Destruction and Reorganization of the Receptor Membrane in Labellar Chemosensory Cells of the Blowfly

Long-Lasting Latent Action of Colchicine

MAMIKO OZAKI, MITSUYOSHI NINOMIYA, YASUHIRO KASHIHARA, and HIROMICHI MORITA

From the Department of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812, Japan

ABSTRACT Electron micrographs of sections of the labellar chemosensilla of the blowfly, *Phormia regina*, showed that treatment with sodium deoxycholate (DOC; 7.2 mM for 2 min) destroyed the distal processes of the receptors from up to 10 μm from the tip of the sensillum, but these processes regenerated almost completely within 0.5 h. However, when DOC treatment was preceded by colchicine treatment (25 mM for 2 min), >10 h was required for complete regeneration. Sugar receptor responses supported these findings and disclosed a more detailed time course of regeneration after DOC treatment: without colchicine pretreatment, the destroyed distal process completely regenerated in 0.3–1.0 h, but with pretreatment, regeneration began at 3 h and reached the chemosensillar tip at 8 h at the earliest. Hardly any depression of the response was observed for 8 h after treatment with colchicine alone, but a transient depression was detected at 12 h. Based on these results, the role of microtubules in the maintenance of the receptor membrane is discussed.

INTRODUCTION

The labellar chemosensillum of the blowfly, *Phormia regina*, contains one mechanosensory and four chemosensory cells. Three of the four chemosensory cells, which extend their distal processes (outer segments of dendrites, according to Altner and Prillinger, 1980; sensory cilia, according to Toh, 1981) into the inner lumen of the sensillum, respond to sugars, salts, and water, respectively, with impulses that are distinguishable from one another. The tip of the sensillum

Address reprint requests to Dr. H. Morita, Dept. of Biology, Faculty of Science, Kyushu University 33, Fukuoka 812, Japan. Dr. Ozaki's present address is Dept. of Biological Sciences, Lilly Hall of Life Sciences, Purdue University, West Lafayette, IN 47907. Dr. Ninomiya's present address is Shionogi Research Laboratory, Shionogi & Co., Ltd., Sagisu, Fukushima-ku, Osaka 553, Japan. Dr. Kashihara's present address is Laboratory of Learning and Memory Research, Dept. of Information Physiology, National Institute for Physiological Sciences, Myodaiji, Okazaki 444, Japan.

opens as a small pore (Tominaga, 1975), so it is easy to investigate the effects of various chemicals on the chemoreceptors, using a simple electrophysiological technique in which recordings are made at the tip (Hodgson et al., 1955).

Shimada (1975) first reported the recovery of the response of the labellar sugar receptor of the fleshfly after treatment with the detergent sodium dodecyl sulfate or sodium deoxycholate. We investigated it in further detail (Ninomiya et al., 1986) and found that the recovery was depressed by pretreatment with colchicine or vinblastine. This fact suggests that tubulin or a microtubule is involved in the recovery process.

In fact, each distal process of the four chemoreceptor cells in a chemosensillum of the blowfly possesses only microtubules as the intracellular structure. In this work, we studied a long-lasting effect of colchicine, and again the evidence strongly suggested that the microtubules were indispensable for the maintenance and repair of the sensory transduction system.

MATERIALS AND METHODS

The blowflies, *Phormia regina*, were reared in our laboratory. Adult flies were used 4–6 d after emergence. They were fed with 0.1 M sucrose.

Electrophysiological Procedures

Since the present experiments were of long duration (10–30 h), each whole fly was kept alive (Getting, 1971). A fly was gripped by the wings, and the proboscis was fixed in an extended state by inserting the base of its haustellum into the gap between two pieces of steel wire (0.6 mm diam), which also served as an indifferent electrode. Thus, the fly showed fairly stable responsiveness for >24 h. Relative humidities were >70% and ambient temperatures were 22 ± 2°C throughout the experiments. The chemosensilla used were of the largest type located on the outer margin of the labellum. The stimuli used were 0.1 M sucrose, 0.5 M glucose, and 0.1 M fructose dissolved in 10 mM NaCl for the sugar receptor, 0.5 M NaCl solution for the salt receptor, and 10 mM NaCl solution for the water receptor. Every solution was taken up in a capillary, which was also used as a recording electrode when applied to the tip of the chemosensillum (Hodgson et al., 1955). The stimulus duration was ~0.5 s, and the interval between stimuli was at least 3 min for disadaptation. A response was defined as the number of impulses during a period from 0.15 to 0.35 s after the generation of the first impulse (Ninomiya et al., 1986).

Aqueous solutions of colchicine and sodium deoxycholate (DOC) were made up at 25 and 7.2 mM, respectively, in 67 mM phosphate buffer adjusted to pH 7.6. For treatment of the receptors, each solution was taken up in a separate glass capillary and applied to the chemosensillum tip for 2 min. Thus, the colchicine or DOC treatment was definable both for concentration and for duration. For example, "colchicine-DOC treatment" means that the receptor was treated first with colchicine at 25 mM for 2 min and then with DOC at 7.2 mM for 2 min. In the figures, the response is expressed as the "relative response," which means the ratio of the response to that before treatment.

Electron Microscopic Procedures

The chemosensilla showing the normal response before treatment and the typical recovery after treatment were used for electron microscopic observation. The responsiveness was checked with 0.1 M sucrose dissolved in 10 mM NaCl, 0.5 M NaCl, and 10 mM NaCl for the sugar, salt, and water receptors, respectively. Within 3 min after the last record of the

response, each labellum was cut off and fixed in 3% glutaraldehyde (buffered with 0.1 M cacodylate buffer at pH 7.4) for 2 h at room temperature to avoid the destruction of microtubules at low temperatures. They were then washed in the same buffer, post-fixed in 2% OsO_4 in the same buffer for 2 h on ice, dehydrated through an ethanol series, and embedded in Epon 812. Cross-sections were cut from the tip toward the base of the sensillum with glass knives using an ultramicrotome (LKB-480, LKB Instruments, Inc., Gaithersburg, MD) and were double-stained with uranyl and lead acetates. The sections were observed with an Hitachi HU-11A electron microscope. Levels along the length of the distal process were estimated by counting the number of sections from the first section containing a part of the cuticle at the tip of the sensillum. The coloration of the sections (silver-gold) implied a thickness of 100 nm.

When treated with DOC only, the samples were fixed with glutaraldehyde immediately or at 30 min after DOC treatment. When pretreated with colchicine, the chemosensilla were fixed with glutaraldehyde immediately or at 5, 10, or 15 h after DOC treatment. To keep the conditions as constant as possible in this case, each fly was held on a stand that had been set up in advance 15 h before fixation with glutaraldehyde.

Chemicals

DOC was purchased from Katayama Chemicals (Kyoto, Japan) and colchicine was obtained from Nakarai Chemicals (Osaka, Japan).

RESULTS

Reversible Effect of Colchicine

Although the colchicine treatment itself hardly affects the response of the sugar receptor, it depresses the recovery after the DOC treatment almost completely for the response to 0.5 M glucose and to less than half of the control values for the response to 0.1 M sucrose (Ninomiya et al., 1986). These effects of colchicine were reversible, as revealed below by long-lasting experiments after the colchicine-DOC treatment.

Fig. 1 shows an example of the responses obtained 20 h after the treatment. The responses in the untreated sensillum were relatively constant throughout the experiment. In the colchicine-DOC–treated sensillum on the same labellum, the responses recovered only partially, or not at all, without the phasic component until 13 h after the treatment. Thereafter, the responses to 0.1 M sucrose began to recover again, in parallel with the recovery of the response to 0.5 M glucose. The rate of this second or late recovery process was clearly slower than that of the first partial recovery. The beginning of the second recovery was also characterized by the reappearance of the phasic component (short arrows). Although the response to fructose was low at the final stage in this experiment, it did recover in others. The recovery of the salt and water receptors was generally poor (see also Fig. 2).

Effective Period of Colchicine

Although no effect of the treatment with colchicine alone was detected by Ninomiya et al. (1986), it might have been latent, lasting for more than 10 h, as suggested by the above experiment. To investigate this possibility, we allowed

various intervals between the colchicine and the DOC treatments. Each fly was prepared 23 h before the DOC treatment and its control responses were recorded 21 h before the treatment. Fig. 2, A and B, shows the results of the experiments, where the intervals were 4 and 8 h, respectively. In each case, the latent effect of colchicine persisted for a long time but became apparent only after the DOC treatment. In a sensillum pretreated with colchicine 4 h before the DOC treatment (Fig. 2A), both the partial depression of recovery of the sucrose response and the complete depression of the glucose response continued for roughly another 4 h. These depressions lessened thereafter, and subsequently all responses, including the water receptor response, which showed a longer delay, recovered fairly well with phasic components. In a sensillum pretreated with colchicine 8 h earlier (Fig. 2B), the depression period was further reduced
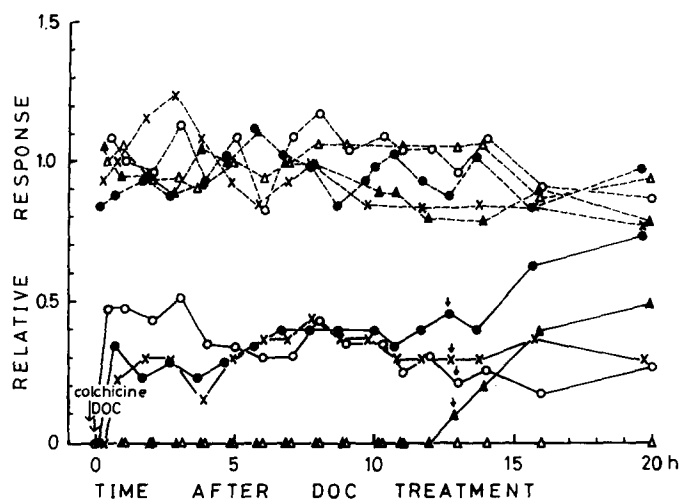

FIGURE 1. Recovery time courses of the sugar, salt, and water receptors after treatment with colchicine (25 mM, 2 min) and DOC (7.2 mM, 2 min). The broken lines connect the control responses in an untreated sensillum, obtained from receptors in the same labellum. The short arrows indicate the reappearance of the phasic response (the same as in Figs. 2 and 5). ●, 0.1 M sucrose; ▲, 0.5 M glucose; ×, 0.1 M fructose; ○, 0.5 M NaCl (salt); △, 10 mM NaCl (water).

to ~2 h in the sucrose and glucose responses. In a sensillum pretreated with colchicine 20 h earlier (Fig. 2C), no depression could be seen in the sugar receptor, so that the recovery time courses were almost the same as in another sensillum treated only with DOC on the same labellum (Fig. 2D). These results show that the latent effect of the treatment with colchicine alone lasts longer than 8 h but less than 20 h in the sugar receptor.

Effect of Colchicine Alone

Such a long-lasting effect of the colchicine treatment could be detected without the DOC treatment. We examined the response up to 16 h after the colchicine treatment and found, as shown in Fig. 3, that colchicine decreased the sucrose response, but only briefly, at ~12 h after the treatment. This decrease was
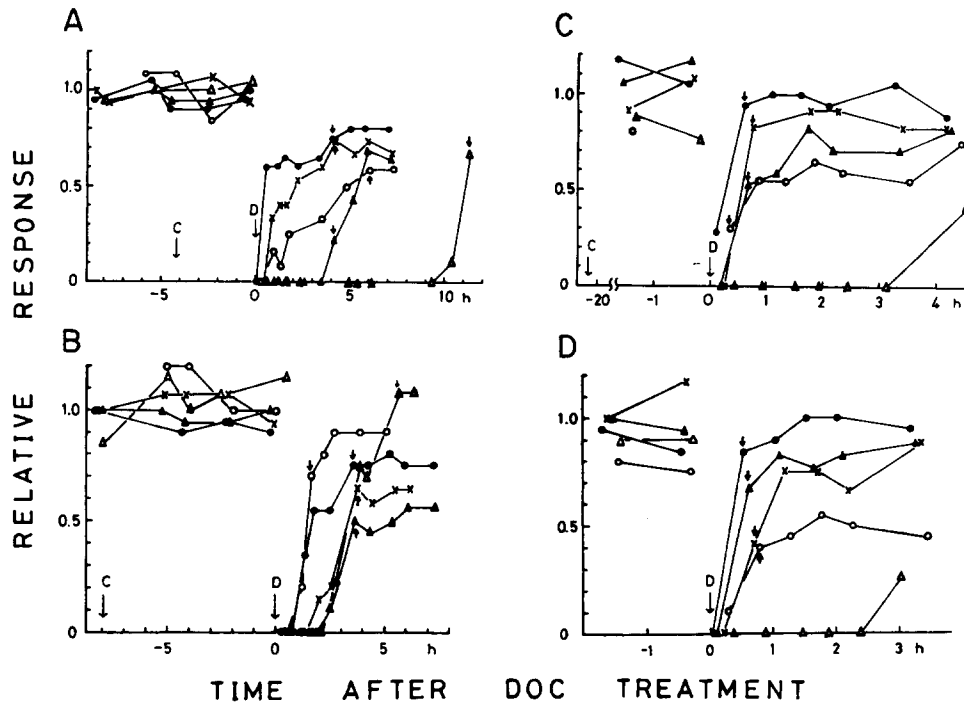
FIGURE 2.   Recovery time courses after DOC treatment preceded by colchicine treatment. (*C* and *D*) Times of the colchicine and DOC treatments, respectively. Notice different time scales for different graphs. ●, 0.1 M sucrose; ▲, 0.5 M glucose; ×, 0.1 M fructose; ○, 0.5 M NaCl (salt); △, 10 mM NaCl (water).

statistically significant (Student's *t* test, <1%), compared with the responses obtained at the same time from the sensilla left untreated as controls in the same preparations. Such a decrease in the response was more pronounced if another colchicine treatment was added before the effect of the first colchicine treatment
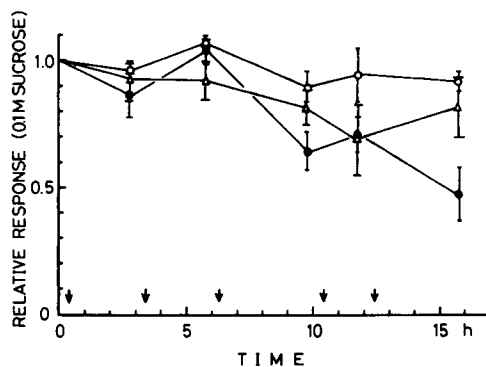

FIGURE 3.   Reduction of the sucrose response by colchicine treatment (arrows). Vertical bars indicate ± standard deviations. Five sensilla were treated with colchicine (△) and five sensilla were left untreated (○) in the same labellum for each series of experiments. The untreated sensilla were pooled as the control.

vanished. Fig. 3 (solid circles) shows the results of this type of experiment, where the sensilla were treated with colchicine five times, as indicated by the arrows. No obvious decrease in magnitude was detected until 5 h after the first treatment, but the response was decreased to about half of the control after 16 h. Such a reduced response was not accompanied by any considerable lengthening of the latent period for the first impulse and still kept the phasic component.

We obtained results which suggested that the salt and water receptors had the same property, but we could not include these because of their poor reproducibility in long-lasting experiments.

Coincidence of Recoveries

Figs. 1 and 2 show that the second recovery step of the sucrose response began at the same time as the recovery of glucose response. This coincidence is shown
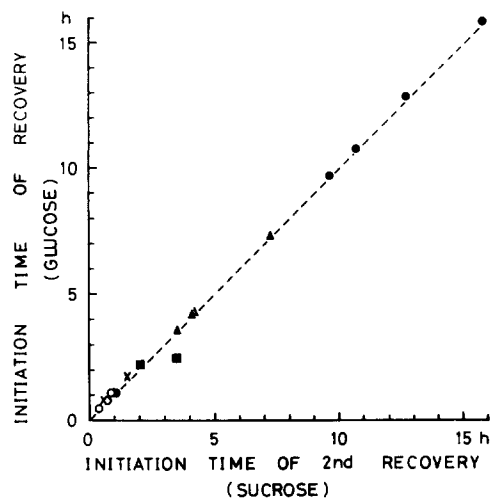

FIGURE 4. Coincidence between the beginning of the second recovery step of the sucrose response and that of the recovery of glucose response (DOC treatment at 0 h). The broken line represents exact coincidence. DOC was added: ●, soon after colchicine; ▲, 4 h after colchicine; ■, 8 h after colchicine; ✕, 16 h after colchicine; ○, DOC without colchicine.

more clearly in Fig. 4, where the initiation time of the recovery of the glucose response was plotted against that of the second recovery step of the sucrose response, which was measured after the DOC treatment. The reappearance of the phasic component that marks the second recovery step was also observed in the recovery after treatment with DOC alone. We were thus able to include the results obtained without the colchicine pretreatment in Fig. 4.

Fig. 4 also shows that the duration of the depression before the second step of recovery was shorter when there were longer intervals between the colchicine and DOC treatments. The similarity of results obtained for the interval of 16 h (✕'s) and without the colchicine treatment (open circles) indicates that the effects of colchicine last for 16 h at most.

As to the recovery in the relative response, it was difficult to find any correlation between the different stimuli except for sucrose and glucose (Figs. 1 and 2). The recovery of the fructose responses in the same sugar receptor cell did not parallel that of the sucrose and glucose responses in time course. However, the phasic part of the response reappeared at the same time in the responses to sucrose, glucose, and fructose, irrespective of the interval between the colchicine and DOC treatments (see arrows in Figs. 1 and 2). There was no relationship among the sugar, salt, and water responses, even in the time of reappearance of the phasic component (Figs. 1 and 2).

Recovery in the Latent Period of Response

The recovery of the phasic response to sugar, salt, or water was always preceded by a shortening of the latent period (the duration from the onset of the stimulus to the first impulse) of the response. Fig. 5 shows the change of the latent period as well as the recovery in the magnitude of the sucrose response after the
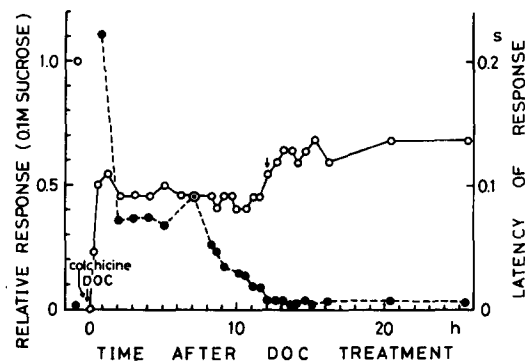

FIGURE 5. Changes in the relative response (O) and in the latent period (●) (from the beginning of stimulus to the first impulse) after the colchicine-DOC treatment.

colchicine-DOC treatment. Starting from an infinitely long period, the latency rapidly decreased during the first step of recovery. Thereafter, it was constant ($\sim$0.1 s) for several hours, and began to decrease again more slowly toward the minimal value. The phasic component recovered when the minimal value was reached, as indicated by a short arrow, and the response began to increase in magnitude as the second step of recovery.

Electron Microscopic Observations

The sensory system used here has been investigated electron microscopically, and its morphology has been established (Larsen, 1962; Adams et al., 1965; Stürckow et al., 1973; Felt and Vande Berg, 1976). The chemosensilla of the largest type, 11 of which are usually located at the outermost margin of the labellar lobe and are 300–400 μm in length, can easily be identified from their locations as those examined electrophysiologically when prepared for electron microscopy.

We first confirmed the morphology established by earlier workers in the intact chemosensillum, and then examined the changes induced by colchicine or DOC treatment with special attention to the microtubules, since colchicine is a well-known antimicrotubule reagent.

Intact Chemosensilla

Fig. 6, A–C, shows the cross-sections of the inner lumen at three different levels along the length of an intact chemosensillum of the largest type, which exhibited normal responsiveness just before the fixation. The most distal section (~1.2 μm from the tip) contained the distal processes of four receptor cells with microtubules on the inside (Fig. 6A). All the distal processes seem to reach the very top of the inner lumen. Fig. 6, B and C, shows the cross-sections at 2.3 and 150 μm from the tip, respectively.

Fig. 6D shows the outer lumen as well as the inner lumen in the section at 2.4 μm from the tip. Usually the outer lumen contains no cell components, but it is seen to contain two vesicles limited by membrane in this section.

In every cross-section of the distal processes, microtubules, though varied in number, were distributed at equal distances from each other with fuzzy structures among them. The fuzzy structures also filled the space between the plasma membrane and microtubules (Fig. 6E). Thus, microtubules form a regular array together with fuzzy structures, and seem to support the distal process as a fairly rigid cytoskeleton. The number of the microtubules decreased toward the tip of the distal process.

Chemosensilla Treated with Colchicine

Some of the colchicine-treated sensilla had the same appearance as the untreated sensilla. Others showed a disordered scattering of microtubules (as seen in Fig. 6F) in the distal process of one or more receptor cells. A few untreated sensilla did show the scattering, but this was exceptional (in 2 of 11 specimens). We could not detect any disruption within a microtubule, but the occurrence frequency of the scattering was greater after the colchicine treatment (in 7 of 10 specimens). This suggests the possibility that the colchicine treatment affected the microtubules in their infrastructure or the microenvironment around them.

Chemosensilla Treated with DOC Only

Fig. 8 shows three cross-sections of one of the sensilla examined just after DOC treatment. This sensillum showed no responses, as shown in Fig. 7A. The responses were confirmed to be normal before DOC treatment (not shown). Fig. 8A is the most distal cross-section of the three presented here, at a distance of 3.2 μm from the tip. This demonstrates the empty inner lumen without any distal processes. In a more proximal section at a distance of 6.0 μm from the tip, fragments of the distal processes or large vesicles in the inner lumen can be seen (Fig. 8B). Below this distorted region, we can see the normal appearance of the inner lumen containing four distal processes with microtubules continuing toward the base (Fig. 8C). From these observations, we can conclude that DOC destroyed the distal processes, leaving only fragments or vesicles within several
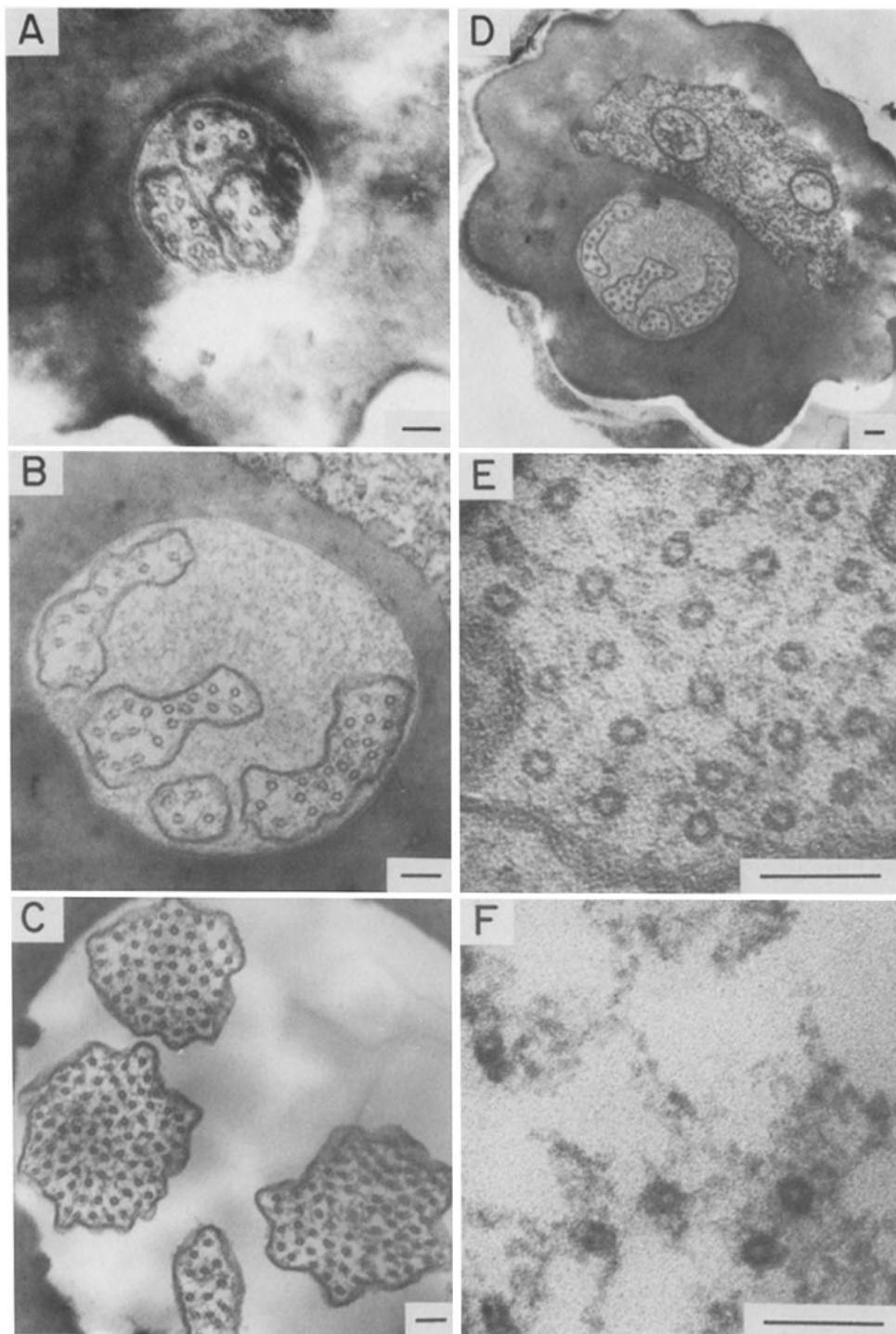
FIGURE 6. Cross-sections of an intact sensillum at distances of 1.2 (A), 2.3 (B), and 150 (C) μm from the tip. Each micrograph shows the inner lumen, containing four distal processes with the uniformly distributed microtubules. (D) A whole cross-section at 2.4 μm from the tip. (E and F) The distribution of the microtubules in the intact and colchicine-treated sensilla, respectively. Scale: 0.1 μm in all plates.

micrometers of the tip. In the sensillum presented here, the empty inner lumen appeared to extend at least 4.2 μm from the tip. The intact tips of the distal processes were located within a distance of 8.5 μm from the sensillar tip, although none of the four distal processes had a normal appearance at the same level.

Fig. 9 shows cross-sections of one of the sensilla examined 30 min after the DOC treatment. Just before fixation, the responsiveness was found to have recovered to ~80 and 50% of the control responses to sugar and to salt, respectively. These responses possessed the phasic components, but the water receptor response had not yet recovered (Fig. 7B). Unfortunately, somewhat slanting sections were cut, but it is clear that two distal processes with microtubules closely approached the tip (Fig. 9A). The other two processes also appeared more proximally, although the level could not be estimated accurately because of the slanting section (Fig. 9, B and C). The two distal processes observed at the level nearest the tip with microtubules might be those of the sugar and the salt receptors, respectively, as judged from the records of responses.

A   immediately after
      DOC treatment

B   30min after
      DOC treatment

sugar
(0.1M sucrose)
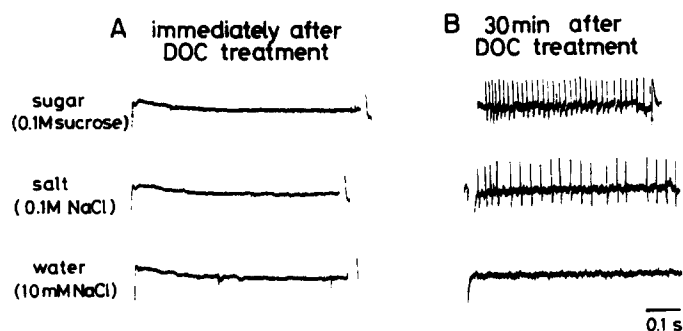
salt
(0.1M NaCl)

water
(10mMNaCl)

0.1 s

FIGURE 7.   Records of responses just before the fixation within 3 min (A) and at 30 min (B) after the DOC treatment. Cross-sections of these sensilla are shown in Figs. 8 and 9, respectively.

Chemosensilla Treated with Colchicine and DOC

Fig. 10 shows three cross-sections of one of the sensilla fixed soon after the colchicine-DOC treatment. Fig. 10A is the most distal section at a distance of ~1.2 μm from the tip, and shows the inner lumen to be almost empty, although faint vesicles can be seen. Fig. 10B shows the section at 5 μm from the tip, where fragments of distal processes or vesicles can be seen. Fig. 10C shows another section at 50 μm from the tip, with most of the normal features of the inner lumen, and a similar normal appearance could be found at distances of <10.7 μm from the tip. These observations are essentially the same as in the sensillum fixed soon after treatment with DOC alone (Fig. 8), and the receptors also showed no response just before the fixation (not illustrated).

Fig. 11 shows three cross-sections of one of the sensilla fixed 5 h after the colchicine-DOC treatment, the responses of which are shown in Fig. 12A. The most distal section, at 2.4 μm from the tip (Fig. 11A), contained nothing but an indistinct, elongated piece in the inner lumen, and another section at 4.5 μm
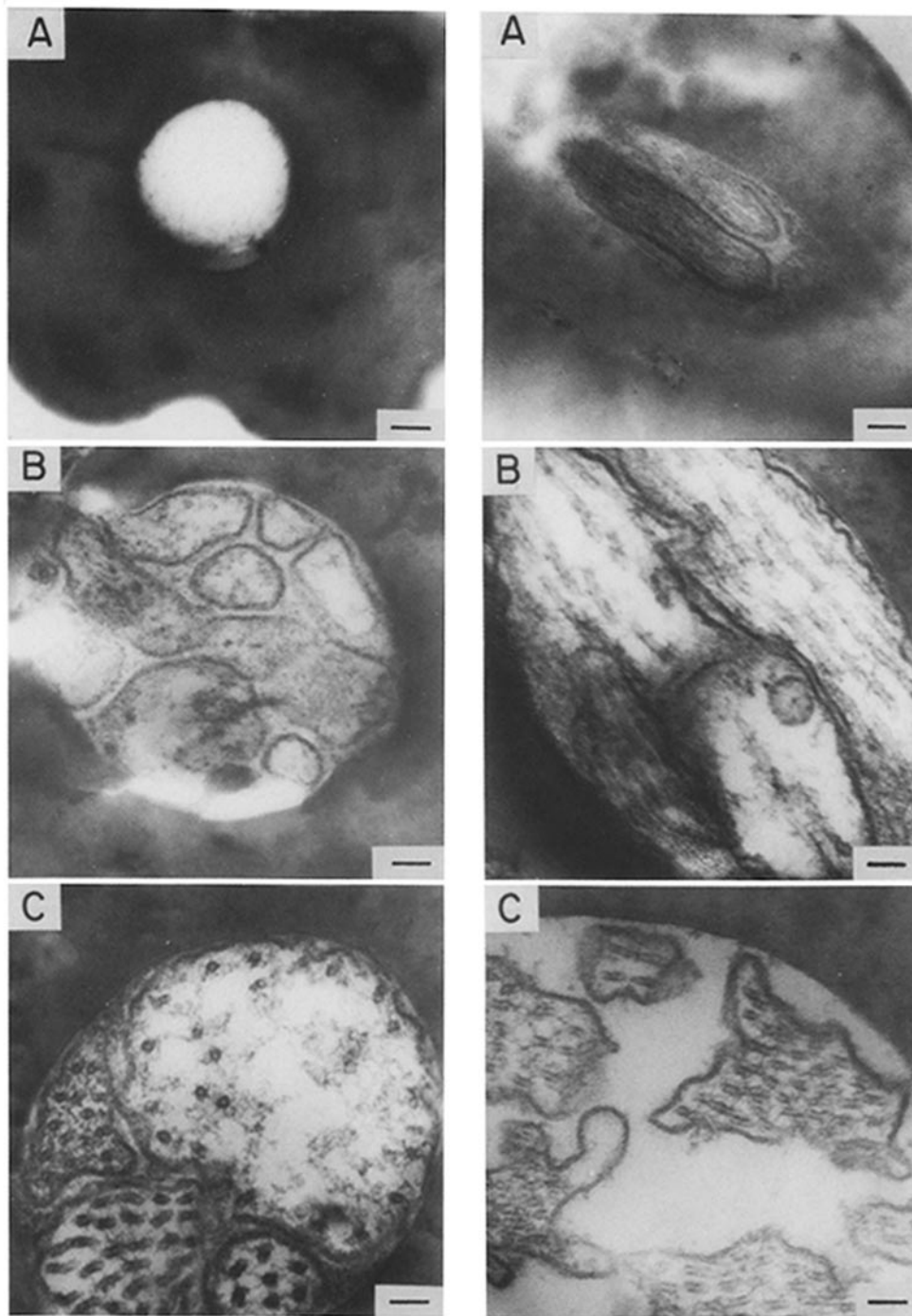
FIGURE 8. (*left*) Cross-sections of a sensillum fixed within 5 min after the DOC treatment, at distances of 3.2 (*A*), 6.0 (*B*), and 50 (*C*) μm from the tip. Distal processes are seen to be destroyed by DOC at the tip.

FIGURE 9. (*right*) Cross-sections of a sensillum fixed at 30 min after the DOC treatment, at distances of <1.5 (*A*), 5–7 (*B*), and >50 (*C*) μm from the tip.
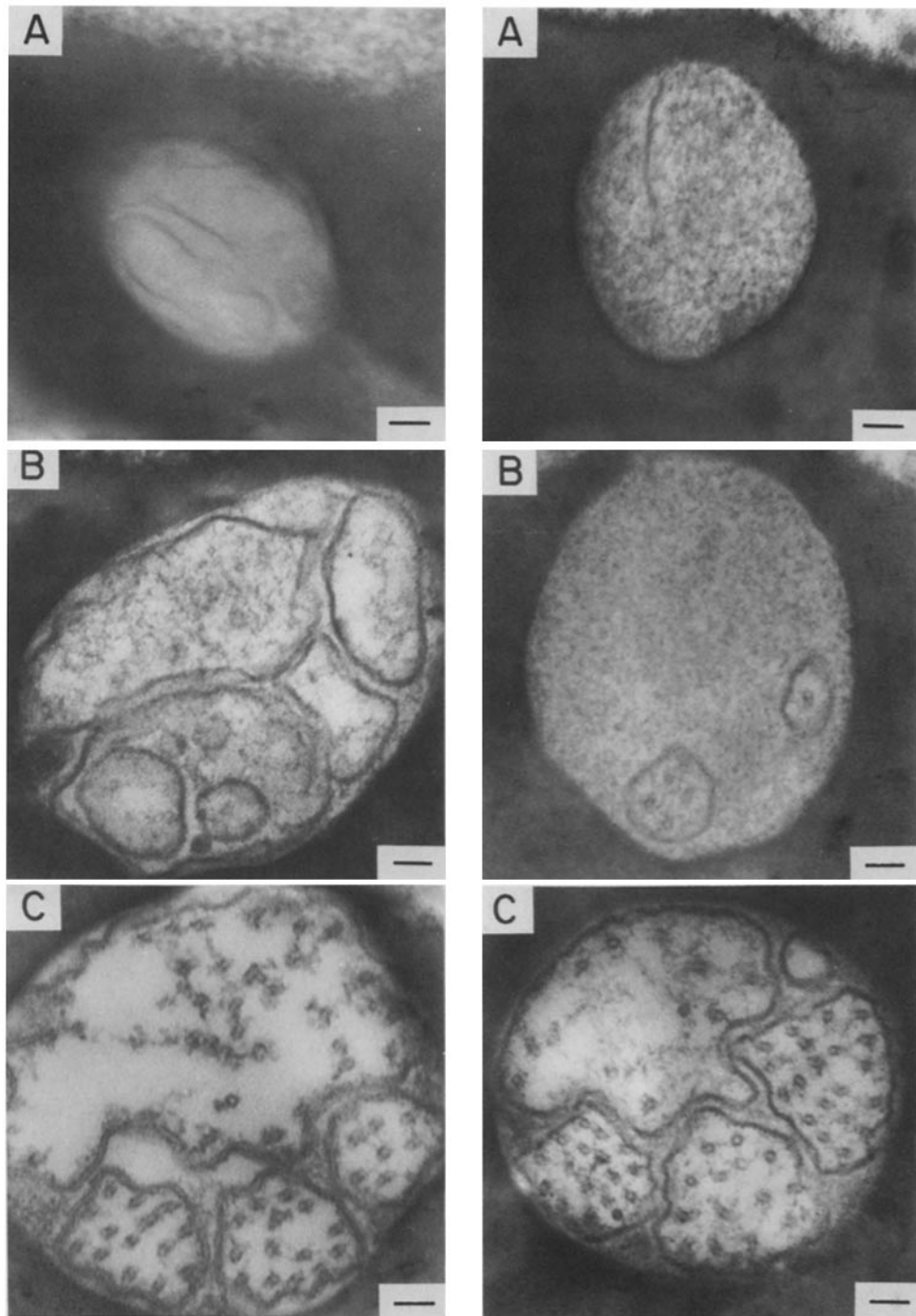
FIGURE 10. (*left*) Cross-sections of a sensillum fixed within 5 min after the colchi-cine-DOC treatment, at distances of 1.2 (*A*), 5 (*B*), and 50 (*C*) μm from the tip.
FIGURE 11. (*right*) Cross-sections of a sensillum fixed at 5 h after the colchicine-DOC treatment, at distances of 2.4 (*A*), 4.5 (*B*), and 20 (*C*) μm from the tip.

from the tip (Fig. 11 *B*) had two distal processes with microtubules, but neither fragments nor vesicles could be seen. All of the four distal processes could be seen at a distance of 20 μm from the tip (Fig. 11 *C*). The smallest additional vesicle or branch near the top is thought to be derived from one of the four processes, since it contained no microtubules. In this sensillum, the first, second, third, and fourth distal processes were observed at distances of <4.1, <4.3, <6.9, and >7 μm from the tip, respectively.

In the same way, Fig. 13 shows three sections of one of the sensilla examined 10 h after the colchicine-DOC treatment. The response did not recover to water
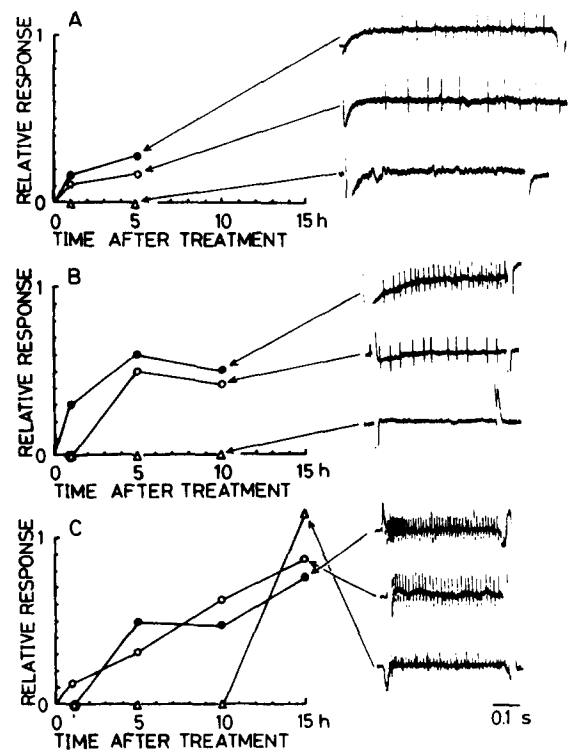

FIGURE 12.   Records of responses just before fixation and recovery time courses up to fixation at 5 (*A*), 10 (*B*), and 15 (*C*) h after the colchicine-DOC treatment. Cross-sections of these sensilla are shown in Figs. 11–14, respectively. O, salt; ●, sugar; △, water.

stimulation but had partially recovered to sugar and salt stimuli just before fixation, as shown in Fig. 12 *B*. Fig. 13 *A* shows the empty inner lumen at 1.5 μm from the tip. Fig. 13, *B* and *C*, shows the sections at 2.5 and 20 μm from the tip, respectively. These pictures are similar to those of the sensillum fixed 5 h after the colchicine-DOC treatment, but the empty space in the inner lumen was confined to more distal levels. We therefore conclude that the distal processes, once destroyed at the sensillum's tip, could regenerate, but did not reach the sensillar tip even 10 h after the colchicine-DOC treatment.
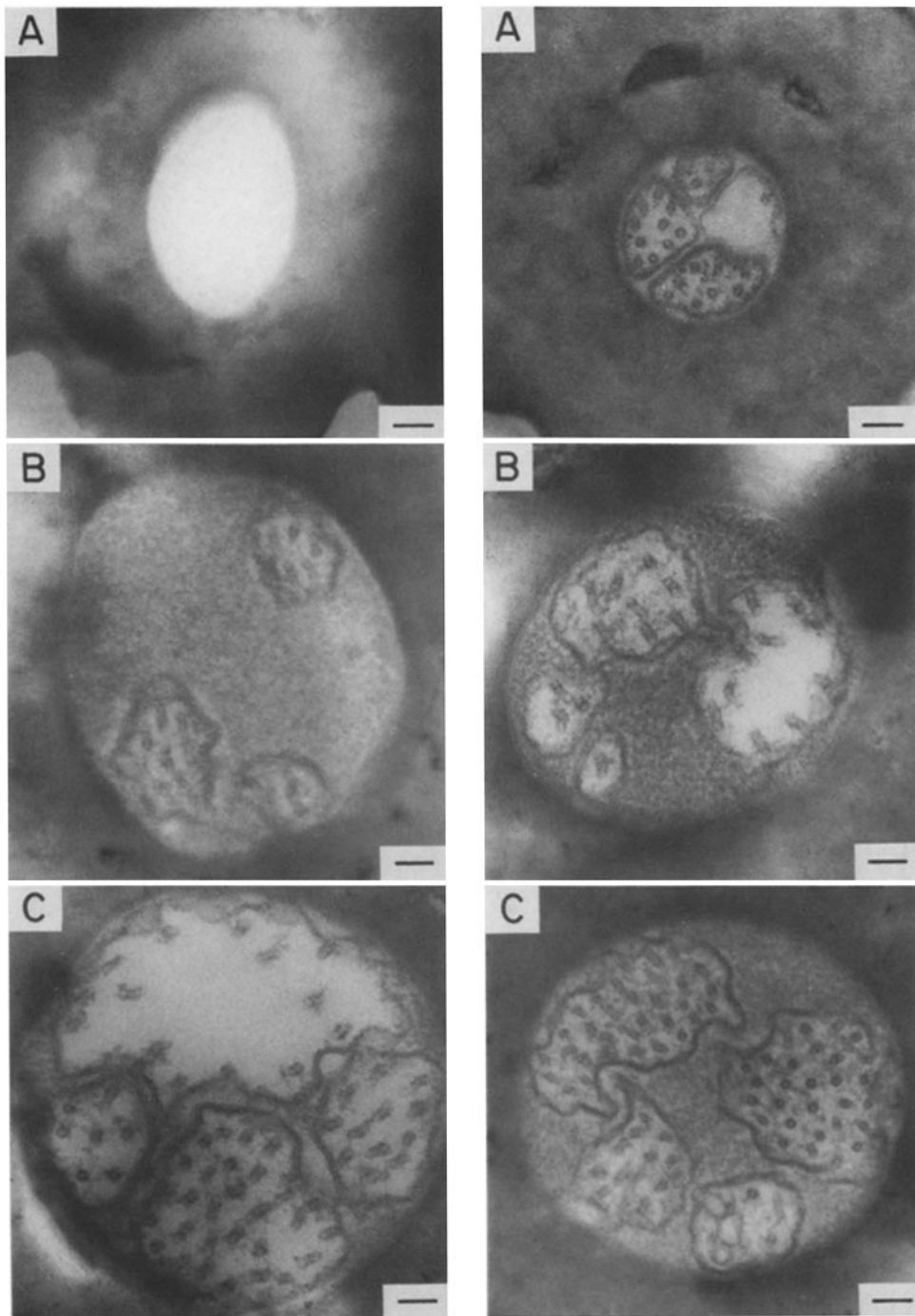
FIGURE 13. (*left*) Cross-sections of a sensillum fixed at 10 h after the colchicine-DOC treatment, at 1.5 (*A*), 2.5 (*B*), and 20 (*C*) μm from the tip.

FIGURE 14. (*right*) Cross-sections of a sensillum fixed at 15 h after the colchicine-DOC treatment, at 1.1 (*A*), 3.4 (*B*), and 21 (*C*) μm from the tip.

However, 15 h after the colchicine-DOC treatment, regeneration of the distal processes with microtubules had been completed and the endings were seen at the level just beneath the tip opening (Fig. 14A), as in the intact sensilla. The levels of the three cross-sections in Fig. 14, A–C, were at 1.1, 3.4, and 21 μm from the tip, respectively. The responses had also recovered almost completely, in both their phasic and tonic components, as shown in Fig. 12C.

DISCUSSION

High Sensitivity to Colchicine: Involvement of Tubulin

The long-lasting effect of 2 min treatment with 25 mM colchicine suggested an extremely high sensitivity of the receptor cell to colchicine. Using a diffusion equation, we can estimate the upper limit of the free colchicine concentration at which the receptor cell began to recover an activity previously blocked by colchicine.

Let us imagine a tube of infinite length on one side filled with a solution, the solute concentration of which is c_0 everywhere in the tube for t (time) < 0. At $t = 0$, the end of the tube ($x = 0$; i.e., x is a distance from the end) is opened to an infinitely large cavity of the solvent, so that the concentration is zero at $x = 0$ all the time for $t < 0$. The concentration c at x is expressed as $c = c_0 erf(x/2\sqrt{Dt})$ for $t > 0$, where erf is the error function, and D is the diffusion coefficient of the solute.

In our situation, the sensillum shaft of a finite length (x_0) opens to a large cavity at the base ($x = 0$), where the concentration of colchicine can be regarded to be practically zero, as in the above case. However, even for $t < 0$ ($t = 0$ at the end of the treatment), $c = 0$ at $x = 0$, $c < c_0$ for $x < x_0$, and $c = c_0$ only at $x = x_0$ ($c_0 = 25$ mM; $x = x_0$ at the sensillum tip). Moreover, there is no supply of colchicine from the outside of the tip after the treatment ($t > 0$). Therefore, the rate of diffusion out from the sensillum shaft (to the basal cavity) should be directly related to the concentration of colchicine remaining after the treatment. Thus, the half-decay time of colchicine at the sensillum tip should be shorter than t^*, which satisfies the equation $c_0/2 = c_0 erf(x_0/2\sqrt{Dt^*})$, and the colchicine concentration should never exceed $2^{-n}c_0$, where $n = t/t^*$.

For the value of t, we have to estimate the effective period of colchicine after the treatment. If the second step of recovery of the response signaled the end of the colchicine effect, the effective period would be 8–16 h (Fig. 4). However, the beginning of recovery of the response latency preceded the second step of recovery by 4–5 h (Fig. 5). Therefore, the direct effects of colchicine can be safely assumed to last for at least 3 h after treatment. Now, adopting 3 h for t, 400 μm for x_0, and 36×10^{-11} m^2 s^{-1} for D (International Critical Table, 1929, for raffinose of 504 mol wt in aqueous solution at 20°C; cf. 400 mol wt for colchicine), we obtain $25 \times 2^{-22.1}$ mM, i.e., 5.6×10^{-9} M, as the upper limit of the free colchicine concentration at the end of its effective period.

Thus, the receptor cell can begin to recover only after the free colchicine concentration is reduced to $<5.6 \times 10^{-9}$ M. This figure is smaller than any of the concentrations at which colchicine is known to be effective on any biological

activities. This can be explained only by the extraordinarily long half-time for the dissociation of the colchicine-tubulin dimer complex (Margolis and Wilson, 1977; Osborn and Weber, 1976). When treated with colchicine and DOC, microtubules near the tip may be disassembled into tubulin dimers, which bind to colchicine. The resultant colchicine-tubulin dimer complex may cap the growing end of a microtubule to prevent its reassembly even at extremely low concentrations of free colchicine, as postulated by Margolis and Wilson (1977).

The present electron micrographs strongly suggest that the recovery of the latency (Fig. 5) results from regeneration of the tip region of the distal process of the receptor cell destroyed by the DOC treatment. They also show that the regenerated distal process always contained microtubules. This regeneration, therefore, is considered to result from a reassembly of microtubules, as in axonal growth (Peters and Vaughn, 1967).

Supply of Receptor Molecules

Another important function of microtubules is suggested by the result that the response was reduced at ≥10 h after colchicine treatment (Fig. 3). As discussed above, such a long-lasting effect of colchicine can be explained only by assuming an involvement of microtubules in the maintenance of responsiveness. However, the response was not reduced at all for several hours after the same treatment (Fig. 2). This clearly shows that microtubules are not parts of the sensory transduction machinery (cf. Matsumoto and Farley, 1978). The simplest picture may therefore be that receptor molecules in the membrane are continually renewed through the microtubule system. The size of the pool of the molecules should be large enough to maintain the responsiveness for several hours during blockade of the renewal process, but should be small enough to reduce the response if the blockade lasts as long as 10 h. This implies that the turnover rate of the receptor molecule is fairly high; i.e., the half-life is in the range of ~10 h. This estimation is based on the simplest of the schemes proposed by Morita (1969), and the necessary constants are given elsewhere (Ninomiya et al., 1986).

The first step of recovery in sucrose response was completed within 1 h after the colchicine-DOC treatment (Figs. 1, 2, and 5). This process is considered to consist of membrane sealing and desorption of DOC from the receptor membrane. The tip of the distal process newly formed by the sealing of the membrane was located at a level several micrometers below the sensillum tip (Fig. 11). We suggest that, under the influence of colchicine, the receptor molecules cannot be supplied to the membrane. It follows that the membrane at a level several micrometers below the sensillum tip may originally contain the receptor molecules. During the second recovery, the newly supplied receptor molecules for sucrose and glucose, but not for fructose, became available (Fig. 1), whereas those for fructose became available before the second recovery (Fig. 2A). This is understandable, since pyranose (sucrose and glucose) and furanose (fructose) sites are different entities (Shimada et al., 1974).

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