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Original article

Pharmacological evaluation of bee venom and melittin

Camila G. Dantas[a], Tássia L.G.M. Nunes[a], Tâmara L.G.M. Nunes[a], Ailma O. da Paixão[a], Francisco P. Reis[a], Waldecy de L. Júnior[b], Juliana C. Cardoso[a], Kátia P. Gramacho, Margarete Z. Gomes [a,]*

[a]*Laboratório de Morfologia e Biologia Estrutural, Instituto de Tecnologia e Pesquisa, Universidade Tiradentes, Aracaju, SE, Brazil.*
[b]*Departamento de Morfologia, Universidade Federal de Sergipe, Aracaju, SE, Brazil*

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A B S T R A C T

The objective of this study was to identify the pharmacological effects of bee venom and its major component, melittin, on the nervous system of mice. For the pharmacological analysis, mice were treated once with saline, 0.1 or 1.2 mg/kg of bee venom and 0.1 mg/kg of melittin, subcutaneously, 30 min before being submitted to behavioral tests: locomotor activity and grooming (open-field), catalepsy, anxiety (elevated plus-maze), depression (forced swimming test) and apomorphine-induced stereotypy. Haloperidol, imipramine and diazepam were administered alone (positive control) or as a pre-treatment (haloperidol). The bee venom reduced motor activity and promoted cataleptic effect, in a similar manner to haloperidol. These effects were decreased by the pretreatment with haloperidol. Both melittin and bee venom decreased the apomorphine-induced stereotypies. The data indicated the antipsychotic activity of bee venom and melittin in a murine model.

Introduction

The bee venom (BV) produced by Africanized honey bee-AHB (*Apis mellifera* L.) is a complex mixture of enzymes, lipids, amino acids, carbohydrates and peptides like apamin and melittin. It also contains dopamine and phospholipase A_2 (Hider, 1988; Sciani et al., 2010; Ferreira-Junior et al., 2010). Melittin constitutes 40 to 60% of dry whole honeybee venom, and this peptide has various biological activities, including high anti-inflammatory activity (Habermann, 1972; Gauldie et al., 1976; Son et al., 2007).

Experimental studies have shown neuroprotective effects of BV and melittin in amyotrophic lateral sclerosis (Yang et al., 2010; 2011) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of Parkinson's disease *in vivo* (Doo et al., 2010; Kim et al., 2011a; Chung et al., 2012; Doo et al., 2012; Cho et al., 2012) and against glutamatergic excitotoxicity in neuronal and glial cell cultures (Lee et al., 2012). Effectiveness of BV acupuncture was also demonstrated for idiopathic Parkinson's disease (Cho et al., 2012).

The subcutaneous administration of BV has also been shown to induce the activation of catecholaminergic neurons in the arcuate nucleus in the hypothalamus of rats (Kwon et al., 2004) and in dopaminergic nuclei, including the

* Corresponding author.
E-mail: margarete_zanardo@itp.org.br; margarete_zanardo@unit.br (M.Z. Gomes).
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nucleus accumbens, substantia nigra, and locus coeruleus. When combined with acupuncture, it was able to reduce methamphetamine-induced hyperactivity (Kim et al., 2011b). In addition, it has been shown that BV exhibited no signs of toxicity when administered within a therapeutic range subcutaneously (*s.c.*) (Kim et al., 2004).

Despite the wide range of proposed uses for BV and melittin, the pharmacological effects in the central nervous system that result from their administration under physiological conditions have been poorly addressed. The development of new therapeutic approaches to mental disorders and neurodegenerative diseases remains a challenge. So, this study aimed to examine the pharmacological effects of BV and melittin in mice, with particular emphasis on dopaminergic related behaviors.

Materials and methods

Animals and ethical statements

Male Swiss albino mice (25-30 g) were maintained in a temperature and light cycle-controlled environment with free access to water and food. All procedures were in accordance with the standards of the Brazilian College of Animal Experimentation, and the experimental protocol was approved by the Ethical Committee for Animal Use of the Tiradentes University, Aracaju-SE, Brazil (approval number 010213).

Drugs

The bee venom (BV) was purchased from an apiary in the city of São Roque-SP, in the Brazilian Southeast region, it was obtained by electric extraction. Other commercial chemicals were used: melittin (Sigma®, São Paulo, Brazil), haloperidol (Janssen-Cilag Pharmaceuticals® Ltda, São Paulo, Brazil), Imipramine hydrochloride (Novartis Biosciences® S.A., São Paulo, Brazil) and diazepam (Union SA National Pharmaceutical Chemistry®, São Paulo, Brazil).

Assays

Pharmacological evaluation of the acute effects

The animals received vehicle, melittin (0.1 mg/kg) or BV (0.1 mg/kg or 1.2 mg/kg). Both BV and melittin were diluted in saline [0.9%, 40 µl], administered subcutaneously (*s.c.*); the doses were chosen based on previous articles (Doo et al., 2010; Kim et al., 2011abc; Lee et al., 2001; Yang et al., 2010; 2011). Behavioral tests were performed 30 min after the treatments. The data were compared with the obtained after treatment with the antipsychotic haloperidol [0.2 mg/kg, intraperitoneal (*i.p.*)], imipramine (10 mg/kg. *i.p.*) and diazepam (1 mg/kg. *i.p.*). In the groups that received haloperidol and BV, haloperidol was injected 30 min prior the BV.

Catalepsy test

Catalepsy, defined as failure to correct an externally imposed posture, was evaluated according to the standard horizontal bar hanging test by placing the animals with both forelegs over a horizontal glass bar with 0.5 cm diameter, and 4.5 cm high from the ground (Sanberg et al., 1988). The time during which the mouse maintained this position was recorded for up to 300 s, with three tries allowed to replace the animal in the cataleptic position. Catalepsy was considered to have ended when the forepaw touched the floor or when the mouse climbed the bar (Del Bel et al., 2010).

The open-field test

To evaluate the motor and emotional state, an open-field test was performed (Whimbey and Denenberg, 1967; Prut and Belzung, 2003). The following parameters were evaluated for 5 min: locomotion or crossings (number of line crosses), rearings (the number of times the mouse stands on its hind legs), grooming (the number of times the mouse "washes" itself by licking during the observation period) and time spent in the central area. The equipment was made of white colored wood, and it consisted of a quadrilateral with a rectangular area of 4830.25 cm^2 and walls 34.5 cm high, with the base subdivided into sixteen quadrants (Hongxing et al., 2007; O'Leary et al., 2013).

Apomorphine-induced stereotipy

The apomorphine-induced stereotypies were measured by a score scale (from 0 to 6 where 0 = asleep or stationary; 1 = active; 2 = predominantly active but with bursts of stereotyped sniffing and rearing; 3 = constant stereotyped activity such as sniffing, rearing or head bobbing, but with locomotor activity still present; 4 = constant stereotyped activity maintained in one location; 5 = constant stereotyped activity but with bursts of licking and/or gnawing and biting; and 6 = continual licking and or gnawing of cage grids) after the application of apomorphine (20 mg/kg, *s.c.*, Sigma-Aldrich Brasil Ltda., São Paulo, Brazil). Each animal was observed for 10 s intervals every 10 min for 60 min (Setler et al., 1976). The results were expressed as the mean sum of scores that were obtained for each group.

The elevated plus-maze test

To evaluate possible action on anxiety, the elevated plus-maze was used. The number of entries and the stay duration of the animals in the open and closed arms (Pellow et al., 1985) were analyzed in an apparatus with a base 45 cm (height) from the ground, with two open arms (50 × 10 cm) and two enclosed arms of the same size, with walls 40 cm in height uncovered on the top. Each mouse was observed for 5 min.

The forced swimming test

The forced swimming test was carried out as previously described (Porsolt et al., 1977) in order to evaluate a possible antidepressant effect. The test consisted of two swim sessions separated by a period of 24 h. In the first session, the animals were placed in a circular water tank (30 cm high) for 15 min. After a period of intense movement, the animals acquire a posture of immobility, with minimal movements to keep the head out of the water. In the second session (test), the animals underwent the same procedure for 5 min. the swimming and immobility times were evaluated.