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

II. Receptors outside the Haller's organ capsule

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Abstract. Bovine odour excites olfactory receptor(s) in a wall-pore olfactory sensillum on the anterior pit of Haller's organ in *Amblyomma variegatum*. Gas chromatography-coupled electrophysiology recordings from this sensillum reveal the presence of 4 active compounds in bovine odour. The two strongest stimulants were identified as 2-nitrophenol and 4-methyl-2-nitrophenol by gas chromatography-coupled mass spectrometry, and by matching electrophysiological activity of synthetic analogues. Synthetic analogues of known vertebrate-associated volatiles also stimulate other olfactory receptors in sensilla on the surface of tarsus I: a lactone receptor responding to γ -valerolactone and 6-caprolactone; different fatty acid receptor types responding best to either pentanoic acid, 2-methylpropanoic acid or to butanoic acid; three receptors responding to NH_3; and one receptor responding to 3-pentanone. Gas chromatography-coupled mass spectrometry analysis of vertebrate volatiles revealed presence of a number of these olfactory stimulants in concentrates of rabbit and steer odour, i.e. 2-methylpropanoic acid, butanoic acid, 3-methylbutanoic acid, pentanoic acid, and γ-valerolactone.

Key words: Tick – Haller's organ – Olfactory receptors – Fatty acids – Ammonia – γ-Valerolactone – Nitrophenols

Introduction

While the previous paper (Steullet and Guerin 1994, companion paper) deals with the identification of vertebrate volatiles that stimulate olfactory receptors in the capsule of Haller's organ on leg pair I of *Amblyomma variegatum* (Acari, Ixodidae), this present study aims to identify host-odour volatiles that excite receptors housed in sensilla located outside the capsule. The 7 olfactory sensilla within the capsule of Haller's organ carry various host-odour receptors, i.e. CO_2-, sulfide-, aliphatic aldehyde-, benzaldehyde-, 2-hydroxybenzaldehyde-, and lactone-receptors (Steullet and Guerin 1992a, b, 1993). Adult *A. variegatum* also possess 12 other wall-pore sensilla on the surface of the tarsus of the first pair of legs (Hess and Vlimant 1982, 1983) (Fig. 1). The latter authors observed that the position of sensilla on the tarsus is conserved between tick species, and distinguished between different morphological types of wall-pore single-walled and wall-pore double-walled sensilla (according to Altner's classification, Altner et al. 1977). Based on detailed ultrastructural studies, Hess and Vlimant estimated that the 12 wall-pore sensilla on the surface of tarsus I in *A. variegatum* carry between 50 and 65 olfactory receptor cells. Only a few of them have been physiologically characterized in *A. variegatum*, or indeed in any other tick species. Receptors sensitive to 2,6-dichlorophenol (a tick pheromone component) were described in the DI.1 and DII.1 sensilla (Fig. 1) of *A. variegatum* (Waladde and Rice 1982; Schoeni 1987), and in a wall-pore sensillum distal to the Haller's organ in *Amblyomma americanum* and *Rhipicephalus appendiculatus* (Haggart and Davis 1981), and *Ixodes ricinus* (Thonney 1987). 2-Nitrophenol, a component of the aggregation-attachment pheromone of *A. variegatum*, excited receptor(s) in a wall-pore sensillum on the anterior pit of Haller's organ of this species (Schoeni 1987). Finally, NH_3-sensitive receptors were discovered in sensilla of the anterior pit, and also in more proximally placed sensilla on the tarsus of *Rhipicephalus sanguineus* (Haggart and Davis 1980).

Materials and methods

Animals. Male *A. variegatum* were reared and maintained in the conditions described previously (Steullet and Guerin 1994).

Electrophysiology. Tick was immobilized on a piece of perspex with double-sided sticky tape. The tip of the sensillum was cut with the

Abbreviations: GC-EL, gas chromatography-coupled electrophysiological recording; GC-MS, gas chromatography-coupled mass spectrometry

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broken tip of a heat-pulled glass rod (1.5 mm diameter) mounted on a micromanipulator and oscillating at ca. 120 MHz induced by a piezo electric transducer disk (n° 4322.020.177721, Philips) (Gödde 1989). A glass electrode (tip diameter: 10–20 μm) mounted on a micromanipulator and filled with 0.2 *M* KCl was brought into contact with the cut tip of the sensillum, whereas the reference glass electrode filled with 0.2 *M* NaCl was introduced into the coxa of the anterior leg. The coxa was previously pinched with blunt forceps to prevent muscle discharge during recordings. Experiments were achieved either under an inverted microscope (Nikon Diaphot TMD) for recording receptor activity in the DI.1, DII.1, DII.5, DII.6, DIII.1, and DIV.1–4 sensilla, or under a stereomicroscope (Olympus SZH) for recordings from the LAII.1, VII.1, and VII.4 sensilla (see Fig. 1). Sensilla are named after Hess and Vlimant (1982). Amplification, data acquisition and spike analysis were accomplished as described in Steullet and Guerin (1992a, b).

Stimulation. Stimulus delivery system as well as the synthetic chemicals used to study specificity of the olfactory receptors were as in Steullet and Guerin (1994). Human breath, human axillary secretion, and bovine and rabbit odours collected on Porapak Q were used as sources of natural stimuli (details in Steullet and Guerin 1992b and 1994).

Gas chromatography-coupled electrophysiology recordings (GC-EL) and gas chromatography-coupled mass spectrometry (GC-MS). GC-EL, with column effluent split, was used to locate active components of bovine and/or rabbit odour extracts. Two thirds of the effluent was sent to a flame ionisation (FID) or an electron capture detector (ECD), and one third to an electrophysiological preparation of an olfactory sensillum as biological detector. Compounds capable of stimulating olfactory receptors in sensilla on the surface of the tarsus were located due to a strong increase in spike frequency of the responding receptors. GC-MS was subsequently employed to identify these active chromatographic peaks. For further details of the GC-EL methodology and GC-MS, see Steullet and Guerin (1994). Identification of an electrophysiologically active peak in an extract was first based on a match between its mass spectrum and that of a known product stored in a computer-based library of the GC-MS. The mass spectrum and Kovat's index of the stimulant to be identified were then compared with those of the proposed synthetic analogue injected under the same conditions and on the same chromatographic phase (DBWAX). The biological activity of the synthetic product was subsequently confirmed by electrophysiology experiments on the olfactory receptor concerned. Thus, synthetic 2-nitrophenol and 4-methyl-2-nitrophenol (>98% purity) were tested on receptors to confirm their identification as olfactory stimulants in GC-EL and GC-MS analysis of odour extracts.

Concentrates of air from rooms without rabbits or steer (blanks) were also analysed by GC-MS to check for the occurrence of identified olfactory stimulants in ambient air, and the amount was compared with that found in bovine and rabbit odour extracts collected under the same conditions. For this purpose, 2-bromophenol (1.64 μg) was added as internal standard into 1.5 ml of rabbit, bovine, and blank extracts before concentration and analysis by GC-MS. Search for nitrophenols in each extract at the retention time of the corresponding synthetic analogues was made with their molecular ions whose mass to charge ratio (M/Z) was respectively 139 for 2-nitrophenol and 153 for 4-methyl-2-nitrophenol. Quantification was achieved by integrating the area under the peak of these characteristic ions. Abundance of each product in an extract was normalized with reference to 2-bromophenol using the peak area of one of its characteristic fragment ions (M/Z = 172).

Results

Human breath, human axillary secretion, and rabbit odour collected on Porapak failed to clearly stimulate olfactory receptors in any of the wall-pore sensilla located on the surface of the tarsus. Bovine odour collected on Porapak evoked a response of receptor(s) in the wall-pore DII.1 sensillum on the anterior pit of Haller' organ (Fig. 1).

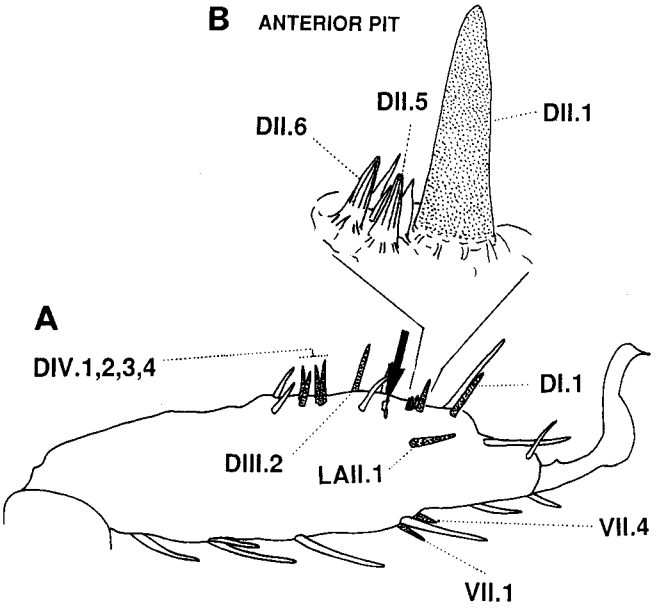

Fig. 1. **A** Tarsus of leg I of an adult *A. variegatum* indicating location and the name ascribed to each olfactory sensillum (*stippled*). *Arrow* indicates the slit-like opening to the capsule of Haller's organ which contains 7 additional olfactory sensilla. **B** Detailed view of the anterior pit sensilla of Haller's organ

Identification of bovine volatiles stimulating receptor(s) in the DII.1 sensillum

Gas chromatography-coupled electrophysiology analyses (GC-EL) with the DII.1 sensillum revealed 4 active compounds in bovine, but none in rabbit odour collected on Porapak (Table 1). Although two only occasional but weak stimulants were not identified, the other two were identified as 2-nitrophenol and 4-methyl-2-nitrophenol. Identification of peak 2 as 2-nitrophenol was based on 1) matching Kovat's index of the GC-EL active peak with the synthetic analogue (Table 1), 2) the presence of the molecular ion (M/Z = 139) of 2-nitrophenol in the unknown in GC-MS analyses at the same retention time as the synthetic analogue, and 3) matching electrophysiological activity of the synthetic analogue (Fig. 2). A full mass spectrum was not obtained because of the small amount of peak 2 in the extract and the presence of coeluting products. Peak 3 was identified as 4-methyl-2-nitrophenol on the basis of 1) matching mass-spectrum with the synthetic analogue, 2) correspondence of the Kovat's index of the unknown in GC-EL with the synthetic analogue (Table 1), 3) matching electrophysiological activity of the synthetic analogue (Fig. 2). Some 10–20 ng of 2-nitrophenol and 200–300 ng of 4-methyl-2-nitrophenol were found in 1.5 ml bovine odour extract after collecting 300 l of bovine odour-laden air on Porapak. Rabbit

Table 1. Identified constituents of bovine odour which stimulated olfactory receptors in the DII.1 sensillum of male *A. variegatum*

Peak number	Olfactory stimulant	a) Identification criteria	b) Odour source	c) Kovat's index in GC-EL	d) Kovat's index in GC-MS	e) Kovat's index of standards in GC-MS	f) Response occurrence
1	unidentified		steer	1803	–	–	1/8
			rabbit	–	–	–	0/5
2	2-nitrophenol	MKE	steer	1812±11	1818	1818	7/8
			rabbit	–	1814	1818	0/5
3	4-methyl-2-nitrophenol	MKE	steer[a]	1914±10	1922	1919	7/8
			rabbit	–	1919	1919	0/5
4	unidentified		steer	1992±15	–	–	1/8
			rabbit	–	–	–	0/5

This table is based on gas chromatography-coupled electrophysiology (GC-EL) and gas chromatography-mass spectrometry (GC-MS) analysis of bovine and rabbit odour collected on Porapak Q. Both types of analyses were made on the same gas chromatographic phase-DBWAX. **a)** Different criteria on which identification of a particular odour constituent was based, M – matching mass spectra, (in the case of 2-nitrophenol identification was based on presence of the molecular ion (M/Z = 139) in peak 2 at the same retention time as the synthetic analogue), K – matching Kovat's index, and E – electrophysiological activity matching with that of the synthetic analogue. **b)** Analyses were made of bovine and rabbit odour as collected on Porapak and [a] indicates that the active compound was also detected by GC-MS in a bovine skin wash. **c)** Mean Kovat's index (±standard deviation) of the active peaks in GC-EL analyses. **d)** Kovat's index of the active peak located by GC-MS. **e)** Kovat's index of the synthetic product corresponding to that of the biologically active peak in GC-MS. **f)** Number of GC-EL analyses in which a response was observed/out of the total number of analyses with the DII.1 sensillum. 2-Nitrophenol and 4-methyl-2-nitrophenol were present at low amounts in rabbit odour extracts, but in insufficient quantity to elicit a response in GC-EL

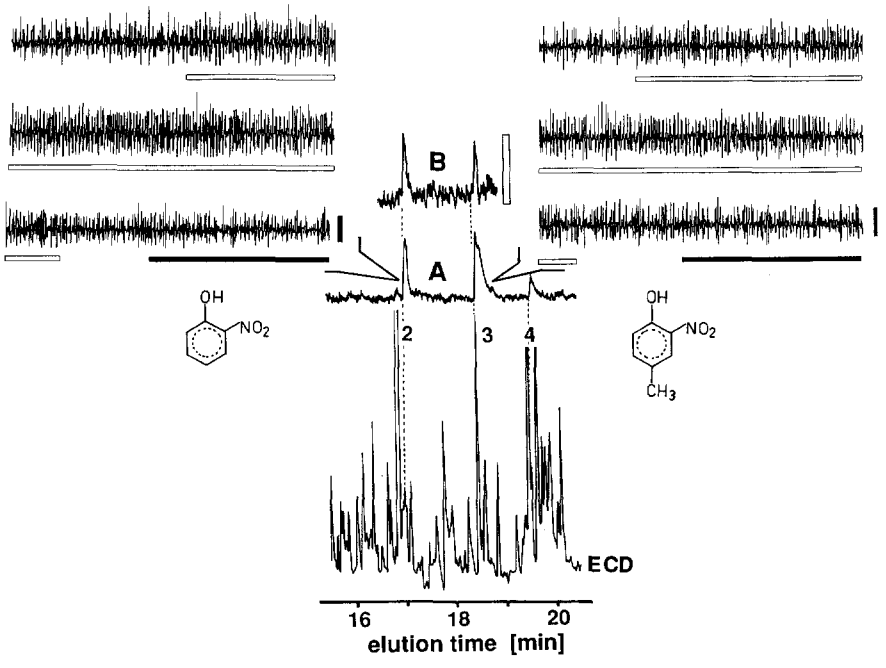

Fig. 2A, B. Section of a bovine odour extract analysis by gas chromatography-coupled electrophysiology of the DII.1 sensillum of a male *A. variegatum* (for details of technique, see text). The *lower trace* details part of the chromatogram of a bovine extract obtained with an ECD (electron capture detector); the *upper traces* (**A** and **B**) represent the summed frequencies of all cells recorded from the DII.1 sensillum (frequency to voltage converted signal) during elution (**A**) of components of the bovine odour extract and (**B**) during elution of 10 ng each 2-nitrophenol and 4-methyl-2-nitrophenol, respectively, at the same retention times as peak 2 and 3 of the extract. Peaks are numbered as in Table 1. Spike trains generated in **A** during elution of 2-nitrophenol (peak 2) and 4-methyl-2-nitrophenol (peak 3) are provided. *Hollow horizontal bars*: approximate elution time of the active compounds; *hollow vertical bar* 50 impulses/s; *solid horizontal bar* 1 s; *solid vertical bar* 0.3 mV. The great complexity of spike trains recorded from the DII.1 sensillum, which contain 14 receptor cells, prohibited us from properly analysing which and how many receptors responded to these nitrophenols

odour extract also contained small quantities of both compounds but in insufficient amount to evoke a response in GC-EL, i.e. 1–5 ng of both nitrophenols in 1.5 ml rabbit odour extract after collection of a similar volume of rabbit odour-laden air on Porapak. By contrast, extracts of room air without steer or rabbits contained no or hardly detectable amounts of either of these substances.

Receptors responding to vertebrate volatiles

Apart from receptor(s) in the DII.1 sensillum which responded to nitrophenols, receptors housed in this and other sensilla were excited by volatiles normally associated with vertebrate odours. The following types of receptors were found:

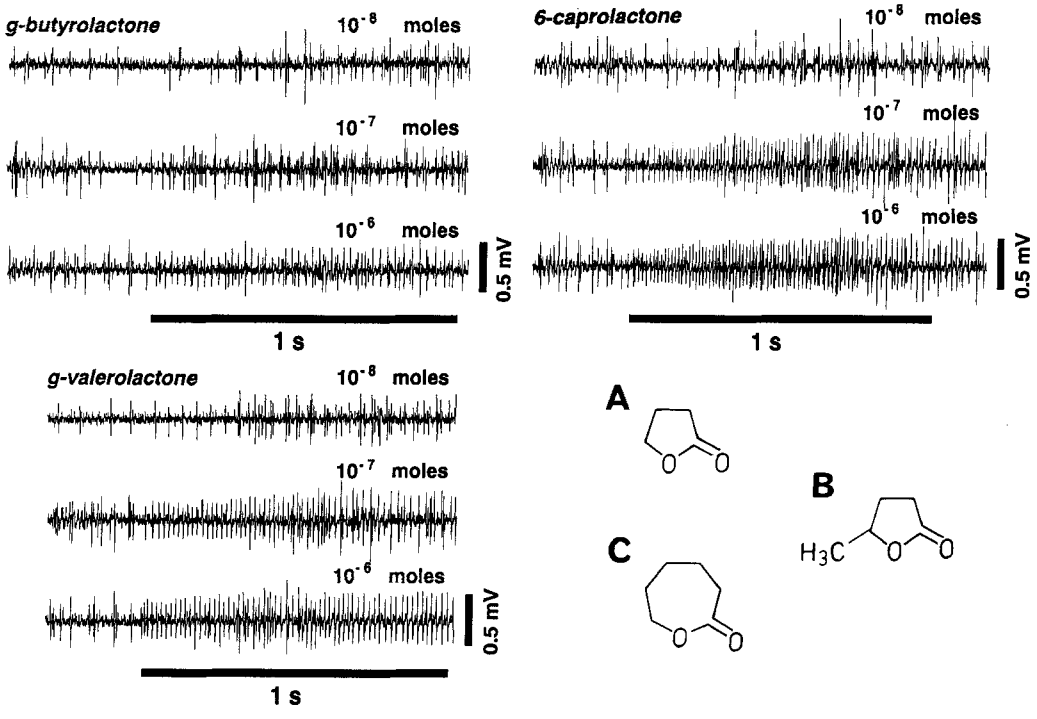

Fig. 3. Responses of an olfactory receptor of the DI.1 sensillum in male *A. variegatum* to γ-butyrolactone (*A*), γ-valerolactone (*B*), and 6-caprolactone (*C*). Stimulus intensity (number of moles in the stimulus cartridge) is indicated above each trace. Due to the multicellular responses, it was often not possible to clearly distinguish the spikes involved in responses to stimulation with low doses of these lactones (10^{-8} moles in the stimulus cartridge). *Horizontal bars* 1 s stimulation

Nitrophenol receptor(s). Both 2-nitrophenol and 4-methyl-2-nitrophenol stimulated receptor(s) in the DII.1 sensillum (Fig. 2). Both nitrophenols were equally active. Since an estimation of 10^9 molecules of 2-nitrophenol/cm^3 air in GC-EL experiments still strongly excited receptor(s) in the DII.1 sensillum, the threshold can be considered at much below this level. However, recordings from the DII.1 sensillum, which contain 14 receptor cells, were extremely complex (Fig. 2). The great number of chemosensitive units firing did not permit either clear determination of the exact number of cells responding or if 2-nitrophenol and 4-methyl-2-nitrophenol stimulated the same receptor(s).

Lactone receptor. One receptor in the DI.1 sensillum was sensitive to γ-valerolactone, found only in traces in bovine and rabbit odour (Fig. 3). Double successive stimulations indicated that this receptor also responded to 6-caprolactone. γ-Butyrolactone was only a weak stimulant at a high concentration (10^{-6} moles in the stimulus cartridge). The difficulty of distinguishing the lactone receptor from the four other receptors housed in the same sensillum during weak stimulation prohibited the establishment of clear dose-response curves (Fig. 3).

Acid receptors. Short-chain fatty acid receptors in the DII.1 sensillum responded best to pentanoic acid (Fig. 4). 3-Methylbutanoic acid and butanoic acid were also active but only at relatively high concentration (Fig. 4). Nevertheless, the complexity of the multicellular recordings from the DII.1 sensillum (with 14 receptor cells) inhibited us from properly studying the responding receptor(s) and from establishing a dose response-curve. Two receptor cells of another sensillum (DII.5) on the anterior pit also responded to C_4 and C_5 fatty acids; hexanoic,

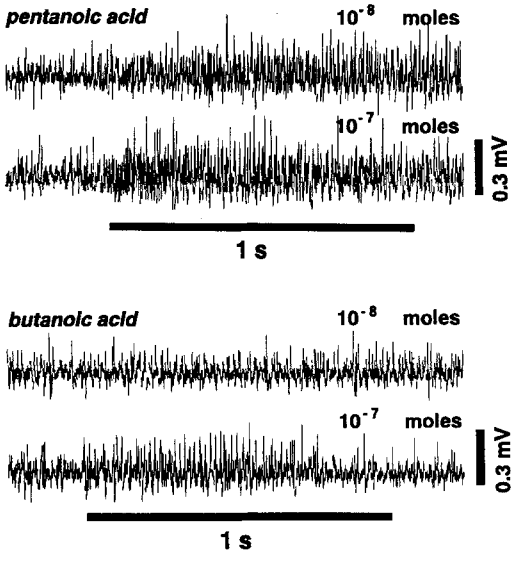

Fig. 4. Responses of olfactory receptors of the DII.1 sensillum of male *A. variegatum* to butanoic acid and pentanoic acid. Stimulus intensity (number of moles in the stimulus cartridge) is indicated above each trace. *Horizontal bars* 1 s stimulation. The great complexity of spike trains recorded from the DII.1 sensillum, which contain 14 receptor cells, prohibited us from properly analysing the receptor(s) responding to these short-chain fatty acids

heptanoic and nonanoic acids only weakly excited these receptors. By contrast with the DII.1 sensillum, the DII.5 sensillum only contains three receptor cells. It was thus possible to differentiate the two fatty receptors of the latter sensillum according to spike shape and amplitude as well as by double successive stimulations (Fig. 5). One receptor in this sensillum (type 1 in Figs. 5 and 6) was most strongly stimulated by butanoic acid but also clear-

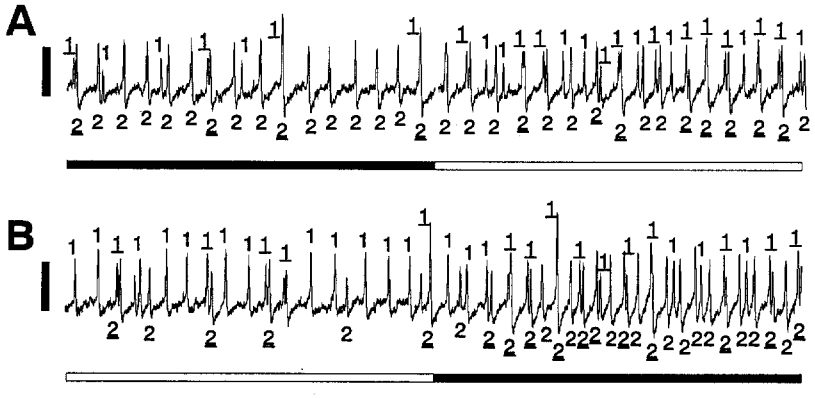

Fig. 5A, B. Double successive stimulations of fatty acid receptors in the DII.5 sensillum with 10^{-8} moles of 2-methylpropanoic acid (*solid horizontal bar*) and 10^{-8} moles of butanoic acid (*hollow horizontal bar*). Note that firing of receptor type 1 increased at the beginning of stimulation with butanoic acid (in **A**), whereas firing of receptor type 2 increased at the beginning of stimulation with 2-methylpropanoic acid (in **B**). Each trace (**A** and **B**) corresponds to a 700 ms spike train. Spikes with underlined numbering are near or overlapping events. *Vertical bar* 0.5 mV

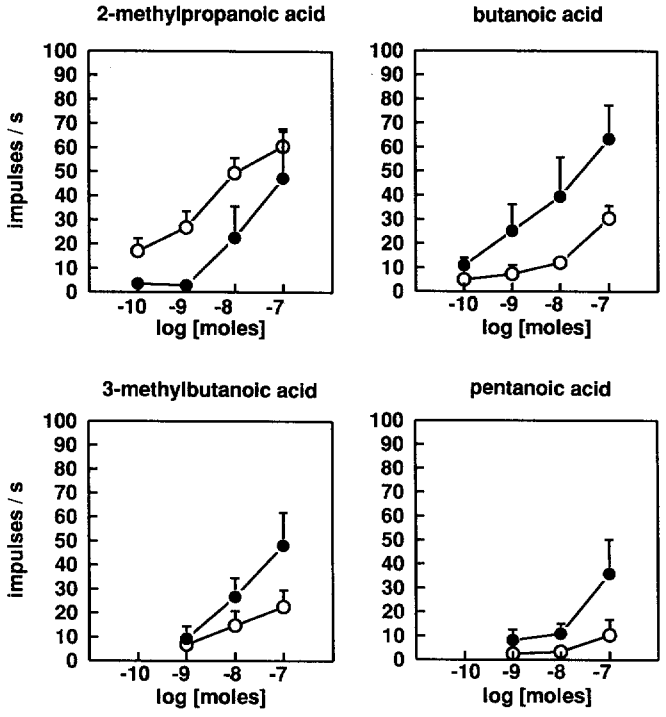

Fig. 6. Responses of two types of fatty acid receptors in the DII.5 sensillum located on the anterior pit of the Haller's organ in male *A. variegatum* to doses (moles in the stimulus cartridge) of 2-methylpropanoic, 3-methylbutanoic, butanoic, and pentanoic acid plotted against spike frequency calculated for the first 400 ms of the responses (n = 8 different ticks). *Trend lines* connect mean values; bars are standard deviations. *Solid circle*: acid receptor type 1 most sensitive to butanoic acid; *hollow circle*: acid receptor type 2 most sensitive to 2-methylpropanoic acid

ly by 2-methylpropanoic acid, 3-methylbutanoic acid and, to a lesser extent, by pentanoic acid. The second receptor was most sensitive to 2-methylpropanoic acid (type 2 in Figs. 5 and 6). To establish if the two fatty acid receptors of the DII.5 sensillum are capable of specifically coding for the different short-chain fatty acids, a firing ratio (Q) between the intensity of the response of receptor type 1 and type 2 to the different acids at various concentrations was calculated (Table 2). Q values for the dose series of 2-methylpropanoic acid were significantly different from those obtained with a similar dose series of 3-methylbutanoic acid ($P \leq 0.05$, ANOVA with the General Linear Model procedure of SAS). Both series of Q values were concentration-dependent ($P \leq 0.05$, Table 2). The butanoic acid and pentanoic dose-series did not elicit divergent Q values ($P > 0.05$), and these Q values were concentration-independent ($P > 0.05$, Table 2). However, Q values for these unbranched fatty acids differed significantly from those due to the branched fatty acids ($P \leq 0.05$, Table 2).

Butanoic and 2-methylpropanoic acids were the most prominent short-chain fatty acids in bovine and rabbit odour, respectively, as collected on Porapak (accounting for > 75% of the total amount of the C_4 and C_5 fatty acids present). However, no increase in spike frequency of the acid receptors was observed when fatty acids contained in bovine and rabbit odour eluted during GC-EL analysis of extracts, most probably because the receptors were not sensitive enough to detect the amounts present. Finally, despite its strong acidic odour, human axillary secretion did also not excite the acid receptors of either the DII.1 or DII.5 sensilla.

Table 2. Firing ratio Q (mean ± SD) of fatty acid receptor type 1 to receptor type 2 in the DII.5 sensillum of 8 different adult *A. variegatum* in response to stimulation with 4 short-chain fatty acids at 4 concentrations

	Number of moles in the stimulus cartridge				
	10^{-10}	10^{-9}	10^{-8}	10^{-7}	
2-methylpropanoic acid	0.7 ± 0.2	0.8 ± 0.3	0.9 ± 0.5	0.9 ± 0.4	§ a
3-methylbutanoic acid	1.4 ± 0.5	1.3 ± 0.6	1.8 ± 0.2	2.0 ± 0.7	§ b
Butanoic acid	2.3 ± 0.3	3.1 ± 1.0	2.9 ± 0.7	2.0 ± 0.5	c
Pentanoic acid	2.8 ± 1.1	3.2 ± 1.2	3.4 ± 0.9	2.5 ± 0.8	c

§ Signifies that Q is concentration-dependent for a defined stimulus, and Q for each stimulus is assigned a different letter when significantly different ($P \leq 0.05$ in both cases, ANOVA with the General Linear Model procedure on SAS)

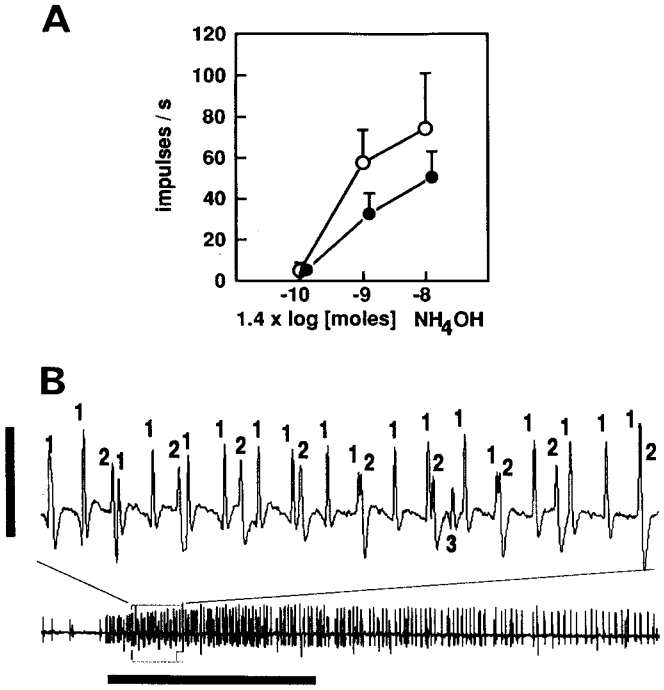

Fig. 7A, B. Responses of two NH_3 receptors of the DII.6 sensillum located on the anterior pit of Haller's organ in male *A. variegatum*. **A** Dose (moles in the stimulus cartridge) of NH_4OH plotted against spike frequency calculated for the first 400 ms of the responses ($n = 6$ different ticks). *Trend lines* connect mean values; *bars* are standard deviations. *Hollow circle*: NH_3 receptor type 1; *solid circle*: NH_3 receptor type 2. **B** Representative response of the NH_3 receptor types 1 and 2 to stimulation with 1.4×10^{-9} moles NH_4OH in the stimulus cartridge. The *upper trace* provides detail of 250 ms of the response. A spike of low amplitude from a third receptor also figures on this trace. *Horizontal bar* 1 s stimulation; *vertical bar* 0.5 mV

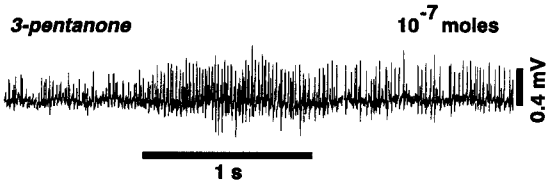

Fig. 8. Representative response of a receptor located in one of the DIV sensilla on the tarsus of adult *A. variegatum* to 10^{-7} moles 3-pentanone in the stimulus cartridge

NH_3 receptors and others. Two receptors responded strongly to the same range of NH_3 concentrations in the DII.6 sensillum such that between 10^{-10} and 10^{-9} moles of NH_4OH in the stimulus cartridge was sufficient to evoke a response (Fig. 7). However, the dose-response curve of the two receptors differed significantly ($P \leq 0.05$, ANOVA with the General Linear Model procedure of SAS). Another receptor in one of the DIV sensilla was also weakly stimulated by high, but not physiologically relevant concentrations of NH_3. This receptor was about 100 times less sensitive to NH_3 than either of the two receptors in the DII.6 sensillum. Except for a receptor in another of the DIV sensilla which responded to 3-pentanone (Fig. 8), no further receptors in the wall-pore sensilla located outside the capsule of Haller's organ on tarsus I of *A. variegatum* were characterized.

Discussion

Olfactory receptors in wall-pore sensilla on the surface of the tarsus of leg I in *A. variegatum* respond to constituents of vertebrate odours. GC-EL analyses of bovine odour presented here demonstrate the response of receptor(s) to nitrophenols in the large DII.1 olfactory sensillum on the anterior pit of Haller's organ. Other receptors, which were found in this and other sensilla, were sensitive to either lactones, short-chain fatty acids, NH_3, or 3-pentanone. However, no responses were obtained from receptors in any of these sensilla to vertebrate odours tested under the headings of human breath, human axillary secretion, or rabbit odour collected on Porapak. This contrasts with the responses of a range of olfactory receptors within the capsule of Haller's organ to different constituents of the same extracts (Table 3, Steullet and Guerin 1992a, b, 1994).

The largest wall-pore sensillum (DII.1) on the anterior pit of Haller's organ presumably bears several receptors for the aggregation-attachment pheromone component 2-nitrophenol as evidenced by the multiunit responses of Schoeni (1987). In the present study, GC-EL experiments employing this sensillum reveals the presence of four active components in odour of tick-naive steer collected on Porapak, i.e. 2-nitrophenol, 4-methyl-2-nitrophenol, and two unidentified volatiles. 4-Methyl-2-nitrophenol is the most abundant of the 4 stimulants in the odour and is also present in skin wash of steer. GC-MS analysis of air from an unoccupied stall (controls) indicates none, or at most traces of these nitrophenols, suggesting that these products are true vertebrate volatiles although neither of them has previously been reported to our knowledge from vertebrates. However, these compounds are known as air pollutants (Welsh and Watts 1990), but as such are likely to be less prevalent in the ambient air of the African habitat of *A. variegatum* than in the suburban environment in which controls were made for this study. There can be little doubt about the biogenic origin of nitrophenols, as 2-nitrophenol is also a major component of the aggregation-attachment pheromone of both *A. variegatum* and *A. hebraeum* (Schoeni et al. 1984; Apps et al. 1988). It is produced in high amounts in dermal glands (type 2) of males after successful attachment and feeding on a host (Diehl et al. 1991), thereby assuring attraction, aggregation, and attachment of conspecifics at the same feeding site (Schoeni et al. 1984; Norval et al. 1989; Delot 1990). The presence of nitrophenols in odours of even tick-naive steer could favour their infestation by pioneer males. *A. variegatum* does show a preference for parasitizing bulls over goats (Barré et al. 1991), and attach better on cattle than on sheep or rabbits (Norval et al. 1992). The present study shows that quantities of nitrophenols found in bovine odour were higher than in rabbit odour extracts, the latter containing 100 times less 4-methyl-2-nitrophenol. Could it be that *Amblyomma* has developed

Table 3. Responses and locations of characterized olfactory receptors on tarsus I of male *A. variegatum* to different vertebrate odours (human breath, human axillary secretion, and bovine and rabbit odour concentrates as collected on Porapak)

a) Receptor location	b) Sensillum type	Human breath	Human axillary secretion	Bovine odour	Rabbit odour	Characterized receptors	References
CI	wp-sw	−	−	(+)	(+)	lactone receptor	Steullet and Guerin (ibid.)
CII	wp-sw	−	−	−	−	methylsalicylate receptor	Hess and Vlimant (1984)
CIII	wp-sw	+++	−	−	−	CO_2-excited receptor	Steullet and Guerin (1992a)
CIII	wp-sw	+++	++	−	−	sulfide receptor type 2	Steullet and Guerin (1992b)
CIV	wp-sw	+++	−	−	−	CO_2-inhibited receptor	Steullet and Guerin (1992a)
CV	wp-sw	+++	++	−	−	sulfide receptor type 1	Steullet and Guerin (1992b)
CVI[a]	wp-sw	−	−	++	+++	benzaldehyde receptor	Steullet and Guerin (ibid.)
CVI[a]	wp-sw	−	−	++	+++	2-hydroxybenzaldehyde receptor	Steullet and Guerin (ibid.)
CVI[a]	wp-sw	−	++	++	+++	aliphatic aldehyde receptors	Steullet and Guerin (ibid.)
DI.1	wp-sw	−	−	(+)	(+)	lactone receptor	this paper
DI.1	wp-sw	−	−	−	−	2,6-dichlorophenol receptor	Waladde 1982, Schoeni 1987, this paper
DII.1	wp-sw	−	−	++	(+)	nitrophenol receptor(s)	Schoeni (1987), this paper
DII.1	wp-sw	−	−	(+)	(+)	pentanoic acid receptor(s)	this paper
DII.5	wp-dw	−	−	(+)	(+)	2-methylpropanoic acid receptor	this paper
DII.5	wp-dw	−	−	(+)	(+)	butanoic acid receptor	this paper
DII.6	wp-dw	−	−	−	−	NH_3 receptors §	this paper
DIV.group	wp-dw	−	−	−	−	receptor sensitive to high doses of NH_3 §	this paper
DIV.group	wp-dw	−	−	−	−	receptor sensitive to high doses of 3-pentanone	this paper

+++ Strong response, ++ medium response, (+) stimulant present in the extract but in insufficient quantity to elicit more than a slight response, − no response. **a)** Name assigned to the sensillum according to its location on the surface of the tarsus (DI-DIV of Fig. 1) or region within the capsule (CI-CVI of Fig. 1 in Steullet and Guerin 1993); [a] signifies presence of two sensilla in the region from which recordings were made within the capsule; **b)** morphological type of sensillum as described by Hess and Vlimant (1982): wall-pore single-walled sensillum (*wp-sw*), wall-pore double-walled sensillum (*wp-dw*). § NH_3 is a common vertebrate-associated volatile, e.g. in urine. No receptors from the DIII.2, LAII.1, VII.1, and VII.4 sensilla (Fig. 1) were characterized

a pheromone system which enhances attractivity of what proves to be a suitable host for pioneer males by secreting high amounts of a host-associated volatile? This would not constitute the first report on use of vertebrate-associated volatiles as components of an aggregation-attachment pheromone in the genus *Amblyomma*. 2-Methylpropanoic acid, nonanoic acid, 2-nitrophenol, and benzaldehyde are components of the aggregation-attachment pheromone of *A. variegatum* and/or *A. hebraeum*, but also vertebrate-associated volatiles to which some tick olfactory receptors are sensitive (Steullet and Guerin 1993).

A receptor in the DI.1 sensillum on the knoll distal to Haller's organ responds strongly to both γ-valerolactone and 6-caprolactone, as confirmed by double successive stimulations. The specificity of this receptor differs from that of another lactone-sensitive receptor found in the capsule of Haller's organ in *A. variegatum* which is more selectively sensitive to γ-valerolactone (Steullet and Guerin 1994). γ-Valerolactone is present in traces in bovine and rabbit odour collected on Porapak. But the quantity of γ -valerolactone in bovine odour extract was not sufficient to evoke a clear response of this receptor in GC-EL analyses employing the DI.1 sensillum, as was the case for the lactone receptor in the capsule of Haller's organ (Steullet and Guerin 1993). Lactones are reported from many vertebrate odours (Goetz et al. 1988; Burger et al. 1990), but are unknown as stimulants for other haematophagous arthropods.

Two wall-pore sensilla on the anterior pit of Haller's organ of *A. variegatum* possess fatty acid receptors: the wall-pore single-walled DII.1 sensillum with receptor(s) most sensitive to pentanoic acid, and the wall-pore double-walled DII.5 sensillum with one receptor most sensitive to butanoic acid and a second most responsive to 2-methylpropanoic acid. Comparison of the spike frequencies of these receptors in response to stimulation with the different acids indicates that the two acid receptors of the DII.5 sensillum can specifically code for 2-methylpropanoic acid and 3-methylbutanoic acid, but cannot discriminate between butanoic and pentanoic acid. However, discrimination between these straight-chain fatty acids may be possible with the acid receptor(s) of the DII.1 sensillum which are most sensitive to pentanoic acid. Equipped with these acid receptors, *A. variegatum* may be able to discriminate between mixtures of short-chain fatty acids which are widespread in vertebrate-associated volatiles (Müller-Schwarze et al. 1974; Ayorinde et al. 1982; Fox 1982; Albone 1984; Goetz et al. 1988; Kanda et al. 1990). GC-MS analysis of the odour extracts collected on Porapak indicated that butanoic was the most abundant fatty acid in bovine odour, whereas 2-methylpropanoic acid predominated in that of rabbit. However, the acid receptors of both the DII.1 and the DII.5 sensilla were not sensitive enough to respond to the fatty acid amounts present in extracts injected for GC-EL experiments. Furthermore, despite of its acidic odour, human axillary secretion did not clearly excite the

fatty acid receptors of *A. variegatum*. This may be due to the fact that human axillary secretion contains an abundance of different branched and unbranched, saturated and unsaturated C_6 to C_{11} fatty acids with (E)-3-methyl-2-hexenoic acid as the major component (Zeng et al. 1991, 1992), but lacks significant amounts of the shorter acids for which the tick possesses receptors. Short-chain fatty acids also act as olfactory stimulants for several blood-sucking insects, i.e. mosquitoes (Lacher 1967) and *Triatoma infestans* (Bernard 1974). Furthermore, these volatiles elicit probing behaviour in *Stomoxys calcitrans* (Hopkins 1964) and attraction in the sheep head fly *Hydrotaea irritans* (Thomas et al. 1985).

Two receptors sensitive to NH_3 are present in the DII.6 sensillum, each showing its own response profile. Another receptor in the DIV wall-pore sensilla group also responds to NH_3, but only at much higher doses. Similar NH_3 receptors exist in another tick species, *Rhipicephalus sanguineus* (Haggart and Davis 1980). NH_3 is known as a kairomone for some haematophagous arthropods, i.e. as a probing stimulus for *Stomoxys calcitrans* (Gatehouse 1970), and attractant for Tabanidae (Hribar et al. 1992). Although NH_3 is widely represented in vertebrate odours, none of the tick NH_3 receptors responded to the extracts tested here. One reason may be that the porous polymer (Porapak), which we used to collect vertebrate odours, is known to have a low affinity for polar low molecular weight volatiles such as NH_3 and amines (Sugisawa 1981).

Finally, as already described by Waladde (1982) and Schoeni (1987), receptors in the DI.1 and the DII.1 sensilla are highly sensitive to the common tick sex pheromone product, 2,6-dichlorophenol (Table 3). No clear responses were obtained from these receptors in single-unit recordings with whole odour extracts of vertebrates, or to individual components of these extracts in GC-EL analyses. These findings confirmed our GC-MS analyses which indicated that the halogenated phenol was not present in detectable amount in the vertebrate odours collected for this study.

Although Lees (1948) suggested that the olfactory receptors responsible for host-odour perception in ticks are mainly located within the capsule of Haller's organ, this study clearly demonstrates that sensilla located elsewhere on the tarsus also respond to some vertebrate-associated volatiles. However, these sensilla do not only contain olfactory receptors. Although human breath delivered directly to the tarsus activated receptors in both the DII.5 and DII.6 sensilla (Steullet, unpublished), the same stimulus delivered in a temperature- and humidity-controlled airflow to the preparation did not excite any receptor. This suggests that thermoreceptors might be involved in the response to breath. In some insects, grooved double-walled sensilla contain olfactory receptors together with a cold unit (Altner et al. 1981; Steinbrecht 1984). Breath components, essential for the arousal of resting *A. variegatum* to initiate host-finding, seem to be exclusively detected by olfactory receptors within the capsule of Haller's organ (Steullet and Guerin 1992a, b), while receptors sensitive to other vertebrate-associated volatiles and/or pheromone compounds are distributed among both the capsule sensilla and those situated on the surface of the tarsus I. Table 3 summarizes responses of all characterized olfactory receptors on tarsus I of *A. variegatum* to the different vertebrate odours tested in our studies (Steullet and Guerin 1992a and b, 1994). This array of characterized olfactory receptors certainly equips *A. variegatum* for a finely tuned image of its odorous environment, and opens a new avenue of research on the behavioural responses of this tick species to the identified stimulants and their role in host-finding.

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