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Active Ion Transport by a Sensory Epithelium

I. Transepithelial Short Circuit Current, Potential Difference, and Their Dependence on Metabolism*

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Summary. Across the sensory epithelium of isolated cockroach antennae, perfused and superfused with identical physiological solutions, a transepithelial voltage (TEP) of about 15 mV (outside positive) has been recorded. It corresponds to somewhat higher transepithelial voltages localized at the sites of sensilla.

Related to this voltage is a transepithelial short circuit current (SCC), which lasts for the hours of survival of the preparation. Density of SCC is near 2.1 $\mu A/cm^2$ at the outer surface of the integument.

TEP and SCC are reversibly reduced by anoxia and by poisons blocking the formation of ATP (Fig. 4–7).

We conclude that an active ion transport located within certain cells of the receptor units is the source of the electrical phenomena observed. Comparative morphological and physiological reasons suggest the electrogenic potassium outward transport to be the special mechanism involved.

Introduction

At epidermal receptors of insects, a potential difference across the epithelium exists; it is confined to the sites of the receptor units (Thurm, 1970, 1974; Thurm and Wessel, 1979). This transepithelial potential difference (TEP) probably is a general phenomenon; though varying in magnitude from 20 to 120 mV (outside positive), its occurrence is independent of the insect species and of the modality of the receptor. In the state of oxygen deprivation, in a N_2- or CO_2-atmosphere, the TEP decreases reversibly to values near zero or becomes more or less negative. Among the arthropods an acutely O_2-dependent TEP at epidermal sensilla is possibly restricted to insects, for Thurm and Wessel did not find this phenomenon with representatives of wood-lice and spiders. In insects this TEP interacts with the generation of the receptor potential (Thurm, 1970, 1974).

This paper examines the hypothesis, that – as the basis for the local transepithelial voltage – an active ion transport might be present in certain cells of the insect integument correlated with receptor units. Results have been preliminarily reported by Thurm (1974).

Figure 1 presents a simplified scheme of an epidermal insect receptor: The dendrite of the sensory cell penetrates the epidermis; the latter is a functionally monolayered, closed epithelium (Thurm, 1970; Thurm et al., in prep.). Low resistance gap junctions connect the epidermal cells to constitute one compartment (Warner and Lawrence, 1973; Popowich and Caveney, 1976). Gap junctions have not been found towards the dendrite (Keil und Thurm, 1979; Foelix, Gaffal, pers. comm.). At least two, usually more specialized epithelial cells surround the dendrite and line off the receptor lymph space covered by the cuticle. The structural basis for an active ion transport mechanism seems to be given by the outermost sheath cell, the tormogen cell. During the intermoult phase and in the imago, this cell is rich in mitochondria and exhibits a considerable enlargement of its apical (i.e., outward directed) membrane by numerous infoldings (Smith, 1969; Thurm, 1970; Felt and Vande Berg, 1976; Gaffal, 1976; Gnatzy, 1976; Weber and Wunderer, 1976; and others).

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Abbreviations: *SCC,* transepithelial short circuit current; *TEP,* transepithelial potential difference

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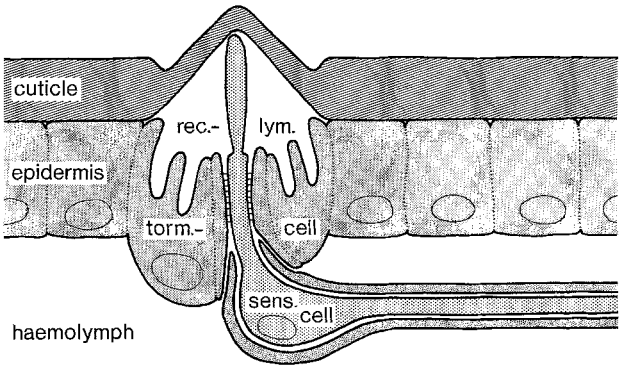

Fig. 1. Simplified and generalized scheme of an epidermal insect receptor with special regard to the functional compartments. For equivalent circuit see Thurm and Wessel (1979)

Material and Methods

Isolated antennae of the adult cockroach *Blaptica* sp. were chosen for the experiments for two reasons: (1) The density of sensilla in that epithelium proved to be high enough to consider it a sensory epithelium, in the sense that its electrical properties are prevalingly determined by the receptor units. (2) The antennae of cockroaches are straight pipes without ramifications, and therefore well-suited for perfusion with artificial media.

In the epithelium of these organs, sensilla of different modalities are incorporated. Their density is about 2,200/mm^2 on the middle segments which we used in the experiments. Figure 2 shows a part of the antenna of *Blaptica* sp. One finds various sensilla looking very similar to those which have been described in detail by Lambin (1973) at the antenna of *Blaberus craniifer* as chemoreceptors at the most.

Figure 3 shows the arrangement of the preparation: The antenna was perfused by a hydrostatic pressure of about 700 mm H_2O. A stopcock with a small dead volume (about 1 µl) allowed the rapid exchange of the perfusing solutions. On a length of 4 mm, the middle part of the isolated antenna was enclosed in a perfusable chamber, tightly sealed with vaseline; thus the length accessible for superfusion was 1–2 mm. In this way it was possible to determine the ionic milieu inside and outside the integument.

Using the data of Pichon (1970) on the ionic content of the haemolymph of the cockroach *Periplaneta americana* and our own measurements of the osmotic pressure of the haemolymph of *Blaptica*, the following "standard solution" was composed (concentrations in mMol/l): Na^+: 126, K^+: 20, Ca^{2+}: 5, Mg^{2+}: 2, Cl^-: 154, HCO_3^-: 5, $H_2PO^-_4$: 1, sucrose: 120, glucose 30, buffered with 10 mMol/l Tris/HCl to pH 7.2. The osmolality was 420 mosm, which is slightly hyperosmotic to the haemolymph.

As it soon turned out during the experiments, the solutions equilibrated with air within the thin polyethylenetubes leading from the stock vial to the preparation. Therefore oxygenating the solutions appeared to be unnecessary; on the other hand, oxygen lack in some experiments was achieved by mounting a column of the oxygen absorber Serdoxid® (from Serva) immediately before the stopcock, leading to the antenna or to the surrounding chamber.

Current and voltage measurements were performed by means of Ag/AgCl-KCl electrodes placed in the chamber and at the open, proximal end of the antenna. Short circuit current was measured by a voltage clamp circuit. In this case the resistance of the electrodes and the internal longitudinal resistance of the antenna were compensated by a negative impedance. The remaining voltage drop within the accessible length is calculated to be less than 2%. For resistance recordings an AC-current was clamped across the integument, the resulting voltage-signal was separated from the TEP by appropriate filters and subsequently rectified.

In addition we measured the transepithelial voltage locally at restricted areas of the integument (down to about 5 µm in diameter), placing a small tapered micropipette directly on the dry surface of the perfused (not superfused) antenna.

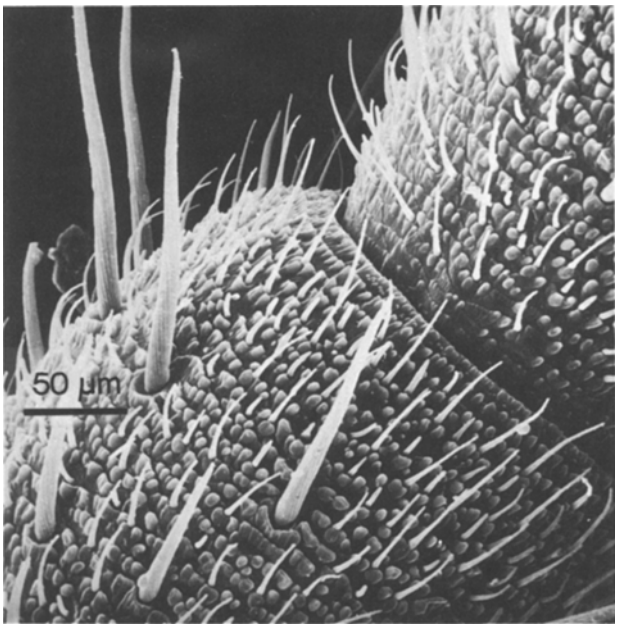

Fig. 2. View on the middle segments of the antenna of *Blaptica* sp. showing a variety of epithelial sensilla (scanning electron micrograph)

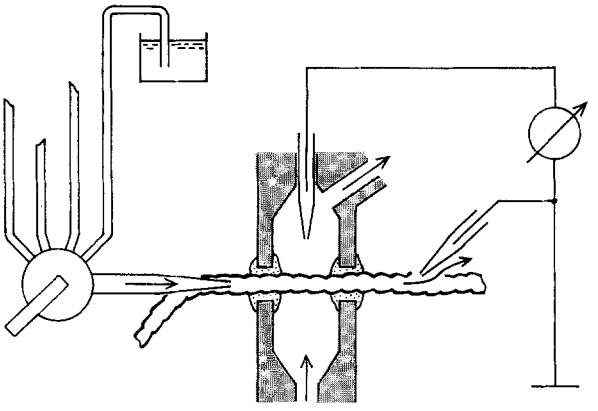

Fig. 3. Scheme of experimental setup. A part of the tube-like cockroach antenna, ready for perfusion, is sealed with vaseline in the superfusion chamber

Results

A. Transepithelial Voltage (TEP) and Short Circuit Current (SCC)

If the chemical gradient across the epithelium and the cuticle is made zero by perfusing the antenna

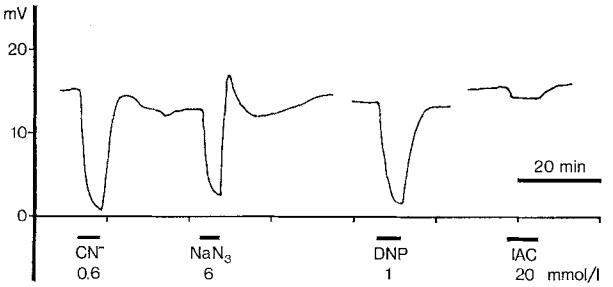

Fig. 4. Effect of CN^-, NaN_3, DNP, and iodine-acetic acid (IAC) on the TEP

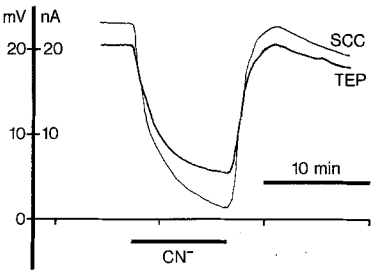

Fig. 5. "Simultaneous" records of TEP and SCC during poisoning with cyanide

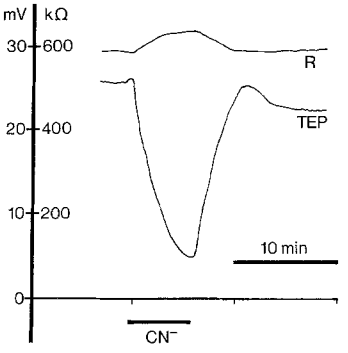

Fig. 6. Transient resistance increase and TEP decay caused by metabolic inhibition

and the surrounding chamber with the same standard solution, a voltage can be recorded which varies between the preparations from +10 to +25 mV with the median at about +15 mV (sign refers to outside). This potential may show slow irregular fluctuations by a few mV, but overall it usually persists for several hours.

If the voltage across the integument is clamped to zero, a short circuit current is measured. It is as stable for survival time of the antenna as the free running voltage is. Three determinations of the current density on different preparations yielded values between 2.0 and 2.3 $\mu A/cm^2$.

Local voltage measurements by the method described indicate that the electrically active sites are the receptor units only:

a. From regions in which sensilla are visibly absent the recorded TEP is around zero. Those regions are (1) the greater part of the surface of the basal segments, and (2) the cuticle at the joint between each segment.

b. At the basis of the largest hairs seen in Fig. 1 (the only sensilla which could be identified by the stereomicroscope) a potential of about +30 mV was recorded. Local measurements on the remaining area of these middle segments yielded values between zero and +25 mV.

By referring the measured current to the mean density of sensilla found in scanning electron micrographs, a current of 10 pA/receptor unit results on the average.

B. The Dependence of Short Circuit Current and Transepithelial Voltage on Oxidative Metabolism

Both the TEP and the SCC acutely depend on oxidative metabolism. If the antenna is perfused with standard solution containing additions of either CN^-, NaN_3 or Dinitrophenol (DNP), the TEP is reversibly reduced to values near zero (Fig. 4). The concentrations of these agents having about equal efficacy on the TEP are different and are given in Fig. 4. Time courses of the TEP changes induced are similar. Iodine-acid (IAC), up to the concentrations of 20 mMol/l, has no or only trivial influence on the TEP (Fig. 4).

Correlated with the TEP, the SCC decays on poisoning. This is demonstrated by Fig. 5 where "simultaneous" records of both parameters are shown by switching between these two states at a frequency of 0.2 Hz.

The decay of the TEP during inhibition of the metabolism is accompanied by an increase of the resistance of the preparation by about 8–12% (Fig. 6).

When cyanide is added only to the outside-medium while the antenna is perfused with pure standard solution, no effect on the TEP can be observed. If the perfusion is stopped, cyanide becomes effective from the outside but its efficacy is much lower than it is when the poison is added to the perfusate within the haemolymph space.

If oxygen is removed from the solutions on both sides by the means described (see Material and Methods), the TEP decreases and increases with a time course as shown in Fig. 7. The slope of TEP increase is steeper and oscillations following metabolic block are more prominent when compared to poisoning by cyanide. The responses of various antennae to oxygen lack on only one side were different from one another. Some preparations could be

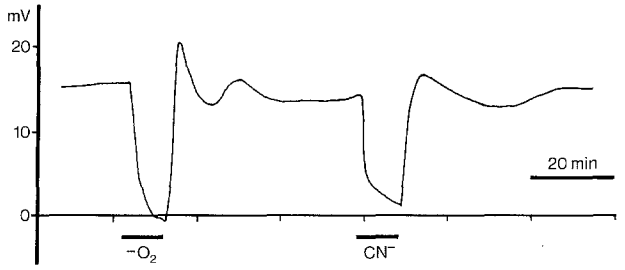

Fig. 7. Voltage change by oxygen lack compared to the action of cyanide

completely supplied by the inside. At others the TEP decreased slowly to some extent when merely one solution was deoxygenated. A total decay of the TEP was achieved only by oxygen deprivation on both sides of the epithelium.

On the other hand, atmospheric air in direct contact with the integument, as usual for these animals, seems always sufficient to maintain the TEP (investigated by local recording method). Preparations aerated from the outside become independent of the oxygen content of the perfusate within the haemolymph space.

Discussion

The transepithelial voltage found in this study at receptor units of the cockroach antenna and its acute dependence on oxidative metabolism correspond to similar results reported for various insect species (Thurm 1970, 1974; Levinson et al., 1973; Thurm and Wessel, 1979). The long-lasting and stable short circuit current which can be recorded from the integument of the cockroach antenna in the absence of any chemical gradient proves the validity of the hypothesis that an active ion transport mechanism is the cause of the locally increased transepithelial voltage. It makes no difference that the structure considered is complex and consists of at least three compartments, the epithelium, the receptor lymph spaces, and the cuticle, and that the chemical gradient across the transporting epithelium itself possibly is not well-controlled by the composition of the perfusing or superfusing solutions as indicated by the low efficacy of cyanide applied from the outside. The electrical phenomena observed cannot easily be explained by concentration gradients (e.g., induced by passive dehydratation of the cuticle exposed to air) and metabolism dependent permeability changes, for instance. If caused by concentration gradients, the total flux of charges observed in longer experiments (up to 7 h) should have led to concentration changes of some Mol/l in the relevant compartments, the receptor lymph spaces, whereas the short circuit current measured has not been seriously affected.

The inefficacy of iodine-acetic acid suggests that the metabolic energy used by the transport derives from fat-decomposition.

In vivo, oxygen seems to have direct access to the cells of the epithelium through the cuticle. That may explain the very short time lag between the exchange of N_2 against air and the rise of the TEP as found in the experiments of Thurm (1970, 1974; Thurm and Wessel, 1979).

Local records indicate that the transepithelial voltage is generated only at the sites of sensilla. The fact that the voltage recorded from single hairs is higher than that recorded by making contact with the whole cuticular surface shows that in the latter cases locally generated voltage is shunted by inactive areas of the integument (see also Thurm and Wessel, 1979).

Within a sensillum, the apical membrane of the tormogen cell seems to be the site of the active transport observed. The area of this membrane is highly enlarged compared to the cross section of the cell within the epithelium (40 times resp. 14 times in different sensilla of flies; see Keil, 1978). A high concentration of mitochondria is associated with the folded membrane area. The membrane itself is studded with 8 nm particles (see below). The reduction of the membrane area during the moulting phase in hemimetabolous insects is correlated with a decay of the transepithelial voltage (Thurm 1974; Thurm and Wessel, 1979; Gnatzy and Thurm, in prep.).

With regard to the measurements of the SCC, the cuticle at the sensilla represents a resistance in series which may greatly differ in magnitude for sensilla of different modalities. Hence it seems difficult to estimate the SCC derivable from the epithelium itself which might easily be higher by a factor of two because the resistance of the cuticle approximately equals that of the epidermis (2–5 $k\Omega \cdot cm^2$ each; Thurm, 1974; Thurm et al., in prep.). The current density of 2 $\mu A/cm^2$ seems rather low when compared to 600 $\mu A/cm^2$ at the haltere of *Musca* (Thurm, 1974); there, however, the receptor density is about twelve times greater than in the present object. At the haltere of *Calliphora* we measured a SCC of $\geqq 200$ pA/sensillum. Yet most of the sensilla of the antenna are smaller than those of the haltere and presumably are provided with a smaller apical membrane area of the tormogen cell. In that membrane current density may be as high as 10 $\mu A/cm^2$, a value Thurm (1974) calculated for the haltere of *Musca*.

As the type of transport present, we suggest an electrogenic potassium transport as described in detail

by Harvey and Nedergaard (1964) and Harvey et al. (1968) for the midgut epithelium of the silkworm. That suggestion is based on the following reasons: The rapid change of the potential induced by anoxia, particularly the reincrease of up to 58 mV/10 s found at epidermal sensilla (Thurm, 1974; Thurm and Wessel, 1979) favour an electrogenic nature of the transport rather than a potential generation by a diffusion potential. In addition, the cytoplasmic surface of the enlarged apical membrane of the tormogen cell is found to be coated with characteristic particles in *Calliphora* (Smith, 1969), in *Musca* (Thurm, 1970), in *Apis* (Weber and Wunderer, 1976), and in *Acheta* (Keil, pers. comm.), a feature we find in various organs of insects known or assumed to transport potassium out of cells (Anderson and Harvey, 1966; Berridge and Oschman, 1969; Oschman and Berridge, 1970). Our investigations on the ionic dependence of the TEP, partly reported in Thurm (1974), also corroborate the assumption of a potassium transport of that kind. In that connection the high potassium concentration in the receptor lymph (100–200 mM/l) which has been found in several insect species (Küppers, 1974, and unpublished measurements) should be mentioned.

The voltage across the apical membranes within sensilla is expected to be relatively high, because the positive transepithelial potential difference points to a higher membrane voltage across the apical membrane than across the basal membrane. Within a tissue of comparable transport activity, the midgut of *Cecropia,* Wood et al. (1969) have demonstrated an apical membrane voltage of more than 180 mV (in the active state of transport), contrasting with about 30 mV across the basal membrane. On the other hand, the concentration gradients of those ions which usually determine membrane conductance may be especially low, as reported above for K^+ and as is also indicated for Cl^- (28 mM/l in receptor lymph of flies: Küppers, 1974). Thus the distribution of these ions across the apical membranes would be far from equilibrium during activity of the transport mechanism. The specific epithelial resistance which we find (Thurm et al., in prep.) is in the range of usual values for "tight" epithelia (between 2 and 5 $k\Omega \cdot cm^2$). Therefore we have to consider that a continuous current is flowing during transport activity within a circuit comprising the apical membranes of the tormogen and neighbouring cells (cells connected by gap junctions) recycling the transported ions. The actual membrane voltage then is determined mainly by the product of transport current times resistance of passive reflux (a high source resistance of transport assumed). This scheme of generation of apical membrane voltage also accounts for the instantaneous changes in transepithelial voltage which are caused by application of serotonin and which have been attributed to an increase in transport activity (Küppers and Thurm, 1975). We hope to discuss the biological significance of such an energy-consuming mechanism elsewhere in connection with results on ion and water metabolism of sensilla.

A second loop of the current circuit is controlled by adequate stimulation of the sensory cell (Thurm, 1974). The actively generated current flows back in this circuit, entering the receptor cell via its stimulus-increased apical conductance, leaving the cell via its basolateral membrane regions, and returning into the tormogen cell via its basolateral membrane.

This transepithelial course of the current loop is indicated by the properties of nervous impulse initiation, pointing to the basolateral membrane of the neuron as the site of receptor current to impulse transformation (Erler and Thurm, 1978, and in prep.), and by the failure to find gap junctions between the neuron and the surrounding epithelial sheath cell (Thurm et al., in prep.; Keil and Thurm, 1979; see also discussion following Thurm, 1974). This second current loop in some sensilla increases receptor sensitivity (Erler and Thurm, 1978; Thurm and Gödde, in prep.).

We suggest that the observed resistance increase during poisoning is an effect of an elevation of the intracellular calcium level that should be expected during metabolic inhibition, either (1) by direct influence of calcium on the membrane permeability or (2) – in the special case – by uncoupling the epithelial cells (Loewenstein, 1972): If the electrical access to the tormogen cell, with its large apical membrane, predominates over that to other epithelial cells, uncoupling might restrict the current carrying membrane area at the basal side of the epithelium and raise in this way the resistance of the preparation.

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