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Electrophysiological responses of the olfactory receptors of the tick *Amblyomma cajennense* (Acari: Ixodidae) to host-related and tick pheromone-related synthetic compounds

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ABSTRACT

In the present study, host-related and tick pheromone-related chemical compounds were tested by means of the tip-recording technique in order to obtain electrophysiological responses in olfactory sensilla of non-fed *Amblyomma cajennense* ticks. The following chemicals were tested on the multiporose sensilla DI.1, located anterior to Haller's organ, and the sensillum DII.1, in the anterior pit of this organ: isobutyric acid, butyric acid, valeric acid, trans-2-heptenal, heptanal, benzaldehyde, salicylaldehyde, nonanal, *m*-, *o*- and *p*-tolualdehyde, 2-furaldehyde, 3-pentanone, γ-valerolactone and 1-octen-3-ol (which are all vertebrate-associated volatiles); and 2,6-dichlorophenol (2,6-DCP), 2-nitrophenol, methyl salicylate and nonanoic acid (tick pheromone components). These were used at 10^{-3} M and 10^{-2} M on at least 10 ticks per substance, and the chemicals that were found to be active at these concentrations were then tested as a series from 10^{-6} M to 10^{-2} M, in decadic steps, on at least 15 ticks per substance. 2,6-DCP was active on both sensilla, with detection thresholds of 10^{-6} M on the DI.1 sensillum and 10^{-4} M on the DII.1 sensillum. The olfactory neurons of this sensillum also responded to nonanal at the highest concentration used (10^{-2} M), while those of DII.1 responded not only to 2.6 DCP but also to 2-nitrophenol (to the same extent as to 2,6-DCP) and to 1-octen-3-ol. These results confirm the importance of 2,6-DCP in the chemical ecology of *A. cajennense* and indicate other compounds that may interfere with the behavior of this tick and which should be investigated.

1. Introduction

Ticks are highly specialized bloodsucking arthropods. They are faced with the need to find suitable hosts several times during their life cycle. Olfaction is an important sensory capability that they use in this challenge (Guerin et al., 2000). The first pair of ticks' walking legs house many sensory structures responsible for detecting olfactory information (Foelix and Axtell, 1971; Hess and Vlimant, 1982).

Among the olfactory stimuli present in the environment, host emanations and tick-produced odors are good cues that guide ticks to locate vertebrate hosts (Guerin et al., 2000). The wall-pore olfactory sensilla located in the capsule of Haller's organ on the tarsus of *Amblyomma variegatum* ticks bear cells responding to vertebrate breath, rabbit and bovine odors, steer wash, as well as to the synthetic analogues of the components of these extracts (Steullet and Guerin, 1992, 1994a). Sensilla located outside the Haller's organ capsule can also detect vertebrate associated volatiles (Steullet and Guerin, 1994b). Additionally, they are especially involved in the perception of tick pheromones. The tick sex pheromone 2,6-dichlorophenol (2,6-DCP) and the 2-nitrophenol, the major component of the aggregation–attachment pheromone in *Amblyomma* spp. and also released by cattle, can be perceived by *A. variegatum* ticks, in their olfactory receptor neurons (ORNs) of the anterior pit sensilla of Haller's organ, the DII.1, named after Hess and Vlimant (1986) (Apps et al., 1988; Bruyne and Guerin, 1994; Haggart and Davis, 1981; Schöni et al., 1984; Waladde, 1982). 2,6-DCP can also be perceived in ORNs in DI.1 sensillum (Bruyne and Guerin, 1994; Steullet and Guerin, 1994b).

Amblyomma cajennense, the Cayenne tick, is a three-host tick that parasitizes a wide range of mammals, including man (Guglielmone et al., 2006a). It can be found throughout the Americas, from the southern USA to northern Argentina (Guglielmone et al., 2006b). In this region it is considered to be a tick of great public health importance, especially because of its major role in transmitting *Rickettsia rickettsii*, the causal agent of Rocky Mountain spotted fever (Bustamante et al., 1946; De Rodaniche, 1953;

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Dias and Martins, 1939; Guedes et al., 2005; Horta et al., 2004). From recent studies, it has become known that females of this tick produce 2,6-DCP, which acts as an attractant and mounting sex pheromone (Gachoka et al., 2011; Louly et al., 2008), contesting previous studies that affirmed that females of this tick did not emit sex pheromones (Rechav et al., 1997). Fed male ticks of this species can also produce a pheromone which enhance attachment by males and females conspecifics to host (Gachoka et al., 2012). However, the compound 2-nitrophenol, the main constituent of this class of pheromone in other *Amblyomma* species, is absent from the chemical composition of male *A. cajennense* pheromone.

In the present study the first electrophysiological results obtained with tarsal sensilla examination in the Cayenne tick, *A. cajennense*, are given. Synthetic compounds related to mammals odors as well as tick pheromone related were the stimuli applied to the two wall-pore single walled sensilla present in the tarsus of this tick: DII.1, in the anterior pit of Haller's organ, and DI.1 sensilla, distal to this sensilar group.

2. Material and methods

2.1. Tick rearing

Fully engorged *A. cajennense* females were collected from naturally infested horses in Goiânia (Brazil) and maintained in incubators at constant darkness ($T = 27\,^{\circ}C$, RH > 80%) in the laboratory, in order to establish a colony. Also fed and newly molted ticks were kept in these same conditions. Immature stages were fed on rabbits, *Oryctolagus cuniculus* (L.). Non-fed adult males were used in the experiments at the age between one and five months after molting.

2.2. Electrophysiology

Ticks were ventrally attached to a circular metal plate (Ø 1 cm) on double-sided sticky tape. Both, the indifferent and the recording electrodes were filled with 10^{-2} M KCl and 1% solution of polyvinylpyrolidone K90 (Fluka, Switzerland). The indifferent electrode, connected to the ground via a chloridized wire, was inserted in the region posterior to the *scutum*, after piercing it with a fine forceps. One of the foreleg was orientated to expose the anterior group of Haller' organ sensilla and immobilized with an adhesive tape. Pedal nerves of the forelegs were destroyed by pinching the *coxa* with fine forceps to prevent muscle activity during electrophysiological recordings. In order to improve contact, the tips of the distal knoll sensilla were cut with metal knives fitted on micromanipulators. The tick preparation and the subsequent recordings were performed under visual control (Leica MZ12 stereomicroscope, at 350× magnification).

Before the recordings, the tip of sensillum was cut with a piece of a razor blade in a holder, mounted in a micromanipulator (NMN25, Narishige) under visual control (Leica MZ12 stereomicroscope, at 350× magnification). Recordings from the olfactory receptors DI.1 and DII.1 were accomplished with glass electrodes connected to a high-input impedance preamplifier (10×) (Syntech INR-5, Hilversum) and brought in contact with the cut tip of the sensillum with the aid of micromanipulators. The recordings were sampled (13,714.3 samples/s) and filtered (10–3000 Hz, with 50/60 Hz suppression) via USB-IDAC connection to a computer (Syntech, Hilversum).

The recordings were done on both the DI.1 and the DII.1 sensilla. At least 10 ticks were tested with all compounds at concentrations of both 10^{-3} and 10^{-2} M in relation to the DII.1 sensillum and 15 ticks in relation to the DI.1 sensillum. To produce D–R curves, at least 15 ticks were tested with all compounds at all concentrations, in relation to both the DI.1 and the DII.2 sensilla.

The action potentials were extracted as digital spikes according to top–top amplitudes, using the Autospike software (version 3.9, June 16, 2009, Copyright © Syntech NL). Spikes from different cells were distinguished according to their amplitudes, but only the total firing rate was analyzed. The recording duration was 10 s, and the stimulus was applied 500 ms after the beginning of the recording. The responses were evaluated according to the difference in the number of spikes between the 500 ms stimulation period and the 500 ms period before starting the stimulation (dsf = difference in spike frequencies).

One-way analysis of variance with repeated measurements (ANOVAR) followed by Bonferroni's post hoc correction test was used to analyze the mean dsf discharged at each concentration of each treatment (substance tested), using the SPSS 17.0 software package (SPSS Inc., Chicago, IL, USA). Statistical significance was achieved when $p < 0.05$. Compounds that triggered dsf significantly higher than the blank stimulus were considered active. To compare the effects of pairs of stimulating compounds, two-way ANOVAR with Bonferroni's post hoc correction test was used. In cases in which the assumption of sphericity was violated, the Greenhouse–Geisser (G–G) or Huynh–Feldt (H–F) corrections were used, if $\varepsilon' > 0.75$ or $\varepsilon' < 0.75$, respectively.

2.3. Stimulus delivery

Synthetic compounds relating to mammal odors and tick pheromone-related substances were tested on the tarsal sensilla of *A. cajennense*. The choice of chemicals was based on the data available on the olfactory receptors of ticks of the subfamily Amblyomminae (Schöni et al., 1984; Steullet and Guerin, 1994a,b; Waladde, 1982). The following chemicals were tested: isobutyric acid, butyric acid, valeric acid, trans-2-heptenal, heptanal, benzaldehyde, salicylaldehyde, nonanal, *m*-, *o*- and *p*-tolualdehyde, 2-furaldehyde, 3-pentanone, γ-valerolactone and 1-octen-3-ol (which are all vertebrate-associated volatiles); and 2,6-dichlorophenol (2,6-DCP), 2-nitrophenol, methyl salicylate and nonanoic acid (tick pheromone components).). All these chemicals were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA) and were >97% pure, except for trans-2-heptenal, heptanal and nonanal, which were 95% pure. They were dissolved in hexane (Sigma–Aldrich, n-Hexane CHROMASOLV) at 10^{-2} M and 10^{-3} M. A 30 μl aliquot of the test solution was applied to a strip of filter paper (6 × 0.5 cm, Whatman No. 40 ashless). After the solvent had evaporated 30 μl of paraffin oil was applied onto the filter paper to reduce volatilization of the odors, and the filter paper strip was inserted into a glass Pasteur pipette. A blank stimulus containing only filter paper plus solvent plus paraffin oil was prepared in order to test for solvent interference with the ORN response. When a receptor responded to a test chemical, graded dilutions from 10^{-6} M to 10^{-2} M were prepared in order to determine a dose–response (D–R) curve. 2,6-DCP was also prepared at 10^{-7} M. The blank stimulus was tested before and after each series of treatment at 10^{-3} M and 10^{-2} M concentrations, so as to observe whether cell responsiveness was preserved throughout the recording session. In the case of dose–response evaluations, the blank stimulus was tested at the beginning of the series of tests on each stimulus. The odors were presented in random order but the concentrations were fixed in an ascending order, starting with the negative control.

A constant flow of charcoal-filtered and humidified air (humidity at the preparation was maintained at over 90%) was passed at 40 cm/s over the preparation, from a metal tube (Ø 1 cm) connected to a stimulus air controller (Syntech, CS-55, Hilversum). The air outlet was positioned around 2 cm from the tarsus. The constant flow included both a continuous (∼15 ml/min) and a complimentary (∼25 ml/min) air stream. Stimulation was performed by inserting the tip of the test pipette into a hole in the metal tube,