Identification of vertebrate volatiles stimulating olfactory receptors on tarsus I of the tick *Amblyomma variegatum* Fabricius (Ixodidae)

I. Receptors within the Haller's organ capsule

P. Steullet, P.M. Guerin

Institute of Zoology, University of Neuchâtel, Chantemerle 22, CH-2000 Neuchâtel, Switzerland

Accepted: 30 July 1993

**Abstract.** Gas chromatography-coupled electrophysiological recordings (GC-EL) from olfactory sensilla within the capsule of Haller's organ of the tick *Amblyomma variegatum* indicate the presence of a number of stimulants in rabbit and bovine odours, and in steer skin wash. Some of these stimulants were fully identified by gas chromatography-mass spectrometry analysis and by matching electrophysiological activity of synthetic analogues as: 1) hexanal, 2-heptenal, nonanal, furfural, benzaldehyde, and 2-hydroxybenzaldehyde (in all extracts); 2) heptanal, 2-, 3-, and 4-methylbenzaldehyde, and γ-valerolactone (only in bovine and rabbit odour). Careful examination of the electrophysiological responses permit characterization of 6 receptor types: 1) a benzaldehyde receptor, 2) a 2-hydroxybenzaldehyde receptor, 3) three types of receptors responding differently to aliphatic aldehydes, and 4) a lactone receptor.

**Key words:** Tick – Haller’s organ – Olfactory receptors – Benzaldehyde – 2-Hydroxybenzaldehyde – Aliphatic aldehydes – γ-Valerolactone

**Introduction**

Adults of the tropical bont tick, *Amblyomma variegatum* (Acaríi, Ixodidae), lie in wait in the litter zone for hosts such as domestic and wild bovidae. The presence of a vertebrate in the vicinity arouses adults of this tick species to initiate active search on the ground in order to locate the host. At the end of the dry season, males of *A. variegatum* are first to find a suitable host, and feed for few days before emitting an aggregation-attachment pheromone (Schoeni et al. 1984), which in turn enhances attractiveness of the host for conspecifics (Norval et al. 1989; Barré et al. 1991). This favours meeting of the sexes on the host. While the aggregation-attachment pheromone together with host odour seems crucial for host-seeking and attachment by females (Barré 1989; Barré et al. 1991), host odour alone is important for infestation of the host by pioneer males.

This paper deals with olfactory receptors housed in wall-pore single-walled sensilla within the capsule of Haller's organ on the tarsus of the leg pair I, considered to contain some of the main host-odour receptors in ticks. This supposition was confirmed by the behavioural bioassay of Lees (1948) on *Ixodes ricinus*, and in electrophysiology experiments in which mouse odour was used to stimulate capsule receptors in *Hyalomma asiaticum* (Sinitsina 1974). In addition, breath components CO₂ and H₂S have been clearly identified as olfactory stimulants for receptors in the capsule of Haller's organ of *A. variegatum* (Steullet and Guerin 1992a, b). The present study aims to extend our knowledge on other olfactory receptors (specificity spectrum) responding to host odour within wall-pore single-walled sensilla of the Haller's organ in this tick species. Gas chromatography-coupled electrophysiology recordings of host-odour receptors are then employed to isolate active constituents in vertebrate odour concentrates.

**Materials and methods**

**Tick rearing.** *A. variegatum*, originating from the Ivory Coast (Adiopodoumé), have been reared since 1981 at the Agricultural Research Centre of Ciba-Geigy Ltd. (St. Aubin, Switzerland). All stages (immature and adult) are fed on Simmental calves at 22 to 24°C and then kept under constant darkness during moult at 28°C/80-90% RH. Unfed males foreseen for these experiments were maintained in an environmental cabinet: 10 h light at 25°C/85% RH, 10 h darkness at 18°C/95% RH separated by 2 h dusk and dawn periods.

**Electrophysiology.** Unfed male *A. variegatum* (under 7 months old) were immobilized on a perspex holder with double-sided sticky tape. Pedal nerves of the anterior leg pair were destroyed by pinching the coxa with fine forceps to prevent muscle activity during
electrophysiological recordings. The narrow opening of the capsule (a slit across dorsal side of the tarsus 50 μm long and 5 μm wide) was enlarged to provide better access to the 7 wall-pore sensilla within by using a piece of razor blade mounted on a Leitz micromanipulator.

Recordings from olfactory receptors were accomplished with glass electrodes connected to a high-input impedance preamplifier and an AC/DC amplifier (UN-03, Syntech, The Netherlands). The reference electrode, filled with 0.2 M NaCl, was inserted into the coxa of one of the anterior legs, whereas the recording electrode (tip diameter <5 μm), filled with 0.2 M KCl and 1% polyvinylpyrrolidone (Fluka, Switzerland), was mounted on a Leitz micromanipulator and gently introduced into the dissected capsule until cell activity was captured. Contact between the electrode tip and the pore-wall of a sensillum was sufficient to capture cell activity. Recordings from different sensilla within the capsule were made by varying the orientation of the recording electrode in the capsule. Cell activity could thus be consistently recorded at 6 distinct locations (Fig. 1). AC and DC signals were stored on video tapes as in Steullet and Guerin (1992a). AC signals were also fed into a 1BM compatible computer and visually analysed using the view option of the spike analysis programme SAPID (Smith et al. 1990), and displayed on paper with a laser printer.

Stimulus delivery. Air scrubbed through charcoal and silicagel, and humidified to 80% RH at 22°C ± 1°C passed continuously at 40 cm/s over the preparation from a 5 mm diameter glass tube, the outlet of which was about 10 mm from the tarsus. Stimulation was achieved by applying a charcoal-filtered air stream to a 5-mm polypropylene syringe containing the stimulus. A solenoid valve permitted displacement of 2 ml of the syringe content in 1 s into the humidified air stream through a septum-covered hole in the glass tube at 3 cm from its outlet. To prevent changes in air flow during stimulation, a solenoid-controlled charcoal-filtered air flow (2 ml/s) was delivered continuously through a blank syringe into the humidified air stream during stimulus off. Stimulations followed at 3 min intervals.

Different concentrates of host odours and the following synthetic chemicals were at first used to study the specificity of receptors located in the different parts of the capsule: ammonia (3.5% and 0.35% NH₄OH in distilled H₂O), acetone (10⁻³ M and 10⁻² M in distilled H₂O), 3-pentanone, 4-heptanone, γ-butyrolactone, γ-valerolactone, 6-capralactone, pentanal, 1-octen-3-ol, propanoic acid, 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentaenoic acid, heptanoic acid, l-lactic acid, and 4-methylphenol (all vertebrate-associated volatiles); nonanoic acid, 2-nitrophenol, 2,6-dichlorophenol and methylsalicylic acid (tick pheromone components); and 1-octene, octylamine, hexyl acetate (others); dichloromethane and distilled H₂O (solvent blanks). Except for ammonia and acetone, all these chemicals (> 98% pure as indicated by GC) were dissolved in dichloromethane (Merck, analytical grade) and tested at 10⁻³ and 10⁻² M dilutions (levels normally evoking clear responses in most responsive receptors). When a receptor responded to a tested chemical, graded dilutions from 10⁻⁵ to 10⁻⁷ M were delivered to the preparation to determine a dose-response curve. A 10 μl aliquot of the stimulus solution was deposited on a piece of filter paper and placed in the stimulus cartridge after evaporation of the organic solvent. Separate cartridges were employed for each stimulus and each concentration. Each cartridge was only used once. Three min were arbitrarily allowed for stimulus evaporation inside the syringes prior to delivering the volatile to the preparation. CH₄ (heat from the mains) and CO₂ (from a gas cylinder of 5% CO₂/95% O₂) were also tested; stimulus syringes were then directly filled with these gases.

Host-odour stimuli. Human breath, human axillary secretion, and extracts of bovine and rabbit odours were employed as host-odour stimuli. Human breath was blown into a 5-ml syringe used as stimulus cartridge (for further details, see Steullet and Guerin 1992b). Human axillary secretion was collected with a dry-acetone-washed cotton pad (7 x 7 cm) rubbed on the axillary area of a 28 year-old male and then enclosed in a stimulus cartridge. The auxillary region was not treated with deodorants or perfumes, and was not washed for 24 h prior to secretion collection. The stimulus blank consisted of a dry-acetone-washed cotton pad.

Air from a metallic cage containing a single tick-naïve rabbit (New Zealand), a white strain sometimes used in this laboratory to feed A. variegatum, was pumped for 24 h at 500 ml/min through ca. 600 mg of conditioned Porapak Q (60–80 mesh) packed in a glass tube 7 cm long × 4 mm diameter (Steullet and Guerin 1992b). The cage was located in an animal room with 20 other rabbits of the same strain. Volatiles were desorbed with 3 ml dichloromethane (Merck, analytical grade) and the extract was then slowly concentrated under N₂ to ca. 50 μl. One or 10 μl of the concentrated extract was enclosed on filter paper in the stimulus cartridge. Air from adjacent rooms without animals (blank control) was also collected as described above on Porapak and analysed by GC-MS.

Air from a 30 ml stall occupied by 2 tick-naïve Simmental steers (about 200 kg each), a race frequently used to rear A. variegatum, was pumped for 24 h at 500 ml/min through 600 mg of conditioned Porapak Q. Solvent desorption and concentration were achieved as for rabbit odour and 1 or 10 μl of the concentrated extract was used as stimulus. Extract of air from a washed stall unoccupied for a month was used as a blank control and analysed by GC-MS. Collection of rabbit and bovine odour was undertaken on several occasions with different rabbits and steers. The concentrated extracts smelled very similar to the natural odours.

Different body parts (head, shoulder, side, dewlap, chest, belly, legs, armpit, and perianal area) of two tick-naïve Simmental steers were rubbed with acetone-washed cotton pads (7 x 7 cm) soaked with dichloromethane (analytical grade). Gloves were used for this operation. The cotton pads were placed in a 500-ml gas-wash bottle, held at 70°C, through which N₂ passed for 1 h at 100 ml/min. Released volatiles were held up in a cold trap (4 mm diameter, 20 cm long glass U-tube steeped in a dry ice/acetone mixture) in a Dewar flask. Dichloromethane (2 ml, analytical grade) was used to extract trapped volatiles, water was removed by lowering the extract to –10°C and removing the solvent from the ice. The extract was subsequently concentrated under N₂ to ca. 50 μl. One or 10 μl of the concentrate was then used as stimulus.

Gas chromatography-coupled electrophysiological recordings (GC-EL). Olfactory receptors, characterized as responding to vertebrate odours, were subsequently employed to locate active product(s) among the many constituents of odour extracts by GC-EL. Components of an active extract (bovine or rabbit odours collected on Porapak, skin wash of steer) were separated on a high-resolution capillary gas chromatograph (chromatograph: Carlo Erba Instruments HRGC 5160 with an on-column injector; fused-silica column: 30 m DBWAX, internal diameter 0.32 mm or 0.25 mm, 0.25 μm film thickness, G&W Scientific, USA; carrier gas: H₂ at 0.5 m/s at 40°C; temperature programmed: 60°C for 5 min, 8°C/min to 230°C, and held for 10 min). The column effluent was split (glass Y-splitter), 2/3 being sent to the flame ionisation detector (FID) and 1/3 (longer arm) to an electrophysiological preparation with receptor(s) sensitive to host odour (biological detector). An air stream (1 l/min), maintained at ca. 80% RH and 22 ± 1°C in a 7 mm diameter glass water-jacketed tube, swept one third of the column effluent to the tick preparation 30 cm away from a heated transfer line (250°C) in the wall of the chromatograph. The outlet of the glass tube (reduced outlet of 3 mm diameter) was 5 mm from the tick tarsus where the air speed was 1.5 m/s. Column effluent was thus simultaneously monitored by the FID and the activity of the receptors recorded to locate possible active component(s) of the extracts being analysed (Wadhams 1982).

All spikes from what usually amounted to multicellular recordings (AC signal) were sorted from background noise with a level discriminator incorporated in the UN-03 amplifier, and the sum of the frequencies of all firing cells was continuously converted to a voltage (time constant of the frequency to voltage converter: 1 s). This signal was printed on a multichannel chart-recorder simultaneous with the FID response. An electrophysiological response was