for Protopolybiakinin-I and Protopolybiakinin-II, respectively (Fig. 5). However, it must be emphasized that these activities are lower than reported for the mCD-peptide from honeybee venom (ED50 = 0.01 μM) [1]. The sequences of both peptides are conserved in relation to Polisteskinin-3 (Table 1). Polisteskinin-3 (at 3.10^-7 M) is 10 times more potent mast cell degranulator than BK [10]. While Protopolybiakinin-I and Protopolybiakinin-II are 7–10 times more potent degranulator than BK, respectively.

The G-protein receptors bound-mastoparan usually adopts an amphiphilic α-helical conformation [30,31]. However, the sequence of BK is too short to fit properly to this type of receptor and to promote a potent activation of degranulation mechanism in mast cells. Protopolybiakinins are relatively long polycationic peptides and can easily assume α-helical conformations in the hydrophobic environment of the membranes where the G-protein receptors are located. Thus, apparently the Protopolybiakinins fill the basic structural requirements suggested by Song et al. [30] to activate the cascade of molecular events, which results in mast cell degranulation with consequent histamine release. These observations could explain the potency differences in mast cell degranulation between Protopolybiakinins and BK.

Kinin-related peptides cause constriction/relaxation in different muscle preparations [5], and are involved in the modulation of inflammatory processes including vasodilatation, increase of vascular permeability and pain [10,26,29]. Kinin-related peptides reversibly block nicotinic transmission in insect ganglion, causing paralysis/death [25]. Thus, arthropods like social wasps use kinin-related peptides in their venom, as toxins both for defense [18] and predation as by solitary wasps [20-26].

In summary, present investigation describes two novel wasp kinins in the venom of neotropical social wasp P. exigua, which may be responsible in part, for the inflammatory and algogenic effects caused by wasp venom on the victims of stinging accidents. These peptides are named Protopolybiakinin-I and Protopolybiakinin-II due to similar pharmacological properties to other wasp kinins. Protopolybiakinin-I, probably the longest waspkinin molecule, is a less potent muscle agonist but more active in causing histamine release from rat mast cells than BK. It is also involved in pain production. While Protopolybiakinin-II is not a muscle agonist or involved with pain production but is a potent mast cell degranulator.

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