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Ultrastructure and physiology of the CO_2 sensitive sensillum ampullaceum in the leaf-cutting ant *Atta sexdens*

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Abstract

The sensilla ampullacea on the apical antennomere of the leaf-cutting ant *Atta sexdens* were investigated regarding both their responses to CO_2 and their ultrastructure. By staining the sensillum during recording, we confirmed that the sensilla ampullacea are responsible for CO_2 perception. We showed that the sensory neurons of the sensilla ampullacea are continuously active without adaptation during stimulation with CO_2 (test duration: 1 h). This feature should enable ants to assess the absolute CO_2 concentration inside their nests. Sensilla ampullacea have been found grouped mainly on the dorso-lateral side of the distal antennal segment. Scanning and transmission electron microscopic investigations revealed that the external pore opens into a chamber which connects to the ampulla via a cuticular duct. We propose protection against evaporation as a possible function of the duct. The ampulla houses a peg which is almost as long as the ampulla and shows cuticular ridges on the external wall. The ridges are separated by furrows with cuticular pores. The peg is innervated by only one sensory neuron with a large soma. Its outer dendritic segment is enveloped by a dendritic sheath up to the middle of the peg. From the middle to the tip numerous dendritic branches (up to 100) completely fill the distal half of the peg. This is the first report of a receptor cell with highly branched dendrites and which probably is tuned to CO_2 exclusively. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Carbon dioxide; Insects; Microclimate; Non-adapting; Sensory organ

1. Introduction

For social insects which live in poorly aerated nests, the perception of CO_2 is of particular interest. Due to respiration, O_2 and CO_2 concentrations inside the nest are linked. Because insects cannot perceive O_2, CO_2 can provide valuable information about the availability of O_2.

Many behavioral studies have shown that in ants (Burkhardt, 1991; Hangartner, 1969; Harkness and Harkness, 1988; Kleineidam, 1999; Kleineidam and Tautz, 1998) and in bees (Lacher, 1967) CO_2 elicits specific reactions. In the context of the control of microclimate, it was shown for bees (Seeley, 1974) and bumble bees (Weidenmüller et al., 1999) that an increased CO_2 concentration inside the nest induces a fanning response which results in an influx of fresh air. A behavioral response with a comparably immediate effect on the nest microclimate cannot be performed by ants. Ants have to rely on passive nest ventilation which they can influence by changing the nest structure (Kleineidam and Roces, in press).

The existence of CO_2-receptor cells has been established electrophysiologically long ago for the antenna of bees (Lacher, 1964) and ants (Dumpert, 1972). However, the sensillum housing the CO_2-receptor cells has been identified only recently in the leaf-cutting ant *Atta cephalotes* (Kleineidam and Tautz, 1996). This sensillum is a typical 'sensillum ampullaceum' (Snod-

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grass, 1935), embedded below the antennomere cuticle and hence not readily detectable at the surface of the antenna. The morphology of the sensillum is very different from other olfactory sensilla. The sensory hair (peg) is located inside an ampulla, which connects to the outside only via a narrow duct.

Investigating the perception of CO_2 is of particular interest for a better understanding of odor perception and adaptation of sensillar structures. Compared to most other odorants, CO_2 is a simple molecule which naturally occurs in high concentrations. This difference to larger and less abundant odorants is expected to be reflected in the morphology and ultrastructure of the CO_2 sensillum. It is tempting to think that the extraordinary structure of the sensillum ampullaceum reflects an adaptation for CO_2 perception.

It has been shown that the CO_2-receptor cells of the leaf-cutting ant do not adapt during a stimulation of several minutes (Kleineidam and Tautz, 1996). A tonic response without adaptation during continuous stimulation was also shown for humidity receptors (Altner and Loftus, 1985; Loftus, 1976; Yokohari et al., 1982) and temperature receptors (Davis and Sokolove, 1975; Ehn and Tichy, 1996; Loftus, 1968). All these studies are based on recordings of neural activity during several minutes of stimulation. However, ants stay in their subterranean nests for hours and have to monitor the absolute CO_2 concentration. So far, no quantitative data have been obtained describing the response of the CO_2-receptor cells in this biologically relevant time scale.

In the present study we give a detailed description of the ultrastructure of the sensillum ampullaceum in the leaf-cutting ant *Atta sexdens*. We present data on the response of the CO_2-receptor cells to stimulation lasting up to 1 h and using CO_2 concentrations that leaf-cutting ants normally encounter inside their nests. We also show the result of an improved staining method for identifying the recorded sensilla.

2. Material and methods

2.1. Animals

Workers of *A. sexdens* were obtained from a laboratory colony collected in Botucatú, São Paulo, Brazil. The colony was approximately 3 years old and its fungus garden occupied a volume of about 12 l. The colony was reared at the Biozentrum of the University of Würzburg in an environmental chamber at 25°C and 50% relative humidity in a 12 h/12 h photoperiod and fed mainly with privet leaves (*Ligustrum vulgaris*). For the experiments, workers were collected from the feeding site, thus it is assumed that only foragers were investigated.

2.2. Electrophysiology

Animals were fixed on a plastic holder with adhesive tape and the scapus was glued onto the holder with water soluble Tipp-Ex®. The recording electrodes mounted on a micromanipulator (Märzhäuser HS-6, Germany) were superficially inserted into the cuticle next to an opening of a sensillum ampullaceum or coeloconicum visible in the microscope. Sharp glass electrodes (similar to those used for intracellular recording with a tip diameter of about 0.5 μm), prepared with a laser electrode puller (Sutter, Model P2000, Switzerland) and filled with 0.15 M KCl were used as an extracellular recording electrode. An electrolytically sharpened tungsten electrode was inserted deep into the last flagellar segment.

The extracellular recordings were made under visual control with a Leitz microscope equipped with a long distance objective (Leitz, NPL-Fluotar L25/0.35). The total magnification was 250 × , and the illumination of the flagellum was from below with a bright-field condensor microscope light.

The recordings were band-pass filtered (60 Hz–3 kHz) and amplified 1000× (Kemo, VBF8, Great Britain). Data were digitized at a sampling rate of 12 kHz (CED, 1401plus, Great Britain) and stored for analysis (CED, Spike2 V2.01, Great Britain) on a PC.

In natural nests, CO_2 concentrations have been found to be about 1% (Kleineidam and Roces, in press). Therefore, the adaptation characteristics of the sensory neurons were investigated by continuous stimulation over 1 h with a concentration of 1.2% CO_2. This CO_2 concentration was obtained by mixing air containing 10% CO_2 ($\pm$1%) from pressure tanks with 'standard' air containing 0.05% CO_2. Throughout the experiment the mean neural activity was measured for periods of 30 s at defined phases:

- before stimulation (standard air current; 0.05% CO_2): 10 min before and immediately before stimulation;
- during stimulation (1.2% CO_2): 1 min after stimulation started and thereafter every 10 min;
- after stimulation (standard air current): 10 min after stimulation was terminated.

2.3. Identification of the CO_2 sensilla

The recording electrode was filled with 1 μl KCl (0.15 M) containing a labeled dextran as fluorescent dye (MW 3000 with texas red, Sigma-Aldrich, USA) and backed up with 0.15 M KCl.

Only sensilla in the last flagellar segment were investigated. The neural activity of a single CO_2-receptor cell was recorded and the sensillum was simultaneously

stained passively (without current injection) for 30 min. Next, the last two segments were cut off with a razor blade and immediately fixated with 5% glutaraldehyde in 0.1 M phosphate buffer (pH 6.9) for 1 h at 4°C. After dehydration in a graded ethanol series the flagellar segments were embedded through propylene oxide in Durcupan ACM (Fluka, Buchs, Switzerland) and sectioned with a diamond knife on a microtome (RMC, MT-7000 Ultra, Germany). The semi-thin sections were then screened with an inverted microscope (Zeiss, Axiovert 405 M, Germany) for regions containing fluorescent dye.

2.4. Morphology

For scanning electron microscopy (SEM) of the cuticular structures, the flagellum was excised and the last antennomere was sectioned obliquely with a razor blade. These tip-fragments were cleaned with KOH-solution, sonicated in order to remove cell particles, dehydrated in a graded ethanol series and critical point-dried (Bal-Tec CPD030, Balzers, Liechtenstein). Then the apical antennomere fragments were glued vertically onto the SEM specimen supports in order to allow an investigation of the inner and outer side of the antenna. Finally, the specimens were gold-coated (Balzers Union MED010 sputter-coating unit, Balzers, Liechtenstein) and examined with a Zeiss DSM962 scanning electron microscope.

For transmission electron microscopy (TEM) observations, the apical antennomeres of three workers were detached to aid fixative penetration. These specimens were immediately immersed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer + 5% sucrose (pH 7.2–7.3), and left at 4°C for 2 h. After rinsing overnight in cacodylate buffer, the specimens were postfixated in 1% osmium tetroxide at 4°C for 1 h and rinsed in the same buffer. Dehydration in a graded ethanol series was followed by embedding in Epon-Araldite with propylene oxide as bridging solvent. The thin sections were obtained with a Diatome diamond knife mounted on a L.K.B. Nova ultramicrotome, collected on collodium-coated 50 mesh grids, stained with uranyl acetate (20 min, room temperature) and lead citrate (5 min, room temperature) and investigated with a Philips EM 400 T eclectron microscope.

3. Results

3.1. Electrophysiology

The preparation allowed us to record the neural activity continuously over a long period (up to 3 h). The mean 'background' activity was 5.8 Hz 10 min and just before stimulation (SD ± 2.9 and SD ± 3.0; $n = 9$; 0.05% CO_2) and 5.2 Hz (SD ± 2.4, $n = 9$) at the same background CO_2 concentration 10 min after stimulation (hence after 70 min). These values are not significantly different (t-test, $p = 0.67$). Fig. 1 shows the mean activity of nine receptor cells for the different phases of stimulation. During stimulation the mean activity was 15.1 Hz (SD ± 5.8, $n = 9$) and significantly different from the activity at 0.05% CO_2 (t-test, $p < 0.01$). Mean neural activity did not change significantly throughout the stimulation (t-test, $p > 0.1$ for all combinations; $p \geq 0.1$: not significant). Thus, we did not find any adaptation of the receptor cells during long-term stimulation.

3.2. Morphology of the sensillum ampullaceum

Sensilla ampullacea belong to the 'peg in pit' sensilla, typically embedded into the lumen of the flagellum and connected to the outside via a small cuticular opening. Of these openings (about 1–2 μm in Ø) about 10 are grouped in a distinct area on the dorso-lateral side of the apical antennomere (Figs. 2 and 3a).

Nevertheless, the presence of such external pores cannot be assigned to the sensilla ampullacea with certainty, as sensilla coeloconica share the same external features and are located in close vicinity to the former. Although there are variations in pore features (e.g. rim structure), transitions exist and therefore classification based on pore structure alone is not possible.

SEM observations of the inner cuticular wall of the last antennomere treated with KOH show the special

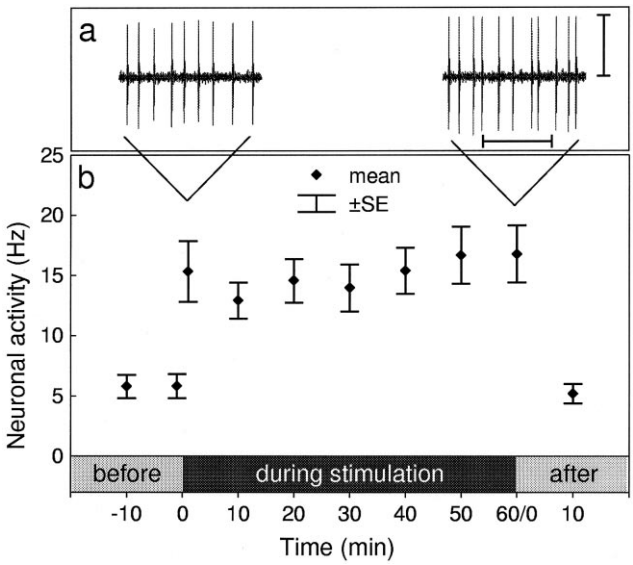

Fig. 1. Response properties of a CO_2-receptor cell during long-term stimulation (1 h) with 1.2% CO_2. A: Example of the extracellular recording at the beginning and at the end of stimulation. Scale bar: horizontal 0.5 s; vertical 0.5 mV. B: Mean values of nine cells with standard deviation and standard error show that no adaptation occurs up to the end of stimulation after 60 min.

feature of the cuticular apparatus of several sensilla ampullacea (Fig. 3b). After removal of the tissue the characteristic cuticular structures of the sensilla ampullacea with their long and narrow ducts (45.5 μm in length; SD ± 4.0; $n = 14$), terminating in an ampulla at the base, become clearly visible.

Serial longitudinal and cross sections of the apical antennomere show that in the sensillum ampullaceum the duct widens distally to form an empty spherical chamber (about 5 μm Ø) that is connected to the outside via the external pore (Fig. 4a). The nearby sensilla coeloconica do not have a duct and are embedded almost completely within the thick antennomere cuticle. Like the sensilla ampullacea they comprise a spherical chamber, but in the sensilla coeloconica the chamber contains an innervated peg of about 5 μm (Fig. 4b). These chambers and (in sensilla ampullacea) the duct and the ampulla are filled with air.

In each of the staining experiments only one single sensillum ampullaceum was marked with the fluorescent dye, indicating the specificity of the method (Fig. 2). In all successful simultaneous staining and recording experiments ($n = 6$) in which CO_2 was the adequate stimulus for the receptor cell a single sensillum ampullaceum was stained, but never a sensillum coeloconicum. We found the fluorescent dye at the base of the peg and along the distal part of the duct.

The ducts of the sensilla ampullacea extend more or less parallel to the antennomere cuticle in the antennomere lumen with a constant diameter of about 1 μm. Proximally, they enlarge into an ampulla 25 μm long and about 4 μm wide (Fig. 3c). Inside the ampulla there is a peg which is almost as long as the whole ampulla (about 20 μm length) (Fig. 4c). The cuticle of the peg is continuous with that of the ampulla, and much thicker at the base (~1 μm) than toward its tip

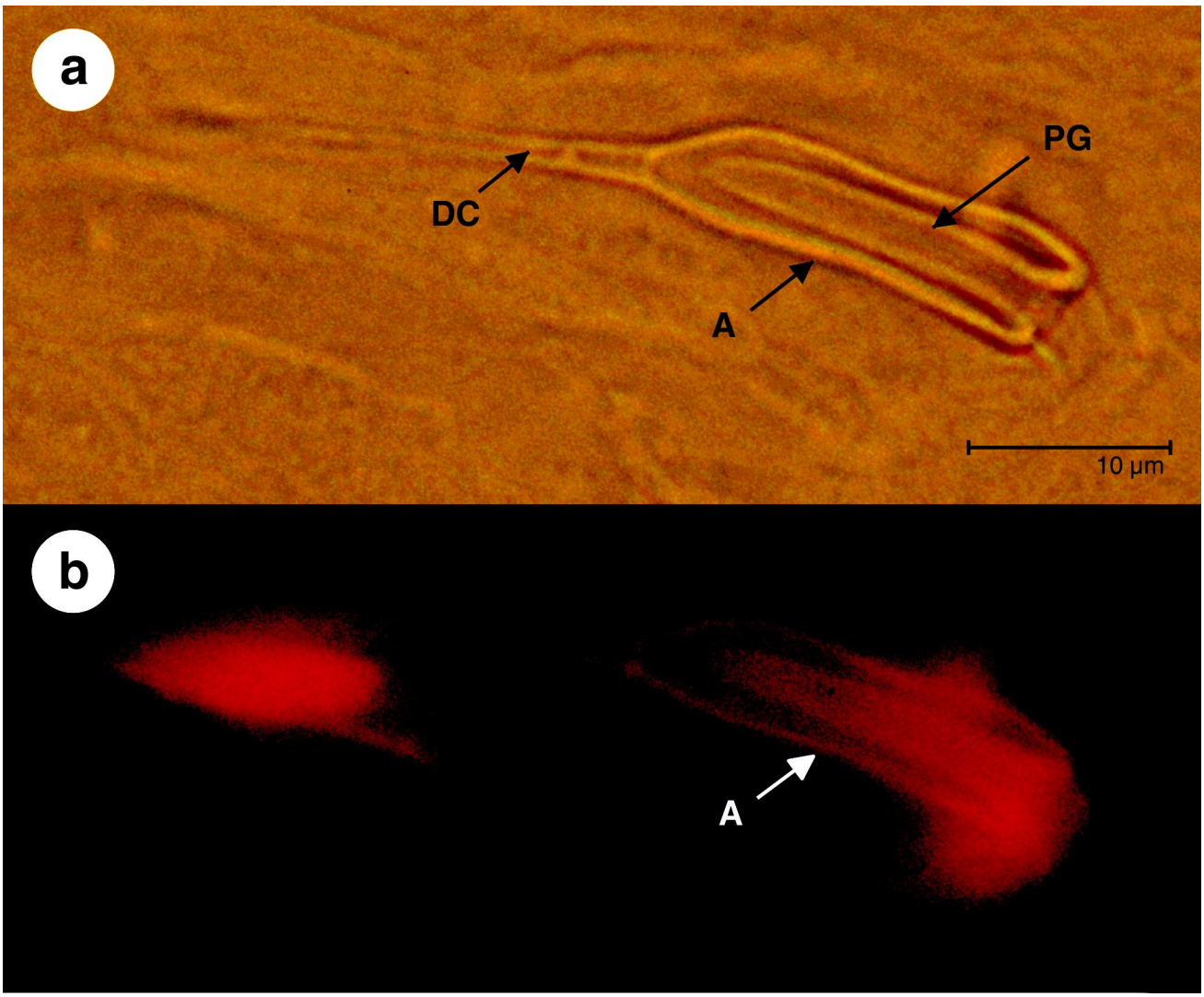

Fig. 2. Photomicrograph showing a longitudinal section of a sensillum ampullaceum. (a) Light micrograph; (b) fluorescent micrograph, stained with a texas red labeled dextran. A, ampulla; DC, duct; PG, peg.

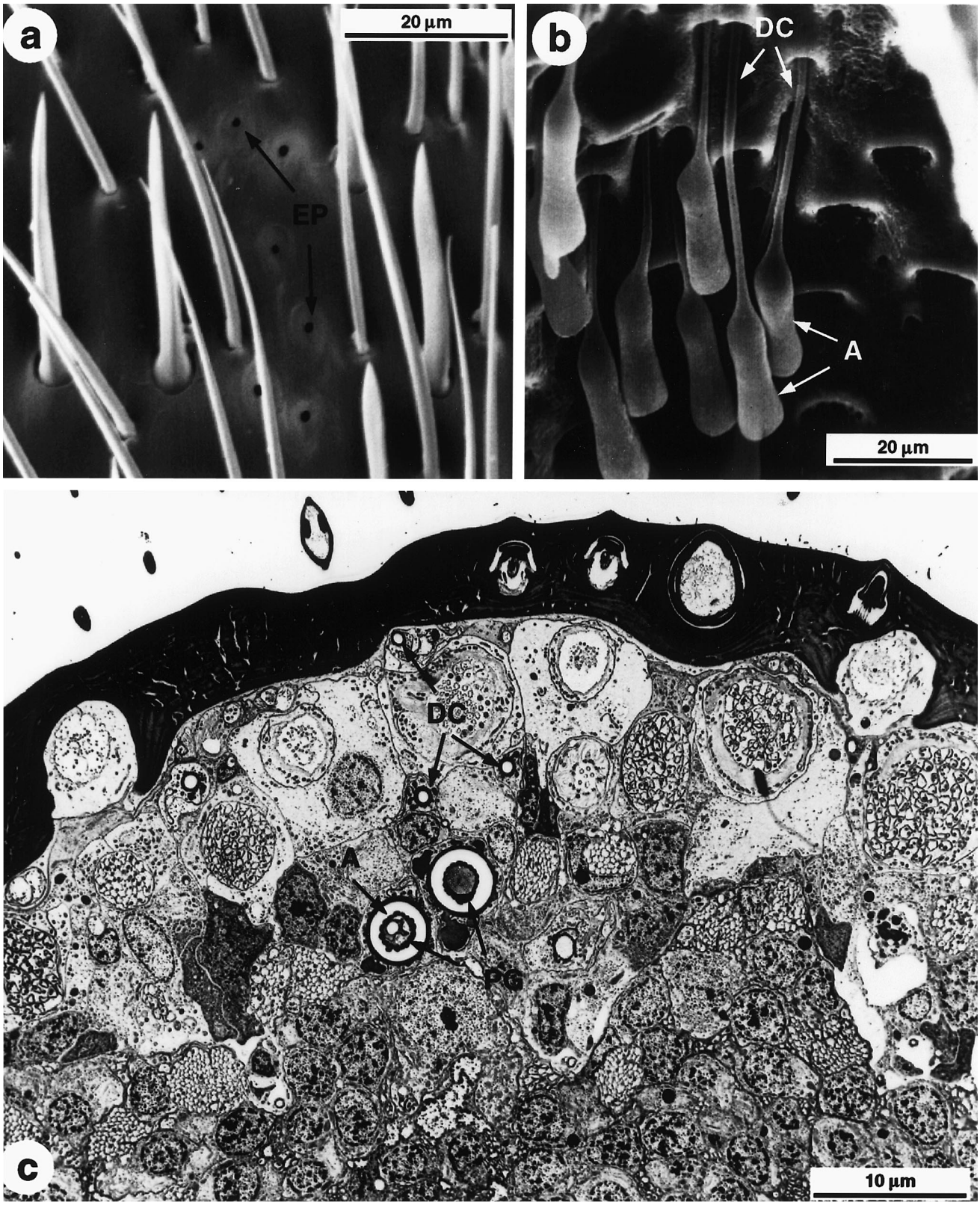

Fig. 3. Scanning electron micrographs showing an overview of the cuticular surface of the apical antennomere with external pores (EP) in (a), and in (b) the internal view of the same area with a group of sensilla ampullacea. (c) Transmission electron micrograph showing a detail of a cross section of the apical antennomere with cuticular and cellular parts of sensilla ampullacea. A, ampulla; DC, duct; PG, peg.

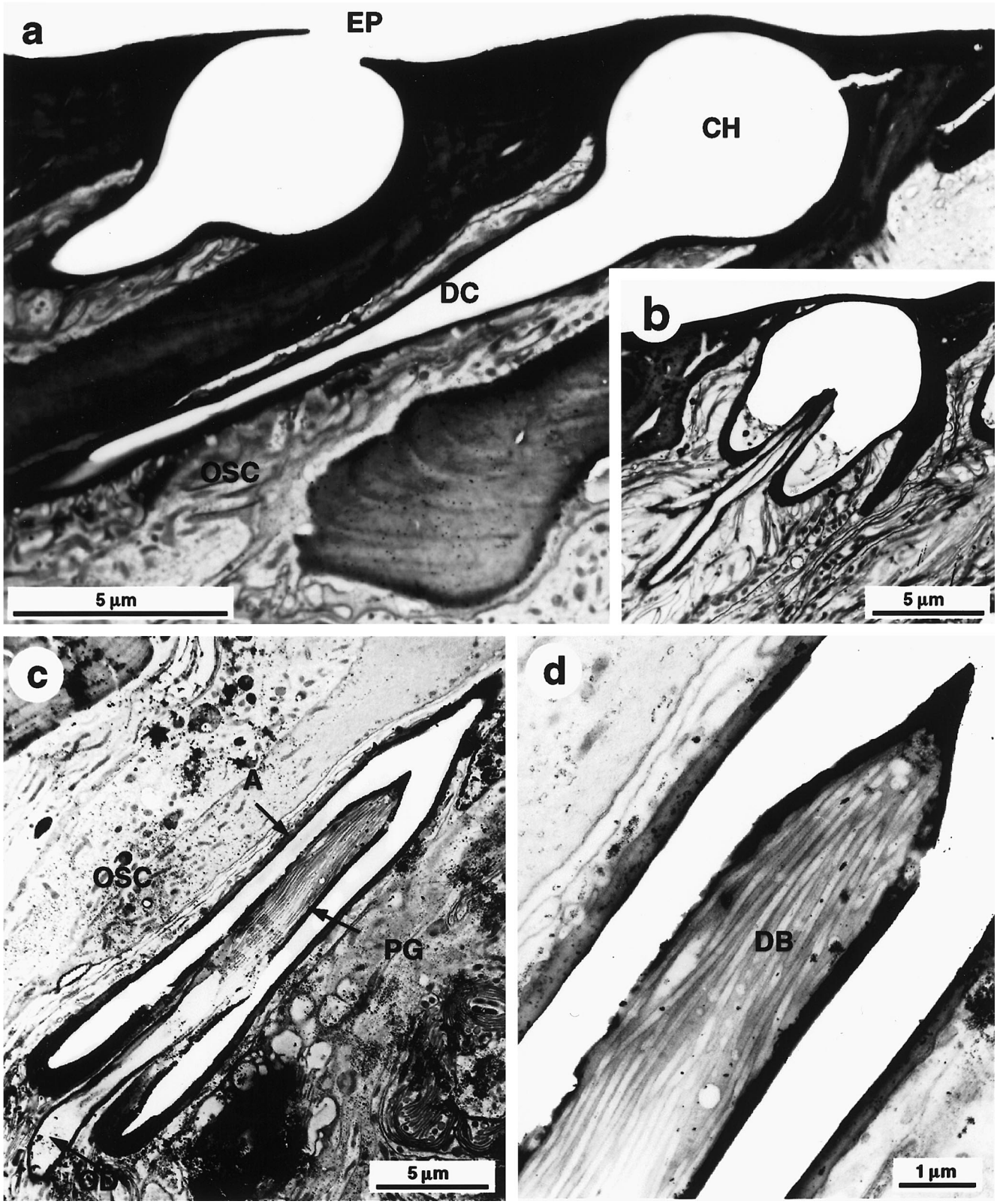

Fig. 4. TEM photomicrographs of sensilla ampullacea. (a) Longitudinal section of two sensilla ampullacea showing the external pore and the chamber in the antennomere cuticular wall. (b) Longitudinal section of a sensillum coeloconicum. (c) Longitudinal section of an ampulla showing the peg innervated by one dendrite which branches at about half length of the peg. (d) Detail of the apical part of the peg with the dendritic branches completely filling the lumen. A, ampulla; CH, chamber; DB, dendritic branches; DC, duct; EP, external pore; OD, outer dendritic segment; OSC, outer sheath cell; PG, peg.

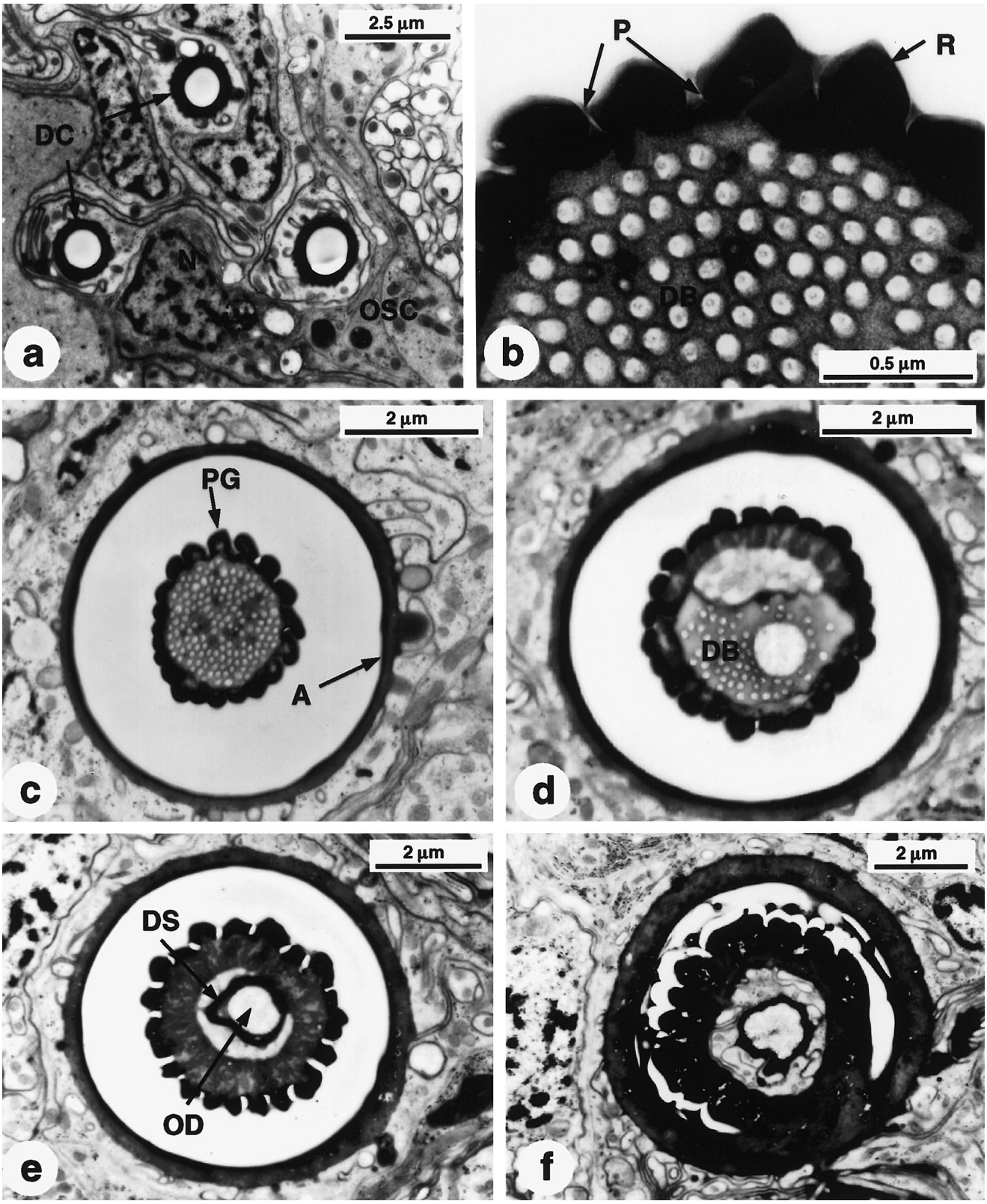

Fig. 5. TEM photomicrographs of cuticular components of sensilla ampullacea: serial cross sections of the ducts (a), and the peg inside the ampulla at a subapical (b and c), intermediate (d and e) and basal level (f). A, ampulla; DB, dendritic branches; DC, ducts; DS, dendrite sheath; N, nucleus of a sheath cell; OD, outer dendritic segment; OSC, outer sheath cell; P, pores; PG, peg; R, ridge.

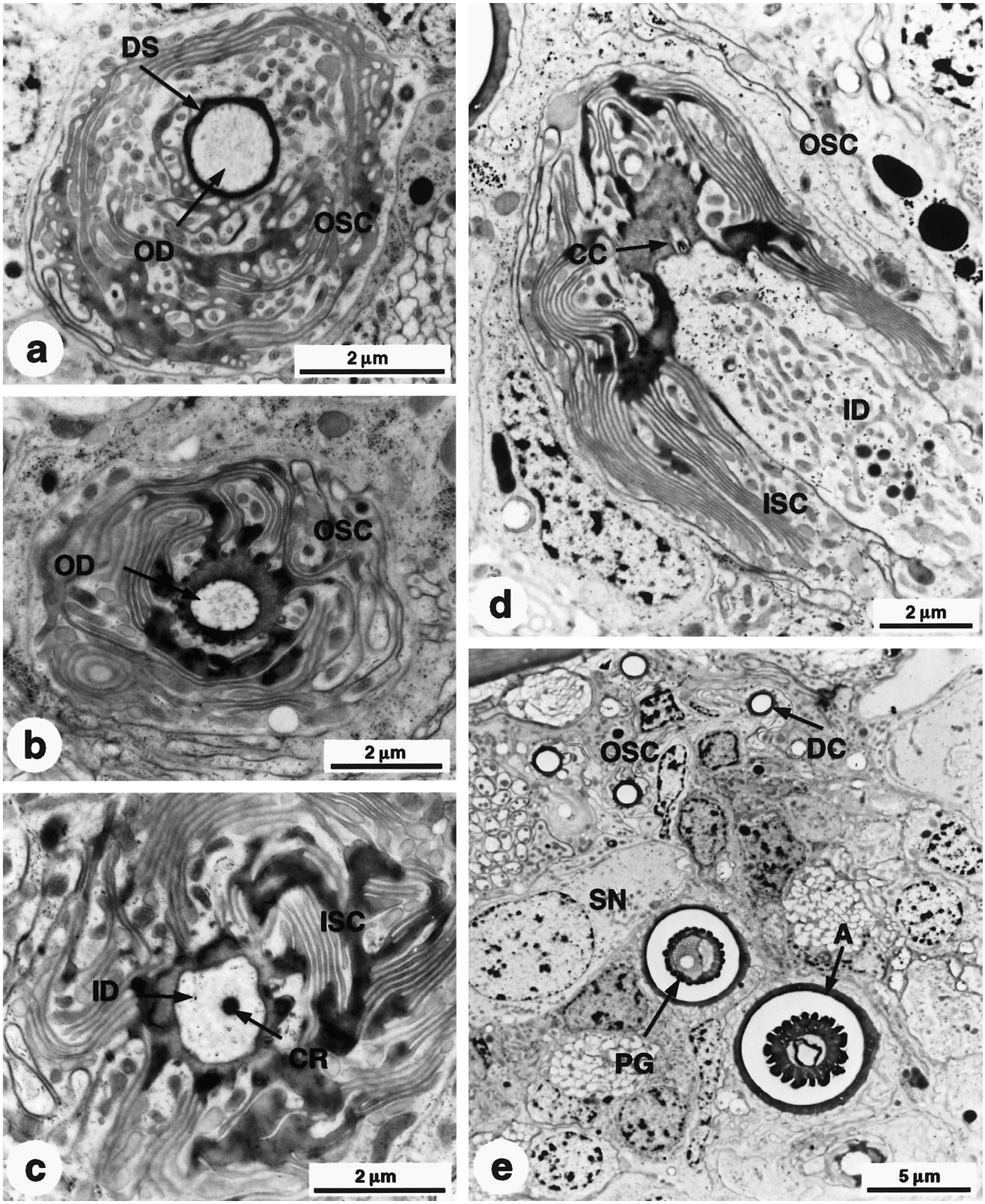

Fig. 6. TEM photomicrographs of cellular components of sensilla ampullacea: (a) cross sections through the outer dendritic segment encased in the dendritic sheath; (b) the region near the ciliary constriction; and (c) the ciliary rootlets. (d) Oblique section through the ciliary constriction and (e) cross section overview showing the large soma of the sensory cell. A, ampulla; CC, ciliary constriction; CR, ciliary rootlets; DC, duct; DS, dendrite sheath; ID, inner dendritic segment; ISC, inner sheath cell; OD, outer dendritic segment; OSC, outer sheath cell; PG, peg; SN, sensory neuron.

(~0.3 μm). The surface of the peg has a constant number of about 20 finger-like cuticular ridges, tapering from the base to the tip and separated by furrows. At the base of the peg the neighboring ridges are fused. Towards the tip of the profile, the ridges become mushroom-like (Fig. 5c–f). Pores are present in the furrows separating the cuticular ridges, which enlarge inside the cuticular wall (Fig. 5b).

The sensillum ampullaceum is innervated only by a single sensory neuron, characterized by the presence of a large sensory soma (~5 μm Ø) with a big nucleus, noticeably bigger than those of the surrounding sensory cell bodies (Fig. 6e). An undetermined number of accessory cells are wrapped around the soma of the sensory neuron. The sensory neuron has a relatively short but thick inner dendritic segment (~3 μm Ø), is rich in mitochondria and tightly wrapped by a lamellated inner sheath cell (Fig. 6d). Since this sheath cell produces the dendrite sheath (Fig. 6c), it represents a thecogen cell. After the ciliary constriction, the inner dendritic segment forms a smaller outer dendritic segment (~1 μm Ø) enveloped by a lamellated outer sheath cell (Fig. 6a,b).

The outer dendritic segment enters the peg at the base of the ampulla enveloped by the dendrite sheath and by one of the accessory cells (trichogen or tormogen cell) (Fig. 5e,f). The dendrite starts branching in the middle of the peg (Figs. 4c and 5d), and the number of dendritic branches increases rapidly toward the tip. Here the whole peg lumen is filled with a conspicuous number of more than 100 branches (Figs. 4d and 5b,c).

4. Discussion

Sensilla ampullacea are common in Hymenoptera. In ants this type of sensillum can be found accumulated at the tip of the antenna, which is described for many species (Dumpert, 1972; Ehmer, 1997; Hashimoto, 1991; Prelinger, 1940; Riedl, 1995; Kleineidam and Tautz, 1996). Besides ants, sensilla ampullacea have been found in several other groups of the Hymenoptera (Ågren and Hallberg, 1996; Martini, 1984; Slifer and Sekhorn, 1961; Walther, 1979). However, this study is the first detailed description of sensilla ampullacea in Hymenoptera. So far the function of the sensilla ampullacea has been elucidated only for the leaf-cutting ant *A. cephalotes* in a previous study (Kleineidam and Tautz, 1996). By staining the sensillum during electrophysiological recordings, we confirmed that, as expected, sensilla ampullacea are responsible for CO_2 perception in another species of the same genus, *A. sexdens*. The staining technique presented in this study allowed a precise identification of the sensillum recorded from. The sensilla ampullacea in *A. sexdens* are innervated by only one single receptor cell. Natural odor blends of the ants (squeezed body parts), green leaves (the foraging substrate of the colony) and humidity did not influence the response of the receptor cell, and temperature changes did so only very slightly. Thus, the sensillum can be assumed to be specialized for CO_2 perception. We are now able to compare the structure of the CO_2 sensitive sensilla ampullacea of the leaf-cutting ant with the few other known sensilla for CO_2 perception in order to find common characters.

In most insect cases, where at least one receptor cell is tuned exclusively for perception of CO_2, the sensilla have been found to reside in a pit. In Lepidoptera they are located on the labial palps in the pit organ (Bogner, 1990; Bogner et al., 1986; Stange, 1992; Stange et al., 1995). In Diptera the CO_2 sensitive sensilla capitula are also located in a superficial pit on the maxillary palps (Grant et al., 1995; Kellogg, 1970; McIver, 1982; Sutcliffe, 1994). However, in the tsetse fly the CO_2 sensitive sensillum on the flagellum is not located in a pit (Bogner, 1992) neither is the putative CO_2 sensitive sensillum of the Queensland fruit fly (Hull and Cribb, 1997).

With their long and narrow duct the sensilla ampullacea resemble the most extreme case of embedding. The question arises: what functional significance might such embedding have? Unlike in all other olfactory sensilla, stimulus perception in sensilla ampullacea takes place below the antennomere cuticle. CO_2 has to pass the long and narrow duct until perception can take place at the sensory peg inside the ampulla.

Prelinger (1940) was the first to discuss the long ducts of the sensilla ampullacea and to compare them among different ant species. He found shorter ducts in species which he suggested lead a 'hidden' life, like e.g. *Solenopsis* and *Leptothorax* and thus assumed a correlation between habitat and duct length (Prelinger, 1940). Unfortunately, the only two categories he used, 'lively and big eyes' vs 'hidden life with small eyes' are somewhat superficial and can be assigned neither to a particular behavior nor to a particular habitat.

In order to discuss the functional relevance of the duct, we hypothesize that the sensilla ampullacea are embedded for isolation from environmental changes e.g. in temperature or humidity. An isolation of the sensory peg against environmental temperature fluctuations should favor a central position in the lumen of the antenna. Although the bulk of thermal resistance is at the surface of the antenna, changes in temperature are expected to be lower in the vicinity of the haemolymph canals inside the antenna than close to the antennal surface. However, the ampullae and the ducts are parallel to the longitudinal axis of the antenna and not centrally oriented, which makes isolation against temperature fluctuations less likely.

A second reason for isolation would be protection against water loss. If the sensory peg has little protection against water loss, long ducts might be favored in order to reduce evaporation.

The longest duct lengths have been described in the ant *Lasius fuliginosus* which often can be seen foraging in open space during sunshine (pers. observ.). In *L. fuliginosus* the ducts are 80–100 μm long, often bent and sometimes even in spirals (Dumpert, 1972) which is not known for any other species. In the desert ant *Cataglyphis bicolor* the sensilla ampullacea also have long ducts (~60 μm) (Riedl, 1995). The duct length of the sensilla ampullacea in workers of *A. sexdens* are of medium size among known duct lengths and with about 45 μm (this study) longer than in e.g. *Proceratium japonicum* (Ponerinae) (Hashimoto, 1990) and shorter than in *L. fuliginosus* (Formicinae) (Dumpert, 1972). If reduction of water loss is an important factor for selection, then longer ducts make much sense for ants foraging in dry habitats.

The category 'hidden life' used by Prelinger (for which he found short duct lengths), often corresponds to a habitat with high relative humidity, which supports our hypothesis. The mean relative humidity in the habitat of *A. sexdens* is high, but when foraging during daytime the workers might be exposed to very low humidity. As soon as a difference in relative humidity between the air in the ampulla and the environment exists evaporation is reduced by the duct. A doubling of the duct length cuts the evaporation in half and thus is a remarkable contribution for protection against evaporation (see Appendix). Diffusion of CO_2 into the ampulla is also affected by the duct. But, only the response onset is delayed. A doubling of duct length doubles the time until a new equilibrium is established inside the ampulla. Diffusion in such dimensions is very fast, yet even a short delay might limit the temporal resolution of the receptor cell by acting as a low-pass filter (Kleineidam and Tautz, 1997).

The peg of the investigated sensillum ampullaceum belongs to thin-walled sensilla (Slifer, 1970), bearing numerous cuticular pores. All sensilla with CO_2-receptor cells investigated so far are thin-walled single-walled sensilla (Bogner et al., 1986; Kaib et al., 1993; Lee et al., 1985). In single-walled sensilla, pore-tubules normally extend from the pore-kettles into the sensillar lymph (Steinbrecht, 1997). However, we could not identify pore-tubules in the sensilla ampullacea of *A. sexdens*. The reason for not detecting pore-tubules might be that the distance between the pores and the dendritic sheath is very small in the investigated sensillum. Thin-walled sensilla are abundant on the antennae of many species of insects and have been found side by side with thick-walled sensilla on the antennal surface (Steinbrecht, 1973). Apparently, these sensilla do not suffer from water loss. The fact that in other insects the CO_2 sensitive sensilla are functional outside pits suggests that their sensillar walls with their pore structures are sufficient barriers against evaporation. Thus, the hypothesis that the ducts are means to protect against water loss cannot be generalized to all CO_2 sensitive sensilla. It may be that the sensilla ampullacea in ants and honeybees represent a special case. Comparative morphology on the sensilla ampullacea of different ant species supports our hypothesis of protection against evaporation, but more studies are necessary to provide evidence.

A common character of CO_2-receptor cells found in insects is an increased surface area of the dendrites. In Lepidoptera the dendrites are lamellated (Lee et al., 1985) and in Diptera there are also some indications that the dendrites are lamellated (Sutcliffe, 1994). We have shown that the single dendrite inside the peg of the sensillum ampullaceum is distally highly branched. Similar to lamellation, branched dendrites supposedly serve to increase surface area, hence to enhance the neuron's sensitivity (Keil and Steinbrecht, 1984; Steinbrecht, 1989). Like other receptor cells for general odorants, the CO_2-receptor cells have a similar range of sensitivity (10^3 units), but at high stimulus intensities (Kleineidam, 1999; Kleineidam and Tautz, 1996). Compared to general odorants, CO_2 occurs in high concentrations (10^{16}–10^{18} molecules/ml). The peri-receptor events might be different in CO_2 perception compared to perception of pheromones or general odorants. While, for example, the pheromone receptors of moths are considered as flux detectors, the CO_2-receptors are expected to function as concentration detectors (Kaissling, 1998). Since CO_2 is water-soluble but also sufficiently lipid-soluble to cross cell membranes it can enter the sensillar lymph and may act directly on the dendritic membrane without involvement of a carrier mechanism (Stange and Stowe, 1999). In this case no odorant binding proteins (OBPs) would be necessary. Binding proteins for CO_2 perception have been proposed in *Cactoblastis* although the homology to other known OBPs is fairly low (Maleszka and Stange, 1997). It remains to be shown whether such basic differences exist between the perception of CO_2 and general odorants.

The characteristic phasic-tonic response of CO_2-receptor cells for short stimuli is not different from that of other olfactory receptor cells. Our results in *A. sexdens* are similar to those attained in *A. cephalotes* (Kleineidam and Tautz, 1996). Neural adaptation is a common feature for olfactory receptor cells and enhances the ability to discriminate intensity differences over a wide range, but the ability to code absolute intensities is reduced (Kaissling et al., 1987; Mustaparta, 1990). In this study we focused on the response to long lasting stimulation as it is relevant for

the ants inside the nest under natural conditions. Our results revealed that the tonic response of the investigated CO_2-receptor cells does not adapt and that therefore the cells are able to constantly monitor the CO_2 concentration. This result has been suggested for other CO_2-receptor cells as well, based on measurements of a few minutes (Bogner, 1992; Bogner et al., 1986; Grant et al., 1995; Lacher, 1964). Since we know that a given stimulus causes slow and persistent changes in the amount of cyclic nucleotides and enzymes which are believed to participate in the regulation of membrane conductances (Stengl et al., 1999; Ziegelberger et al., 1990), only a quantitative analysis of long lasting stimulation allows an evaluation of adaptation properties. Besides the CO_2-receptor cells investigated here, such a stable response during continuous stimulation has been described for no other olfactory sensory neurons so far. In most cases the tonic response of chemosensory neurons adapts almost immediately. This is also the case in the CO_2-receptor cells of *Cactoblastis* (Lepidoptera) (Stange et al., 1995) which the authors consider as an exception.

We have shown that olfactory receptor cells do not necessarily need to adapt to continuous stimulation. It is possible that the large soma and nucleus of the CO_2-receptor cells in the sensilla ampullacea reflect their ability to continuously generate action potentials at high frequencies.

The only other insect order where sensilla ampullacea have been described in detail is Diptera (mosquitoes and biting midges), where their function for temperature and/or humidity perception has been discussed (Cribb, 1997; McIver, 1982; Sutcliffe, 1994). However, the peg of the sensilla ampullacea in the investigated Diptera is thick-walled and aporous with unbranched dendrites (McIver, 1982) and these sensilla are described as deeply sunken sensilla styloconica elsewhere (Keil, 1999). At least in these properties the Dipteran sensilla ampullacea are different from the sensilla ampullacea investigated in the current study and presumably do not represent CO_2 sensitive sensilla.

In conclusion, our findings show that the coding of absolute CO_2 concentration is attained at the level of the receptor cells in leaf-cutting ants. We provide a detailed description of the sensillum ampullaceum and show that it is innervated by a single neuron with highly branched dendrites. Our proposed hypothesis for duct function as protection against evaporation is supported by comparative morphology but remains to be justified.

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Appendix

Under steady state conditions, the concentration gradient across the length of the duct must be uniform, implying that the diffusive flux (J_m) is inversely proportional to duct length (l_c). It is described by: $J_m = D_m \times A_c \times \Delta C \times l_c^{-1}$ where D_m is the diffusion coefficient for H_2O and CO_2, respectively ($m^2 \times s^{-1}$), A_c is the cross area of the duct (m^2), ΔC is the difference in concentration between the inside of the ampulla and the ambient air above the antennomer cuticle (mol $\times$ m^{-3}) and l_c is the length of the duct (m) (Denny, 1993).

Example for diffusive flux of water at steady state conditions: For a given duct (1 μm in diameter, 50 μm long, open to the environment with 40% rH at one end and connected to an ampulla of a volume of 250 μm^3 and 100% rH at the other end; 1 atm and 30°C) the diffusive flux is 41×10^{-14} mol $\times$ s^{-1}. With a duct lenght of only 25 μm the diffusive flux is twice as large (82×10^{-14} mol $\times$ s^{-1}). For an estimated volume of 140 μm^3 the peg contains less than 78×10^{-13} mol of H_2O. Thus, diffusive flux would drain the peg in only 10 s if no diffusion barrier and no water supply exists at the peg.

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