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Ultrasound Elicits Tonic Responses and Diminishes the Phasic Responses to Adequate Stimuli in Thread-Hair Mechanoreceptors of *Acheta domesticus*

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Abstract. Single mechanoreceptor cells in filiform hair sensilla on the cercus of *Acheta domesticus* were stimulated adequately by steplike deflections in their plane of least restraint and inadequately by ultrasound. Ultrasound was fed either into the cercus or into the thread-hair as substrate-borne sound of 110–120 kHz. The receptor responds to deflections of the thread-hair (adequate stimuli) with phasic receptor potentials which can be picked up transepithelially. These responses can be either depolarizing (excitatory responses) or hyperpolarizing (inhibitory responses). Ultrasound applied simultaneously or shortly preceding the adequate stimulus reduces both kinds of responses in a graded way. The receptor can respond to ultrasound alone. At small intensities the responses are predominantly inhibitory; with increasing intensity they may become excitatory. In both cases the responses to ultrasound are tonic and do not reach the peak responses to saturating adequate stimuli. The "off-effect", which follows adequate stimuli and leads to inhibitory responses after excitatory stimuli and vice versa, typically does not occur or is exitatory at the end of sonication. The observed effects of sonication are totally reversible. The correlation between transepithelial voltage, spike frequencies and spike amplitudes in the unsonicated and sonicated sensilla allows the responses to be attributed to sonication to the same conductance, which is also modulated by adequate stimuli. A model is discussed, according to which ultrasound exerts its effects by facilitating dissipative relaxation in the dendritic membrane, which is assumed to be involved in stimulus-energy transfer.

Key words: Receptor potential – Ultrasound stimulation – Mechanoreceptor – Insect – Mechanoelectric transduction

Introduction

Of the many mechanisms proposed for mechanoelectric transduction (see e.g., Atema 1973; McIver 1975; Rice et al. 1973; Wensler 1977; Wiederhold 1976), the model which bases the mechanically controlled inhibitory and exitatory conductance changes on gating events in directly or indirectly mechanically sensitive channels appears most attractive. Recently, Hill (1983) has found, with intracellular recording, discrete depolarizations in auditory receptor cells of the locust, which may represent such gating events. The same assumption has been made by Corey and Hudspeth (1983) for the vertebrate hair cells, although the channel nature of the mechanically controlled conductance has not been established so far. Yet, in accordance with channel-related terminology, all changes in the mechanically controlled conductance are referred to as "gating" in this paper.

Gating in electrically excitable membrane channels is considered to result from membrane-potential-dependent changes in the difference in free energy between the closed and open channel state(s) (Hodgkin and Huxley 1952; Schwarz 1978). It has been proposed (Corey and Hudspeth (1983); Thurm (1982); Thurm et al. 1983) that similar stimulus-induced differences in Gibbs free energy account for changes in the average open-probability in mechanically sensitive channels. However, such models alone cannot explain the adaptation inherent in phasic responses. The adaptation could be due to viscoelastic properties of the stimulus-transmitting apparatus [e.g., Loewenstein and Mendelson (1965) for the Pacinian corpuscle]. Alternative adapting mechanisms are conceivable, in which each channel attains a closed state by some inactivation mechanism such as the classical one described by Hodgkin and Huxley (1952).

Inadequate stimuli can provide information about the mechanoelectric transduction if they elicit specific responses and by interfering with the transduction of adequate stimuli. Receptor potentials as primary electrical responses can easily be studied in the cercal filiform hair sensilla of *Acheta*. Their interpretation in terms of conductance changes is facilitated by the knowledge accumulated on the morphology and electrical circuitry in a number of epithelially organized insect sensilla (Erler and Thurm 1981; Gnatzy and Tautz 1980; Kaissling and Thorson 1980; Küppers 1974; Thurm and Wessel 1979; Thurm 1974a, b, 1982; Thurm and Küppers 1979, 1980; Thurm et al. 1983; Wieczorek 1982). Therefore only a brief description, summarized from the works listed and stressing the particularities of the cercal filiform hairs of *Acheta*, will be given here.

The sensillum contains one sensory neuron. Its dendrite protrudes from the hemolymph side through the electrically tight epithelium into the receptor-lymph cavity at the apical side. Mechanical stimuli modulate a conductance in the membrane of the dendrite and thereby the receptor current. Normally this current flows from the receptor-lymph cavity through the sensory cell and its glial sheath to the hemolymph space (sensory pathway) and through the auxiliary cells, mainly the tormogen cell, back to the receptor-lymph cavity (auxiliary pathway). The transepithelially measured receptor potential reflects the voltage drop, which this current produces along the resistive components of the auxiliary pathway. The receptor current appears as a change in the clamp current, if the voltage between the receptor-lymph and hemolymph is clamped. The mean stationary current, on which these changes are superimposed, is determined by the auxiliary pathway.

Receptor potentials and receptor currents as responses to identical mechanical stimuli are proportional to each other and equal in their time-course if recorded at similar transepithelial voltage.

The conductance of the unstimulated sensory pathway is extremely low in some mechanically (and chemically) sensitive epithelial sensilla in insects. This can be shown in campaniform sensilla on the haltere and thoracal macrochaetae of *Calliphora* by the lack of elicitability of spikes by a fast increase in transepithelial voltage (Erler and Thurm 1978; Gödde 1983). (Slow changes in transepithelial voltage could be overcome by the quickly adapting spike generator.) The existence of depolarizing and hyperpolarizing responses to deflections in different directions, as found in some hair-like sensilla, is an indicator for a stimulus-dependent conductance of the sensory pathway, which is non-zero when the sensillum is at rest (Gödde 1983; Gödde and Thurm in preparation). Such responses have been shown for cercal filiform hair sensilla in several species – for *Periplaneta* by Nicklaus (1965), for *Gryllus* by Gnatzy and Tautz (1980) and Kniazew (1976) and for *Acheta* by Gödde (1983). The stimulus-dependent resting conductance of the sensory pathway should not be confused with an electrically sensitive, stimulus-independent one which can shunt the stimulus-dependent conductance [Erler and Thurm (1981) for the tibial thread hair sensilla in *Acheta*].

More recent measurements (Gödde 1983; Gödde and Thurm, in preparation) indicate for the filiform hair sensilla in *Acheta* that the de- and hyperpolarizing deflections, which are of opposite direction in the plane of least restraint (Gnatzy and Tautz 1980), control the same resting conductance of the sensory pathway, which is exclusively mechanically controlled. Therefore stimuli which decrease this resting conductance and the resulting responses will be called inhibitory and those which increase it excitatory.

The existence of a fraction of open sensitive channels in the filiform hair sensillum's resting state offers the best chance to test non-adequate stimuli for their influence upon gating, because both potentially closing and opening effectiveness of such stimuli can be tested.

In this paper it is shown that mechanical oscillations in the ultrasound frequency range (110–120 kHz) can elicit specific responses of the sensory neuron and diminish its sensitivity for adequate stimuli. Implications for the mechanisms of gating and adaptation are discussed.

Material and Methods

Measurements were performed on lateral hairs within the proximal third of the cercus of crickets (*Acheta domesticus,* both sexes). In these hairs the plane of least restraint is parallel to the cercus and there is least risk of moving the whole cercus by deflecting the hair. Only freshly hatched animals of the last instar were used because tanning clogs the hair lumen, which had to be conductive in our experiments (see below, electrodes). After brief narcotization with CO_2 the animals were paralysed with 0.015 ml of 0.73 mol/l procaine hydrochloride (injected through the cervix) and mechanically fixed with wax. The relatively high dose of procaine (roughly LD50) has no appreciable effect on the responses of the sensory neuron, but interferes with tanning. Thereby it suppresses clogging of the hair lumen even in prolonged experiments.

Electrodes

Receptor potentials were measured transepithelially by a variation of the method whichWolbarsht and Dethier (1958) developed for chemoreceptors and which Erler and Thurm (1977) adapted for mechanoreceptors. Access to the hemolymph side of the epithelium was made by the reference electrode in the thorax. The filiform hair under study was cut to a length between 300 and 500 μm, the surrounding hairs to less than 100 μm. Then a polyethylene pipe, which was part of the recording electrode, was slipped over the hair with its 1-mm-wide orifice (Fig. 1). The width of the pipe allowed for deflections of the hair by more than 10° without touching the wall of the electrode. Because of the relatively large surface between electrolyte and ambient air, the development of crystals is a critical problem in long-term experiments (Gnatzy and Tautz 1980), which Erler and Thurm (1977) circumvented by adding over 1 mol/l choline chloride to the electrolyte. In the present experiments an electrolyte of only 0.05 mol/l KCl without hydrophilic additives was used instead. Losses by evaporation were replaced from a closed vessel. The hydrostatic pressure in this vessel determined the shape of the meniscus at the orifice of the electrode. It could be controlled by movements of the open leg of a 10-mm-wide, water-filled U-shaped hose to which it was connected by another 0.5-mm-wide gas-filled pipe.

Adequate Stimulation

In all experiments reported here, deflections of the hair within its plane of least restraint (adequate stimulus) and sonication (inadequate stimulus) could be applied simultaneously and independently. The tip of a glass capillary, mounted on an aluminium rod of an electromagnetic stimulator, deflected the hairs, which were attached to the tip by a highly viscous fluid (Oppanol B3 and Oppanol B10 or a mixture of the two; both kind gifts of BASF, Ludwigshafen, FRG; Oppanol is the tradename for polyisobutylenes of different lengths) (Fig. 1). The stimulator (developed in this laboratory) contained a feedback loop to compare the actual position of the aluminium rod (measured by a pair of photodiodes) to the stimulus program. The position signal was recorded. The raising time (90%, no overshoot) was less than 2 ms.

Ultrasound Stimulation

Ultrasound was generated by a transducer disk (20 mm diameter; 1.3 mm thick of the material Sonox P8; article no. 35120-00.3; kind gift of Rosenthal Technik

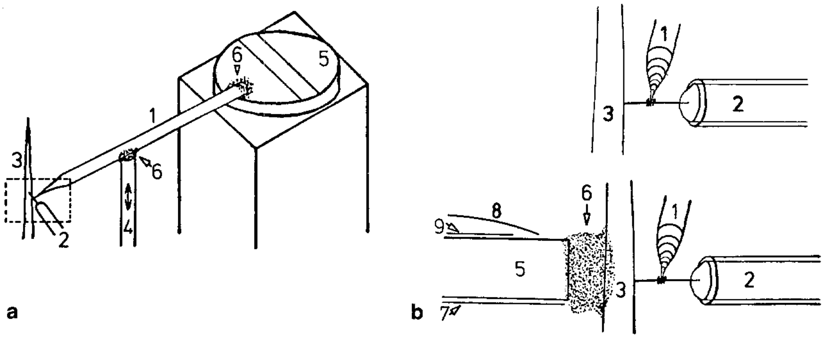

Fig. 1. Setup for stimulation. **a** sonication of the hair; *1:* glass capillary, *2:* electrode, *3:* cercus, *4:* aluminium rod of stimulator, vertically moving, *5:* transducer disk with strip of soldering tin, *6:* connection of wax. **b** *top:* detail from **a**, *bottom:* same detail, but drawn for sonication of the cercus (in these experiments the wide end of the capillary was not connected to the transducer, but moved freely) *5:* margin of transducer-disk with grounded electrode (*7*, bottom), grounded screening (*8*), and "hot" electrode (*9*)

AG, Lauf/Pegnitz, FRG. The transducer was developed as a thickness vibrator for liquid-atomizer applications, in which the resonance frequency would have been roughly 2 MHz. For the experiments it was used in the resonance of a flexural vibration mode at 115–120 kHz, which was reinforced by soldering a strip of soldering tin across one of the fired-on silver electrodes. Only with this resonance could the necessary intensities be reached with low-driving voltages (less than 24 V peak-to-peak). Driving signals were derived from a function generator (model 129CG, Exact Electronics Inc. Hillsboro, Oregon, USA) and a driving amplifier (built in this laboratory). Because of its interior impedance the output voltage of this amplifier dropped by up to 50% when the frequency was wobbled through the resonance frequency of the transducer. The rectified output of the driving amplifier was recorded to monitor the intensity of the ultrasound. The intensity is proportional to the square of the driving voltage, which is the parameter indicated in the figures. In the first series of experiments the resonance frequency of the transducer was reached by a ramp on the FM-input of the function generator. In later experiments the frequency was continuously wobbled with a triangular low frequency swing at a rate of 10 kHz. Both measures were intended to overcome slow frequency drifts of the function generator from the resonance frequency of the transducer. In control experiments wobbling gave a reduced effectiveness if compared to a driving signal of constant (resonance-) frequency.

The ultrasonic oscillations were fed into the sensillum as substrate-borne sound in two ways (about 20 experiments with each method): either into the cercus or into the hair (Fig. 1). The cercus was sonicated via a wax-bridge (roughly 1 mm wide) opposite the hair under study. The other end was melted to the point of maximal distance to the soldering-tin strip on the margin of the transducer disk (Fig. 1). The axis perpendicular to the plane of the disk was parallel to the cercus. The short distance between the transducer and the recording electrode in cercus sonication experiments occasionally caused some cross-talk of the transducer driving voltage to the probe amplifier, which, however, remained below 1 mV (tested with dead preparations).

The hair was sonicated via the capillary, which also was used to deflect it (Fig. 1). In this setup, the tip of the capillary oscillated vertically, i.e., parallel to the adequate deflections of the hair. The oscillation of the capillary could be directly observed as vertical blurring of the tip (up to 200 μm), if the transducer was excited without a hair as load. This blurring was independent of the wobble-sweep repetition rate and therefore no artifact-reflecting fluctuations in the mean position elicited by wobbling.

Absolute values of intensities or oscillation amplitudes cannot be given.

Registration and Evaluation of Signals

All recordings were done on an FM-tape (store 4D, Racal Thermionic Ltd., Hythe, Southhampton, GB) at DC to 650 or 1,250 Hz bandwidth. The repetition rate for stimulus combinations was 2 s. Data were digitized with an 8-bit- 12-bit (time) transient recorder (TR-42, Haag, Langen, FRG) in connection with a computer (8032, Commodore) with a diskette station (8050, Commodore) and a printer-plotter (X80SP, ADCOMP, Munich, FRG) as periphery. The pixel size of this printer-plotter determined the resolution of all the figures. Assembler-written routines handled sets of usually 4,000 samples of stimulus and response (sampling times depending on the requirements of the respective analysis from 0.01 to 0.5 ms). These routines were embedded in programs written for the (modified) basic interpreter of the computer.

Results

Responses to Adequate Stimuli

The sensilla react to steplike deflections in their plane of least restraint with predominantly phasic receptor potentials or receptor currents (Figs. 3, 4, 6, 7). Typical phasic stimulus-response characteristics (peak amplitudes versus angles of deflection) as shown in Fig. 2, are sigmoidal. Near the ends of the operating range these curves decline. This decline is probably due to mechanical restraint of the hair movement by its socket. Concomitant with the adaptation, which diminishes the response amplitude to small tonic values during steplike stimuli, the dynamic characteristic is shifted along the stimulus axis without changes in the saturating amplitudes or the steepness (Fig. 2). [Similar shifts of the dynamic characteristics have been found in other mechanoreceptors in insects; see Thurm (1965) for hair-plate receptors of Apis, Gödde (1983) for thoracal macrochaetae of *Calliphora.*] Restoring the former position of the hair after a stimulus results in an "off-response", which has the opposite sign to the response to the onset of the stimulus, though not necessarily symmetrical in amplitude or time course (Figs. 3, 4, 6, 7, 11). These off-responses are typical for the cercal filiform hairs

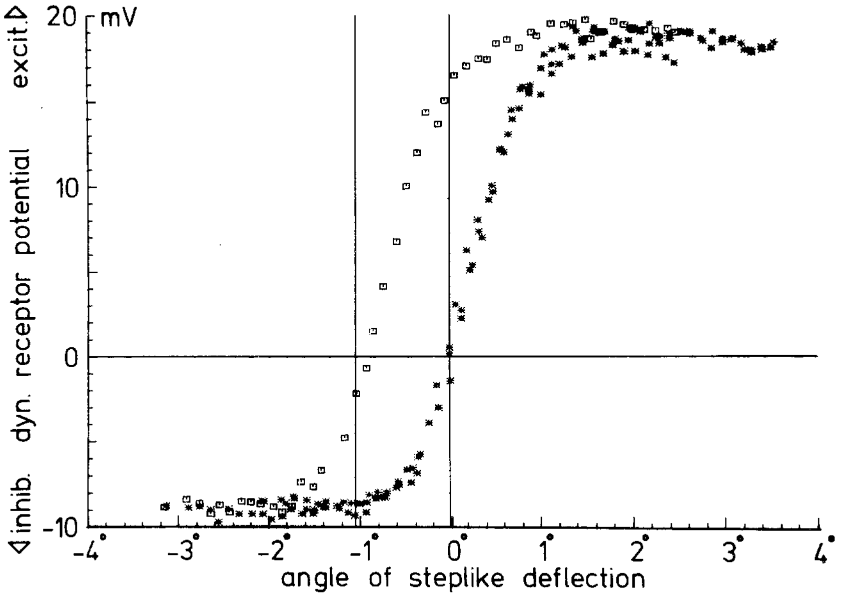

Fig. 2. Dynamic stimulus response characteristics for steplike deflections and their adaptation by adequate stimuli. *Asterisks:* responses to deflections from resting position. *Rectangles:* responses to deflections which were superimposed on an inhibitory 300-ms conditioning stimulus: steplike deflection of −1.05° (indicated by the left vertical bar). Excitatory conditioning stimuli would have caused a shift to the right

of *Acheta.* The amplitude of the off-response increases with the duration of the preceding stimulus which provokes it. A descriptive formalism and its interpretation can be found elsewhere (Gödde 1983; Gödde and Thurm, in preparation).

Responses to Sonication

Sonication alone can elicit predominantly tonic electric responses with a positive and negative sign in the sensilla (Figs. 3, 4, 7). The accompanying change in the spike frequency is the same as in adequately elicited responses of the same sign. The achievable response-amplitudes were generally smaller in experiments in which the sound was transmitted via the cercus than for sound transmission via the hair. Measurements of the intensity response characteristics showed that for cercus sonication the intensities which led to the highest obtainable response amplitudes irreversibly abolished the sensitivity for smaller intensities and, occasionally, for adequate stimuli. Whether sonication of the cercus elicited inhibitory or excitatory responses (see Introduction) depended unpredictably on the individual preparation.

Sonication of the hair was also able to elicit inhibitory or excitatory responses (Fig. 4). In these experiments response versus intensity characteristics could be

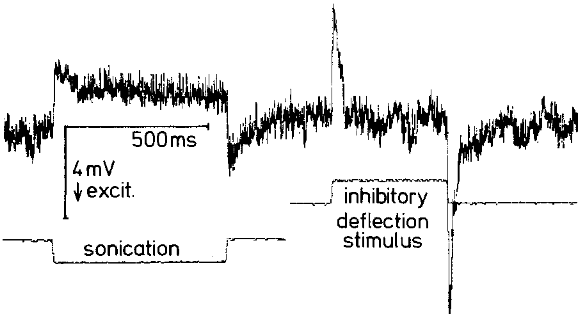

Fig. 3. Comparison of responses to inhibitory deflection stimulus and to sonication of the cercus; excitatory (negative) responses downward

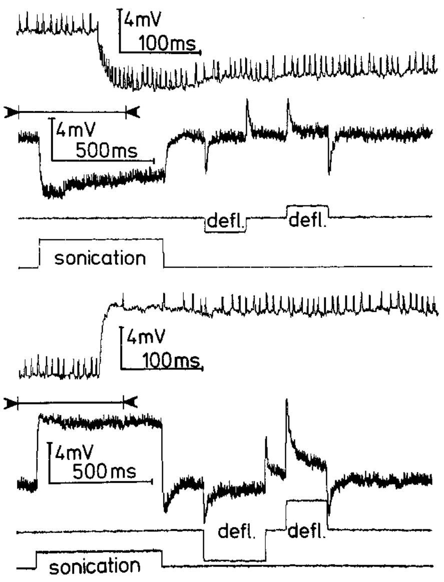

Fig. 4. Large inhibitory and excitatory responses to sonication of the hair in the same cell. *Upper half:* excitatory response; note tail after end of sonication and off-responses after deflections. *Lower half:* inhibitory response, same cell; 133 min later; note excitatory off-response after sonication; different arbitrary scales for intensity and deflection registration in both parts. Uppermost trace in upper and lower half, respectively: time-spread section below cursor (region between arrows) in respective registration; the spike amplitudes in these time-spread traces were magnified by adding to the original trace its (digitally) high-pass filtered, rectified, amplified, and normalized copy

measured repeatedly for single sensilla (Fig. 5). Although these characteristics are highly variable, even in the same sensillum, there is a general trend: the characteristics tend to be non-monotonous. Small intensities mostly elicit inhibitory responses. Increasing the intensity of the sonication may lead to a decrease of inhibitory responses and eventually to excitatory ones (Fig. 5, but also Fig. 7). The sensitivity of the sensillum for sonication of the hair changed only slowly, so that within 15 min some hundred combinations of deflection- and ultrasound stimuli could be tested without marked differences in the effectiveness of sonication.

All responses to mere sonication adapt more slowly and less completely than adequately elicited responses of comparable phasic amplitude (Figs. 3, 4, 7). Independent of the sign of the ultrasound-response itself, only excitatory off-responses could be found after sonication. The end of excitatory responses to sonication seems to elicit no off-response, although the response returns to the level before sonication, typically with a tail (Figs. 4, 7).

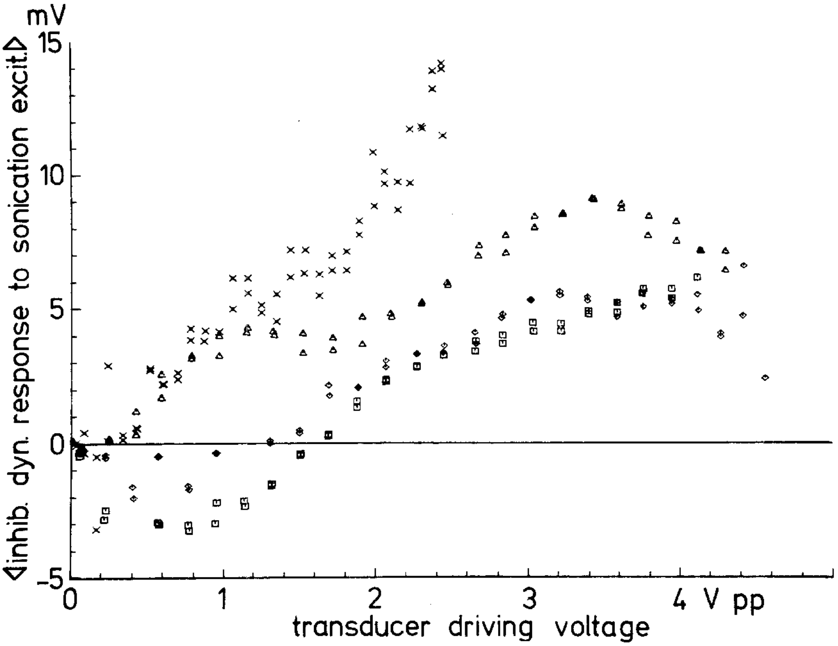

Fig. 5. Dynamic response versus driving voltage of the transducer. Comparison of four characteristics of the same preparation at different times. *Crosses:* first registration; *triangles:* 5 min later; *rhombus:* 119 min later; *rectangles:* 123 min later; twinned points were measured consecutively, all curves taken in ascending order

The amplitudes of responses to mere sonication in no case reached the saturating phasic amplitudes obtainable with adequate stimuli, not even at ultrasound intensities, which irreversibly destroyed the sensitivity for adequate stimuli.

The shape and the mean amplitudes of the spikes, which are superimposed on the receptor potentials (or the receptor current) are alike before, during, and after sonication (Fig. 6, insets).

In some experiments, after the normal experimental program had been performed, the intensity of hair sonication was raised to the upper limit of the driving amplifier and transducer. This led to sudden, irreversible drops in transepithelial voltage by roughly 30–50 mV, more than the largest stimulus-inducible voltage changes. Concomitantly the frequency and the amplitude of the spikes increased. The simplest explanation for this is that the apical membrane of the sensory neuron was destroyed.

Interaction Between Sonication and Adequate Stimulation

Sonication in all experiments diminished the excitatory and inhibitory on- and off-responses to adequate stimuli. This decrease, which is totally reversible (up to several hundred times tested on one sensillum), is shown in Figs. 6 and 7.

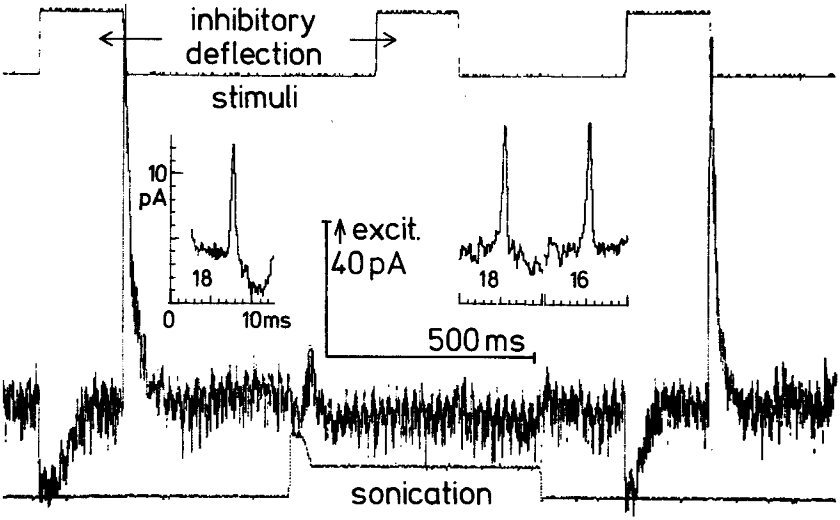

Fig. 6. Suppression of responses (recorded in voltage clamp) to inhibitory deflection stimuli by simultaneous sonication of the cercus; insets: comparison of mean spike amplitudes before, during and after sonication. After the onset of the sonication driving signal the frequency was tuned to the resonance of the transducer. The resulting drop in the envelope signal (due to source output impedance) coincides with an increase in intensity of sonication; "noise" in receptor-current registration mainly due to superimposed spikes. *Upper trace:* deflection stimulus; *medium trace:* receptor current, excitatory upward; *bottom trace:* sonication mark. *insets:* mean shape of all spikes from 200 ms-intervals before, during, and after sonication; numbers of averaged spikes indicated, inverted with respect to current trace

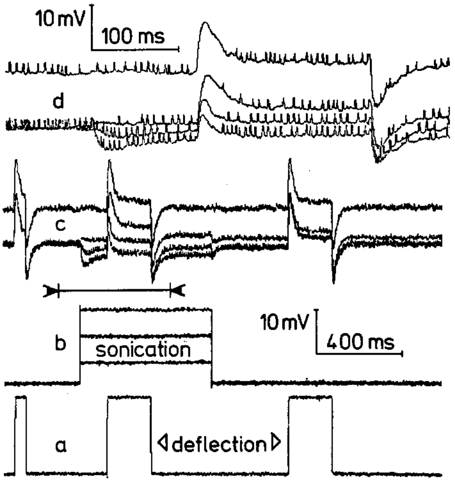

Fig. 7. Partial suppression of responses to inhibitory deflection stimulus by sonication of the hair; **a:** deflection, **b:** sonication, **c:** receptor potentials, *top:* control, **d:** detail above region between arrows in **c:** spike amplitudes in these traces were amplified as indicated in Fig. 4. Note that sonication with increasing intensity first elicits inhibitory responses, but then increasingly excitatory ones, whereas the reduction of the responses to deflection depends monotonously on the sound intensity

However, sonication of the cercus and sonication of the hair (see Methods) diminished the responses to deflection with varying effectiveness and in different ways.

Sonication of the cercus reduced the saturation levels for inhibitory and excitatory stimuli by suppressing the responses to all deflection amplitudes to

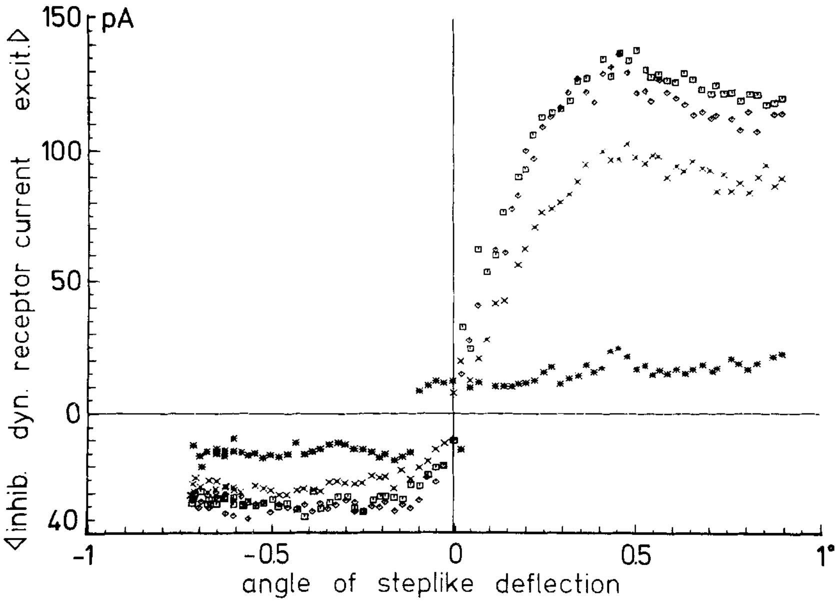

Fig. 8. Dynamic characteristic for two intensities of cercus sonication; stimulus program as in Fig. 6. *Rectangles and rhombi:* control responses without sonication; *crosses:* responses during low-intensity sonication; *asterisks:* responses during high-intensity sonication; remaining deviation from zero (and gap around zero in the other curves) due to noise-peak detection by the evaluating computer program

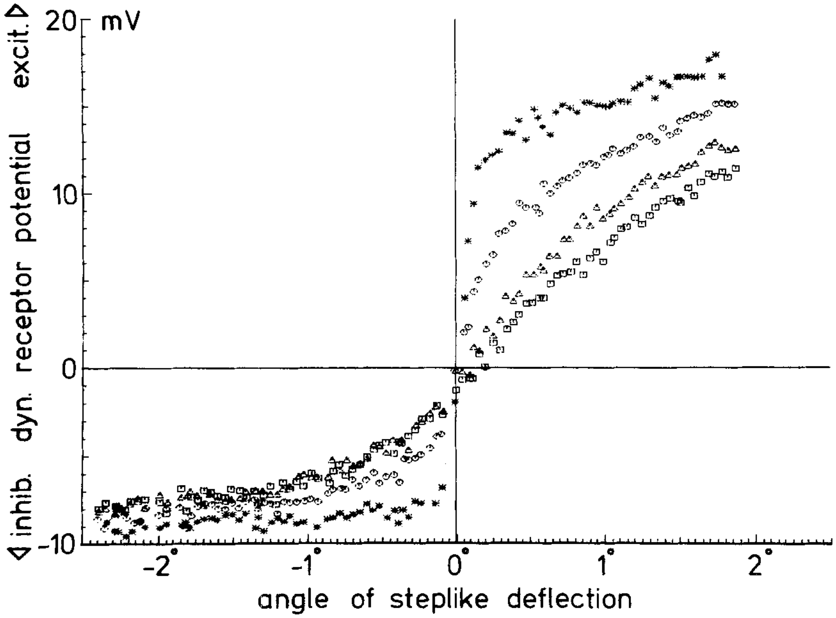

Fig. 9. Dynamic characteristic for three intensities of hair sonication; stimulus program as in Fig. 7. *Asterisks:* control; ellipses, triangles, rectangles: three intensities of sonication during deflection

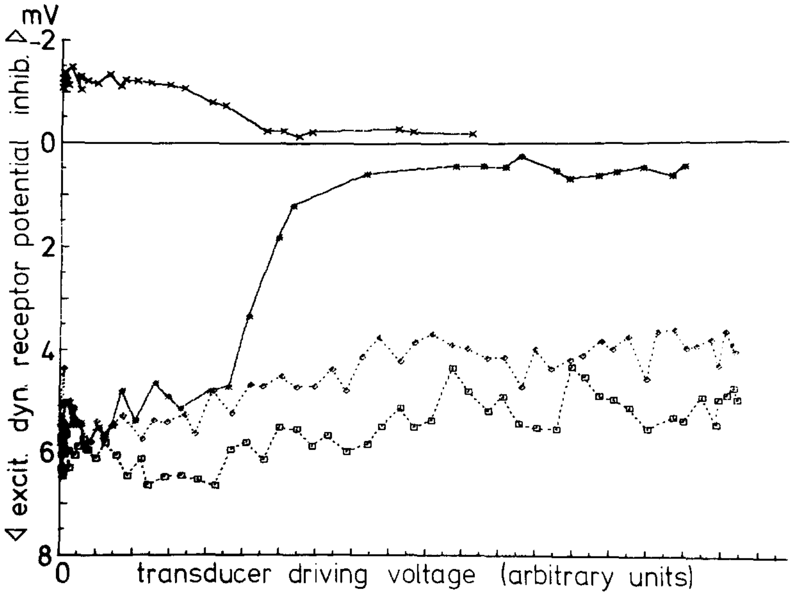

Fig. 10. Suppression of deflection responses as function of intensity of cercus sonication, tested with excitatory deflection stimulus and its inhibitory off-responses. *Crosses in (upper) solid line:* off-response during sonication. *Asterisks in (lower) solid line:* dynamic peaks during sonication, remaining levels due to peak-detection program. *Rectangles:* control before each sonication. *Rhombi:* control after each sonication. Inclination in control curves due to incomplete recovery, proved by repetition of registration

zero (Fig. 8). Sonication of the hair flattened the dynamic response versus deflection characteristic (Fig. 9), but the diminished response amplitudes could be compensated for by greater deflections (tested with deflections of over 5° to each side for the preparation shown) so that the saturation levels remained constant.

The effectiveness of cercus and hair sonication in reducing the responses to adequate stimuli could not be measured as a function of absolute intensities.

Ultrasound intensities, which do not elicit significant responses, are sufficient to suppress the responses to simultaneous adequate stimuli totally, if applied to the cercus (Fig. 6). In the sonicated cercus, the diminution of the dynamic responses to adequate stimuli is graded only within a narrow band of intensity (Fig. 10).

If the hair was sonicated instead, phasic-tonic responses to deflections during sonication and the corresponding off-responses could still be seen when the static receptor potential due to ultrasound had reached several millivolts (Fig. 7). No matter whether the ultrasond response versus intensity characteristic was monotonous or not, the flattening of the resulting response versus deflection characteristics increased monotonously with the intensity of the sonication (indicated for one angle of deflection in Fig. 7). Response versus deflection characteristics taken without and during sonication of the hair give almost equal

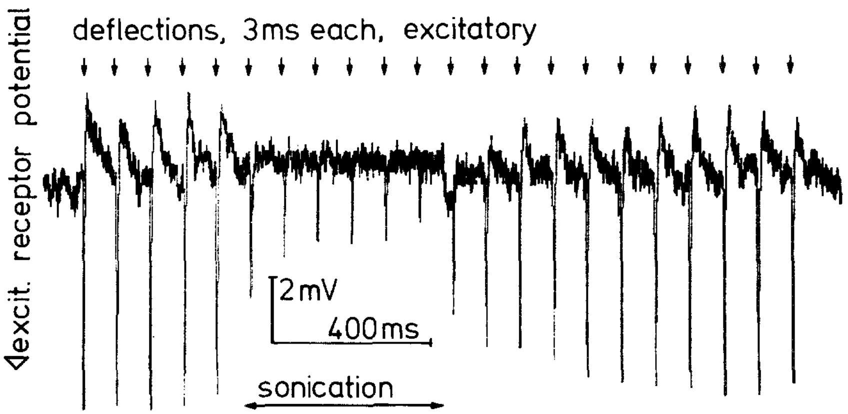

Fig. 11. Time course of suppressing effect of cercus sonication, tested with excitatory deflections of 3 ms duration

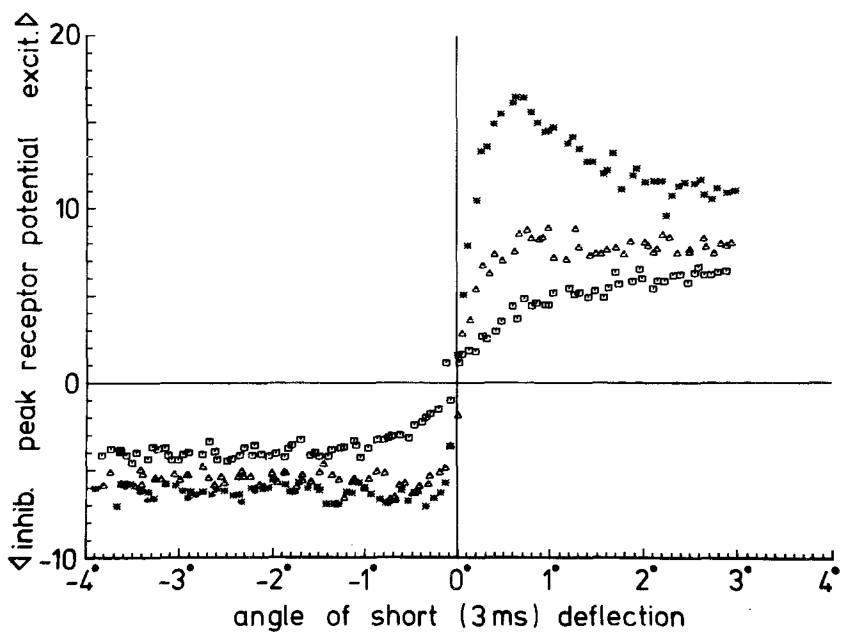

Fig. 12. Suppression of responses to short (3 ms) deflections at two times after onset of cercus sonication; stimulus program as in Fig. 11, evaluation of first control response before sonication (*asterisks*), first (*triangles*) and last (*rectangles*) deflection during sonication. Note that within 3 ms the responses to deflection do not reach their dynamic peak amplitude

saturation levels independent of the intensity of the sonication, the amplitude of the responses to the sonication and the time between the onset of sonication and the deflection stimulus.

The time-course of the ultrasound-dependent suppression of sensitivity for adequate stimuli was tested with trains of short deflections which started before sonication (control) and ended some time after the cessation of sonication of the cercus (Fig. 11). To avoid continuous adaptation to the mechanical stimuli, the deflections had to be short (3 ms) due to the repetition rate (80 ms). The repetition rate determines the temporal resolution of the method. Longer-lasting deflections would have been more desirable, because 3 ms is too short for the responses to reach their peak amplitude. Figure 11 shows the efficiency of suppression at different times after the beginning of sonication and indicates that the suppression is not relieved abruptly at the end of sonication, but decays within several hundred milliseconds. The time-dependent increase in suppression is effective for all angles of deflection, as Fig. 12 shows.

The suppression of mechanical sensitivity produced by sonication of the hair (instead of the cercus) also lasted longer than the sonication, but time-courses have not yet been studied in detail.

Discussion

General Effects of Ultrasound

In the search for the mechanism of adaptation the use of ultrasonic vibrations was intended to stimulate the filiform hair sensilla. Generally ultrasound is used for diagnosis (see e.g., Linzer and Norton 1982), but also for homogenizing tissues. The destructive potential of sonication could possibly cause reversible damage with electrical epiphenomena anywhere in the sensillum. Ruling out such damage and relating the electrical responses to sonication to the system for mechanoelectrical transduction is therefore a prerequisite for meaningful interpretation. The ultrasound intensities in diagnosis and homogenization differ greatly. Intensities below 0.1 W/cm^2 are regarded as harmless for the sonicated tissues, but the setup used in the experiments does not even allow a rough estimate of the absolute intensity near the sensillum. Therefore information within the observed responses themselves had to be used to classify the sonication as being harmless for the sensillum but having an effect on the transducing mechanism. The following five findings, taken together, characterize the observed electrical responses as changes in the same conductance, which is modulated by adequate stimuli:

i) The ultrasound effects are repeatedly and relatively quickly reversible.

ii) Suppression of the responses to adequate stimuli can be graded by the ultrasound intensity.

iii) A transient change in passive electrical properties of the epithelium during sonication is unlikely, because even at intensities that suppress all responses to deflections of the hair, the mean amplitude and shape of the spikes remain constant.

iv) Gradedly destructive, but repeatedly resealing alterations of the permeability of membranes or of the whole epithelium, should cause a decline in transepithelial voltage. Yet, at low ultrasound intensities (applied to the hair) an increase in transepithelial voltage was typically observed, which is described above as inhibitory response. This interpretation is justified by the initial drop in spike frequency that accompanies these responses to ultrasound (Figs. 4, 7).

Such drops in frequency indicate a closure of the sensory pathway, because any rise in transepithelial voltage (due to events in the auxiliary pathway) at constant conductance in the sensory pathway would tend to increase the spike frequency by augmenting the depolarizing current. The only residual conductance within the sensory pathway, which can be subject to lowering by sonication, is the one which is also modulated by adequate stimuli.

v) The saturating levels for phasic responses to deflection stimuli are not changed by superimposing the responses to deflection on responses to sonication of the hair. (However, sonication of the cercus, which suppresses all responses to adequate stimuli, does reduce the saturating levels.)

So far it is not quite clear why the sensilla react to ultrasound at all, especially why the reaction can be excitatory and inhibitory. The generally known effects of ultrasound may give further clues. Cleaning, disruption, and disintegration by ultrasound are mostly based on cavitation, which occurs in liquids when the sound pressure becomes more negative during one half-wave than the atmospheric- plus hydrostatic- minus vapour pressure. The negative pressure gives rise to bubbles, the collapse of which is destructive because of their abruptly stopped backstroke.

Cavitation as a cause of the observed effects cannot be excluded, but, at least at sonication of the hair, it should have disrupted the connection between the capillary and the hair before the hair could transfer sufficient intensity for cavitation to the sensillum. In one of the previously mentioned cases of intentional destruction of the sensillum, cavitation occurred within the dropled of Oppanol B3, by which the filiform hair was coupled to the capillary, and which was relatively large in that experiment (roughly 50 μm; in many other experiments only a film of Oppanol on the capillary was used). The resulting oscillation of the hair caused atomizing of the electrolyte from the orifice of the electrode.

In sonicated media a mean sound radiation (over)pressure is built up, which is proportional to the mean energy per volume. Spatially inhomogeneous sonication causes pressure gradients and flows of the medium if it is fluid. Sound is partly reflected at boundaries of structures with different acoustic impedance. The resulting differences in mean energies per volume and radiation pressure between the two media generates a continuous mean pressure on the boundary between the media.

Indications of both effects were observed in the preparations. In one experiment, in which the illumination was brighter than usual, rotational movement of the cells in the hemolymph indicated a continuous gyration around the cercus axis during sonication of the cercus. Hydrostatic pressure in the cercus (1000 Pa) deflects the filiform hairs (Gödde 1983). During sonication at high intensities (as compared to the intensities needed for suppression of responses to adequate stimuli), similar deflections can be observed. Even if the ultrasound was fed into the cercus via a hair, static deflections of other filiform and club-shaped hairs could be induced. These sonication-dependent deflections are probably due to radiation pressure. The optical setup allowed no distinction between movements of the whole socked apparatus or mere deflection of the hair within its articulation.

Possible Causes of Responses to Sonication

1. Responses of the receptor to sonication can be due to gating events which are reversed within each oscillation. In this case only deviations of the mean gating state from its resting value could be resolved because of amplifier bandwidth and shunting capacities within the preparation. However, for steplike deflections the time from the onset of the response to the dynamic peak is in the order of magnitude of 10 ms. If the time to peak were the same for adequately and ultrasound-induced gating and if these times reflect gating kinetics, then the resulting oscillations (at ultrasound-frequency) of the response should be negligible.

2. Forces which are caused by radiation sound pressure are continuous. They can develop in many parts of the cercus far from the sensillum (e.g., shearing forces between rotating hemolymph and the basal side of the epithelium) or within the dendritic membrane itself. Several such effects with different intensity dependencies possibly superimpose to give the non-monotonous response versus intensity characteristic. Clearly, further investigations are necessary.

The Stimulus-Transmitting Apparatus

Further interpretation of the experiments requires at least a rough knowledge of the stimulus-transmitting apparatus, which is schematically reported according to Gnatzy and Tautz (1980), Gödde (1983), Thurm (1982), Thurm et al. (1983) and is also shown in Fig. 13. The relatively flat tubular body in the dendrite of the sensory neuron together with the cell membrane and the dendritic sheath is compressed during excitatory deflections of the hair. Between a fibrous cap and a protrusion in the base of the cuticular hair (cuticular peg), the dendrite is arranged as between a pair of nippers of different materials.

Adaptation

If specific responses of the sensory neuron (specific in contrast to mere deletion) could be elicited, it was expected that time dependencies in the transfer function of a possibly viscous (plastic) stimulus-transmitting apparatus could be circumvented by ultrasound. The specific and highly static reactions of the sensillum to sonication confirmed this expectation. This does not, however, prove that the stimulus-transmitting apparatus is the sole source of adaptation to adequate stimuli, as will be discussed below.

The terms phasic and tonic refer to adaptation as seen in the decline of responses to sustained stimuli. In this sense the adaptation in responses to sonication is slower and less complete than in deflection-induced responses of equal phasic amplitude, but sonication responses are not altogether tonic (Figs. 3, 4, 7). The cause of this adaptation to sonication is unknown. Perhaps it is an artifact due to some softening of the coupling between the capillary and the hair, for instance. In some cases, especially excitatory responses to ultrasound (of

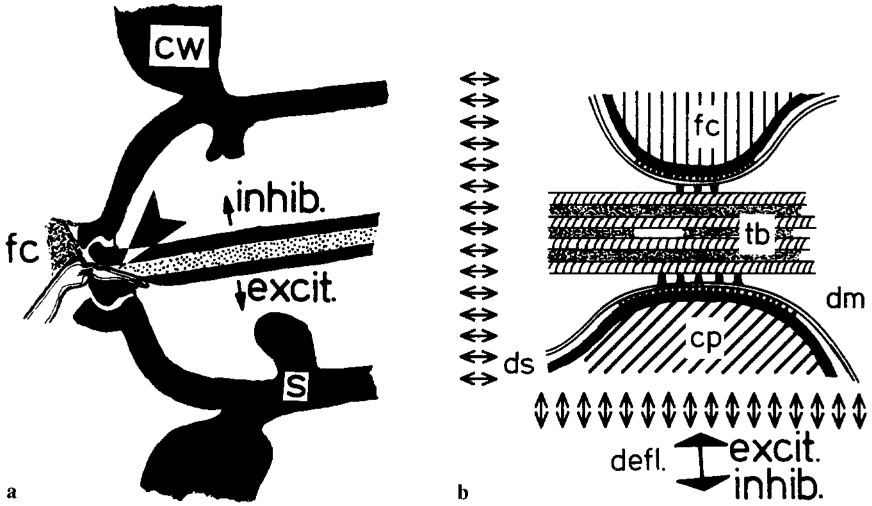

Fig. 13a and b. Diagram of the base of the filiform hairs, drawn according to Gnatzy and Tautz 1980 and Thurm et al. 1983. **a** overview with broad arrow towards presumable pivot point. **b** detail of the sensitive region with arrows indicating presumable direction of particle oscillation; horizontal arrows: for cercus sonication– vertical arrows for hair sonication. *cp* cuticular peg in hairbase, *cw* cuticular wall of the cercus, *defl* arrow, indicating deflection-induced movement, *dm* dendritic membrane, *ds* dendritic sheath, excit. (inhib.) direction of movement in excitatory (inhibitory) deflection stimuli, *fc* fibrous cap, *s* "cup"-shaped socket, *tb* tubular body

relatively high intensity) even increased during sonication. Another explanation is the induction of net movements in the cercus. Phasic responses to such more or less adequate stimulation would then be superimposed on the tonic sonication responses and make them seem phasic-tonic.

Adaptation can also be expressed by the influence of a stimulus on the sensitivity to subsequent (sets of) stimuli. Thus the sonication-induced loss in sensitivity for adequate (deflection-) stimuli may be called ultrasound adaptation. In contrast to ultrasound adaptation, "adequate adaptation", as induced by steplike deflections, does not alter the maximum steepness of the dynamic stimulus-response characteristic nor its saturation levels. Onset and recovery of ultrasound- and deflection-induced adaptation for subsequent adequate stimuli have similar timecourses.

Deflection stimuli can shift the dynamic stimulus-response characteristic over 5°, which corresponds to a movement of more than 150 nm at the hairbase, if one assumes the reported distance between the pivot and the contact point (Gnatzy and Tautz 1980; Thurm 1982; Keil and Thurm, in preparation). Such movements cannot be taken up by thickness modulation of the tubular body (about 400 nm thick) or even its membrane and therefore must constitute deformations elsewhere within the stimulus-transmitting apparatus. If these deformations are plastic instead of elastic, they could explain adaptation to standing deflection stimuli and the subsequent off-responses. However, the resting fraction of the stimulus-dependent conductance and the time-course of adaptation to adequate stimuli acutely depend on oxygen supply (own unpublished results). Attributing the adaptation to adequate stimuli exclusively to the stimulus-transmitting apparatus would therefore require its (mechanical) properties to depend on the oxygen supply, which seems unlikely. Consequently membrane-(or gating-) related events in adaptation to adequate stimulation must be postulated.

One may compare the membrane-borne portion of adaptation to an inactivation-like mechanism. Inactivation in the Na-channel (Hodgkin and Huxley 1952) can be abolished by pronase treatment (Bezanilla and Armstrong 1977). Attempts to explain the adaptation to adequate stimuli by a corresponding inactivation mechanism, which might be blocked during sonication, must fail for two reasons. According to the formalism Hodgkin and Huxley developed for inactivation, (unless a surplus of normally silent spare channels is postulated) the decline in the phasic deflection response to the tonic level would require decreased saturation responses for superimposed stimuli (because the channels which are inactivated by the conditioning stimulus would not be instantaneously susceptible to the test stimulus). However, the saturation responses remain unaffected by adequate adaptation. The results further indicate that sonication (at intensities which elicit tonic responses) does not block the adaptation to adequate stimuli because this should lead to a reduced decline in responses to deflections, which are superimposed onto a (hair-) sonication stimulus. However, as Fig. 7 shows, such stimuli still elicit (diminished) phasic responses with normal adaptation and are followed by off-responses. According to these data all models which try to explain the adaptation in the filiform hairs (for instance by events which act stochiometrically at the channel site) should fulfil an additional postulate: the adapting event must fail to appear if the previous gating was elicited by sonication. A less complicated model for the transduction and adaptation is presented below.

A Model for the Transducing Mechanism and Adaptation

The dynamic response versus deflection characteristic indicates that the gating process (opening and closing of stimulus-dependent conductance, see Introduction) is a highly anisotropic reaction because it senses the direction of the mechanical stress. However, this characteristic does not tell whether stimulation induces the conformational changes of the channel protein (complexes) directly or via a chain of coupled reactions (as in visual transduction for instance). In any case, the primary reaction appears to be a stimulus-dependent shift in some equilibrium, because gating is possible with blocked oxidative metabolism (Vohwinkel, personal communication – own unpublished results). The integrity of the tubular body is not required for responses to deflections as Erler (1983) has proved for the tibial thread hairs in *Acheta*. For this reason and because of the very short latency of the onset of reactions to a deflection (50–70 μs; Thurm et al. 1983), the membrane of the dendrite or its vicinity is the most likely site for the primary event in transduction. Discrete "sensor-channel units" have been proposed by Thurm et al. (1983) as sites which receive the mechanical stress and control gating. However, the stimulus energy may just as well primarily induce conformational changes within the (whole?) membrane, which so far has been regarded as passive. Conformational changes in membrane proteins (channels) can induce changes in the packing of boundary lipids (Beurer et al. 1983; Klausner et al. 1979). On the other hand, the activity of channels (or other membrane proteins) can be modulated by conformational changes in the lipids around them (Armond and Staehelin 1979; Heremans 1982; Israelachvili et al. 1980; Sandermann 1978; Vogel 1983). Thus, even channels which float in a membrane without having contact to rigid supporting structures can react indirectly to the stimulus by sensing stimulus-induced conformational changes of the lipids within a "receptive domain" around them. The margins of such "receptive domains" may be smeared or overlap. The minimal energy needed for switching is independent of the size of the domains. It is 1 kT per domain in Gibbs free energy for an 3-fold increase of the equilibrium constant, provided the coupling within the domain is rigid enough. The same concept is applicable to thermoreceptors, if temperature instead of mechanical stimuli alters the conformation of the membrane.

For mechanoelectric transduction two processes would determine the behaviour of such a system: (*i*) propagation of stimulus-induced disturbances of the lipid conformation towards the next channel, and (*ii*) dissipative relaxation of the disturbance. Sufficient survival times for non-equilibrium elastic changes following external mechanical perturbation in membranes have been discussed by Israelachvili et al. (1980). If the kinetic, by which the incoming energy is handed to the channels, is faster than the dissipative relaxation, then this model could also account for the dynamic stimulus-response characteristics of the filiform hairs, and at least partially for the adaptational shift of these curves and for the off-responses. Macroscopically well-defined concepts such as "rigidity" and "stimulus-transmitting apparatus", and "site of transduction" lose their meaning somewhat on this scale. As a convention the structures outside the dendrite and the bulk of microtubuli within the tubular body are called stimulus-transmitting apparatus in this discussion.

Application of the Model to the Data

The previously mentioned, but so far highly speculative model for mechanoelectric transduction is consistent with the adequately elicited responses, their membrane-borne component in adaptation, and the responses to sonication. Further, it can explain the different time-courses of the response to sonication and the sonication-dependent suppression of responses to adequate stimuli. This will be shown in the following.

For large deflections most of the deformation by a steplike deflection is taken up by the stimulus-transmitting apparatus. Simultaneously, the dendritic membrane receives anisotropic pressure and undergoes non-equilibrium elastic (shape or conformational) changes, which cause gating. Plastic properties of the stimulus-transmitting apparatus and dissipative relaxation of the conformational change can produce the adaptation to adequate stimuli. Ultrasound should be able to pressurize the membrane directly and continuously by virtue of radiation sound pressure. This pressure ceases at the end of sonication, as do the responses to it.

In terms of the proposed model sonication can disturb the order of the dendritic membrane and thereby facilitate dissipative relaxation of stimulus-induced non-equilibrium states. This corresponds to a diminution of the effective size of the "receptive domains", the nuclei of which may remain sensitive. However, the whole system would require more stimulus energy than normal for the same response, be it excitatory or inhibitory. As predicted thereby, sonication reduces the sensitivity for both inhibitory and excitatory adequate stimuli. The time-course of the diminution of the sensitivity and its recovery within some 100 ms after the end of sonication may reflect the time-course of the disturbance of the membrane order. This disturbance alone should not be effective for gating (and therefore not elicit the observed responses to sonication), because then the time-courses of the responses to sonication and the diminished sensitivity to adequate stimuli would have to be correlated, which is not the case.

Diffraction, scattering, and reflection influence the sound propagation within the cercus, but in the bulk of it (with the exception of the cuticle) longitudinal oscillations ought to be favoured over shear vibrations. For the experiments with sonication of the cercus it seems reasonable to assume a particle oscillation preferentially parallel to the plane of the sensitive membrane (Fig. 13). With sonication of the hair, in which flexural vibrations within the plane of least restraint were induced, the oscillation should have been more perpendicular to this membrane. The membrane as a whole must be fairly resistant to forces perpendicular to its plane, because such forces represent the adequate stimulus, whereas oscillations parallel to the membrane should be more effective in interfering with lateral interaction between lipids. This agrees with the observation that sonication of the cercus almost totally suppresses the sensitivity for adequate stimuli before eliciting responses itself, whereas with sonication of the hair adequately elicited responses can be superimposed on significant responses to sonication.

Comparative Aspects

Recordings from auditory interneurons of the cricket *Teleogryllus oceanicus* revealed suppression by airborne sound frequencies between 3 and 8 kHz, but strong excitation by unmodulated ultrasound in the range between 15 and 100 kHz (Moiseff and Hoy 1983). The present experiments show that the transduction mechanism can generally work in the range of ultrasound frequencies, even in those sensilla which physiologically never receive these frequencies from the stimulus-transmitting apparatus.

In auditory receptor cells of locusts, Hill (1983) recently found depolarizations after the end of sound stimuli of low intensity, whereas at high intensities the depolarization starts with the onset of the stimulus. This is in accordance with the inhibitory reponses to hair sonication at low intensities, followed by an excitatory off-response and the excitatory responses at higher intensities in the filiform hair sensilla. Inhibitory responses cannot be resolved well in the locusts' auditory receptor cells because of their low spontaneous depolarization rate.

In the experiments with sonication of the hair, the triggering of specific responses occurred at intensities of less than 5% of those which destructed the sensilla. Such triggering of specific responses by solid-borne ultrasound at intensities far below the threshold of destruction merits further investigation in the medical field because of the frequent use for diagnosis.

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