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Evaluation of Thr<sup>6</sup>-bradykinin purified from *Polybia occidentalis* wasp venom in the choline uptake of mammal cortices

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**ABSTRACT**

**Context:** Thr<sup>6</sup>-bradykinin is a peptide found in the venom of social and solitary wasps. This kinin, along with other bradykinin-like peptides, is known to cause irreversible paralysis in insects by presynaptic blockade of cholinergic transmission. However, this activity has never been tested in mammals.

**Objective:** As such, the objective of this study was to evaluate the effect of Thr<sup>6</sup>-bradykinin on the cholinergic system of rats.

**Materials and methods:** The peptide was isolated from the venom of the Neotropical social wasp *Polybia occidentalis* Olivier (Vespidae). After correct identification and quantification by ESI-MS and MS/MS, the peptide was tested in [<sup>3</sup>H]-choline uptake using rat cortical synaptosomes. Each uptake assay was accompanied by lactate dehydrogenase (LDH) activity measurement to evaluate synaptosome integrity in the presence of six increasing concentrations of BK or Thr<sup>6</sup>-BK (0.039, 0.156, 0.625, 2.500, 10.000 and 40.000 μM).

**Results:** Data revealed that neither BK nor Thr<sup>6</sup>-BK at any of the six concentrations tested (from 0.039 to 40.000 μM) affected [<sup>3</sup>H]-choline uptake in synaptosomes. Moreover, there was no increase in LDH in the supernatants, indicating that BK and Thr<sup>6</sup>-BK did not disrupt the synaptosomes.

**Discussion and conclusion:** In contrast to previous reports for the insect central nervous system (CNS), Thr<sup>6</sup>-BK had no effect on mammalian cholinergic transmission. Nevertheless, this selectivity for the insect CNS, combined with its irreversible mode of action may be relevant to the discovery of new sources of insecticides and could contribute to understanding the role of kinins in the mammalian CNS.

**Introduction**

The presence of peptides similar to bradykinin (BK) in animal venom has been widely reported (Jacques & Schachter 1954; Kishimura et al. 1976; Griesbacher & Legat 1998). BK-related peptides present in Vespid wasp venom are grouped as wasp kinins (Nakajima 1986). The most active kinin isolated is Threonine<sup>6</sup>-Bradykinin (Thr<sup>6</sup>-BK), found in the venom of social wasps such as *Polistes rothneyi iwatai* Vecht (Watanabe et al. 1976), *Polistes jadwigae* Dalla Torre, *Polistes chinensis* Fabricius, *Vespa analis* Fabricius, *Vespa lewisi* Smith (Nakajima et al. 1984), *Polybia occidentalis* Olivier (Mortari et al. 2007), and solitary wasps *Megascolia flavifrons* Fabricius, (Yasuhasha et al. 1987), *Colpa interrupta* Fabricius (Piek et al. 1990), *Camposomeriella annulata* Fabricius, *Carinoscola melanomaso* Saussure, and *Cyphononyx dorsalis* Lepeletier (Konno et al. 2002).

The neurokinin Thr<sup>6</sup>-BK is a small linear peptide with nine amino acid residues, namely Arg-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg-OH. The neurotoxic action of Thr<sup>6</sup>-BK was described in a model that mimics a wasp sting. This involves the microinjection or microperfusion of the toxin into the cockroach central nervous system (CNS) (Piek et al. 1993a, 1993b). This kinin causes irreversible paralysis in insects via the presynaptic blockade of cholinergic transmission induced by the noncompetitive inhibition of choline uptake, similar to the effect of hemicholinium (Piek 1991; Piek et al. 1993b). In the insect CNS, this peptide was 10 times more potent than BK, despite a single conservative substitution in the sequence (Hue & Piek 1989). Its strong paralyzing and irreversible effect on insects has prompted researchers to investigate the development of new insecticides from its chemical structure (Piek et al. 1990; Schwartz et al. 2012).

Despite being one of the first chemically characterized social wasp venom peptides, little is known about the pharmacological activity of Thr<sup>6</sup>-BK and its mechanism of action in mammals (Picolo et al. 2010). There are several reports of paw edema and nociceptive behavioral responses after local (intraplantar) injection in unanesthetized rats (Griesbacher & Legat 1998). Thr<sup>6</sup>-BK caused contraction in guinea pig ileum smooth muscle preparations and was completely blocked by a B<sub>2</sub> receptor antagonist (Picolo et al. 2010). In addition, central anti-nociceptive effects were assessed after intracerebroventricular injection in rats (Mortari et al. 2007). However, the effect of this peptide on the cholinergic system of mammal CNS remains unexplored. Thus, the aim of the present study was to isolate and determine the
activity of Thr⁶-BK in the choline uptake system, using rat cortical synaptosomes.

Materials and methods

P. occidentalis nests were collected between August and October of 2014 at the Ribeirão Preto Campus of the University of São Paulo in São Paulo, Brazil, and immediately submitted to low temperatures in a cooler containing ice. The nests were stored in the laboratory at −20 °C for 5 h to euthanize the wasps. The venom reservoirs were manually removed, crushed in Milli-Q grade water/50% acetonitrile and centrifuged at 5000 g for 10 min, at 4 °C. Supernatants were carefully collected.

The peptide was separated and isolated according to the methodology proposed by Mortari et al. (2007). The supernatant was filtered using a Microcon 3 centrifugal filter (Millipore). The filtered extract was resuspended in 1:1 acetonitrile/water (ACN/H₂O; v/v) and 0.07% trifluoroacetic acid (TFA). The solution was chromatographed in an ODS C18 RP-HPLC column (Jupiter 15 μm, 20 × 250 mm, Phenomenex, Torrence, CA) at a flow rate of 5.0 mL/min, and eluted using a linear gradient from 2% ACN/H₂O (v/v) (containing 0.07% TFA) for 20 min, followed by 2–60% for 40 min and 60% for 20 min. The fraction containing Thr⁶-BK was re-chromatographed in an ODS C18 RP-HPLC column (Jupiter 5 μm, 4.6 × 150 mm, Phenomenex, Torrence, CA) at a flow rate of 1.0 mL/min and eluted by a linear gradient from 2% ACN/H₂O (v/v) (containing 0.07% TFA) for 10 min, followed by 2–60% for 30 min and 60% for 10 min. Molecular mass spectral analyses of peptides were performed on a Micromass Quattro-LC mass spectrometer from Manchester, UK. Solutions were infused into the ESI source at 10 μL/min, using a Harvard Apparatus model 1746 syringe pump (Holliston, MA). Deionized water (Milli-Q, Millipore, Billerica, MA) was used throughout the study. The mass scan range was from m/z 1000 to 2000. Experiments were performed with a cone voltage of 30 V, capillary voltage of 3 kV and a desolvation gas temperature of 80 °C. A single charged (protonated) molecule was submitted to collision-induced dissociation (CID) with argon gas at collisions energies of 10–50 eV. All MS/MS experiments were conducted in continuous acquisition mode, scanning from m/z 50 to 2000, with a scan time of 5 s. Peptide sequences were obtained based on the countings efficiency of 85% for 14C. Results were expressed as % of control averages with their SEM. Nonspecific uptake was determined from samples incubated in the presence of hemicholinium-3 (500 μM, Sigma). These values were subtracted from the total uptake. Statistical analyses were performed using Student’s t-test.

Results

Two phases of chromatography produced high purity Thr⁶-BK, whose sequence was confirmed by mass spectrometry (data not shown). Figure 1 shows that neither BK nor Thr⁶-BK affected [14C]-choline uptake. Each uptake assay was accompanied by parallel lactic acid dehydrogenase (LDH) activity measurement. No increase in LDH was observed in the supernatants, indicating that the synaptosomes maintained their integrity in the presence of different concentrations of BK and Thr⁶-BK.

Discussion and conclusions

It is well known that arthropod and animal venom in general are an important source of bioactive compounds. Moreover, the neurotoxins present in these venoms have proved to be extremely useful tools in understanding synaptic transmission events, in addition to contributing to the development of new drugs for the use in synaptic transmission events, in addition to contributing to the development of new drugs for the treatment of neurological diseases.
treatment of neurological disorders (Nakajima 1986; Harvey et al. 1998; Beleboni et al. 2004).

The present study isolated, identified and tested the effect of a neurokinin on rat cortical synaptosomes. Previous investigations using the insect CNS have shown that Thr⁶-BK is 10 times more potent than BK in the irreversible blocking of nicotinic receptors of cholinergic transmission (Piek et al. 1990). The author attributed the blockade to depletion of the readily releasable store of acetylcholine (ACH), similar to the activity of hemicholinium-3 (HC3), a classic choline uptake inhibitor (Hue & Piek 1989; Piek et al. 1993a). Nevertheless, our in vitro assays demonstrated that neither BK nor Thr⁶-BK inhibited choline uptake in mammalian cortical synaptosomes.

Choline transport has two main purposes in the nervous system, namely to provide supply for the synthesis of phosphatidylcholine or ACh. The latter, is mediated by a high-affinity choline uptake and ACh synthesis in all cholinergic neurons of the mammalian CNS.

In this respect, unlike HC3, the Thr⁶-BK showed no activity in mammal cholinergic uptake, proving to be selective for the insect nervous system. Therefore, our results strongly support the theory that it may be a subfamily of insect CHT, increasing the opportunity of developing compounds that specifically target insect proteins. In addition to the peptide’s irreversible mode of action, this effect may be relevant to the discovery of new sources of insecticides. Moreover, neuroactive kinins from wasp venom may also contribute to research on the poorly understood role of kinins in the mammalian CNS.

Disclosure statement
The authors declare no conflicts of interest.

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References


