brief communication

Single unit recording from olfactory cilia

Stephan Frings and Bernd Lindemann
Department of Physiology, Universität des Saarlandes, D-6650 Homburg/Saar, Federal Republic of Germany

ABSTRACT Sensory cilia from olfactory receptor cells can be pulled into a patch pipette located above the mucus layer of an olfactory mucosa. While the pipette does not form a tight electrical seal with the ciliary membrane, it nevertheless allows to record current transients driven by action potentials arising in the olfactory neuron. This method is an alternative to single-unit-recording with electrodes pushed into the mucosa and, in some respects, to patch clamp recordings from isolated olfactory cells. Its advantage is technical simplicity and minimal disturbance of the neuron from which signals are derived. Less than 5% of the chemosensitive apical surface of the neuron is covered by the pipette. The neuron remains in situ and its cilia remain covered with some mucus. (However, mucus is in part dissolved by the bathing solution). Odorant thresholds in the picomolar range were thus obtained.

INTRODUCTION

To study vertebrate olfactory receptor neurons by patch clamp methods, the cells are often dissociated from the mucosa and kept in isolation during stimulation with chemicals dissolved in the bathing solution (1, 4, 6, 7, 11–13, 16, 18, 20, 21). Whereas responses to second messengers and agents like forskolin and xanthines can be obtained from such preparations, disappointingly large concentrations of odorants (in excess of 500 nM cineole) are required to stimulate isolated olfactory cells. We therefore attempted to estimate odorant thresholds from cells remaining in situ, by recording currents from the sensory cilia contained in the mucus layer of the olfactory mucosa. We found that a loose-patch situation can be achieved without difficulty and that trains of capacitive current pulses can regularly be recorded. These current pulses are driven by action potentials which change frequency in response to low concentrations of odorants.

METHODS

Frogs (*R. esculenta/ridibunda*) were killed by decapitation. The skin covering the nose was removed and the two dorsal olfactory mucosae were excised by cutting out the triangular plate of bone which supports the epithelium on each side. After transfer to Ringers, the mucosae were carefully cut from the bone plates and used immediately or stored at 4°C. The recording chamber was a standard glass slide for microscopy, to which a flat silicon ring was glued. The chamber volume was near 500 μl. Two needles (200 μm thick) held the tissue, which was folded around one of them so that the mucosal surface was accessible (Fig. 1 *A*). A capillary (i_2) was positioned near the mucosal edge, directing its outflow toward the point of recording. By gravity, it constantly delivered a stream of Ringers solution, which could be switched to an odorant-containing solution by electrically actuated valves. The delay due to dead space was 8–15 s, depending on flow rate. The interstitial surface of the tissue was constantly washed with oxygenated Ringers through a separate inlet capillary (i_3). Thus chemical agents applied to the mucosal surface could not reach the interstitial side (see Results). A third inlet (i_1) served to flush the chamber and thus accelerate washout of stimuli. Fluid was removed by overflow into a suction tube (o). Patch pipettes were pulled from borosilicate glass, and fire-polished to have resistances of 25–35 MOhm when filled with Ringers. They were filled with Ringers and positioned with a hydraulic 3-D micromanipulator. An inverted microscope with a 40× objective was used. For recording of electrical signals, a patch clamp amplifier was set to voltage clamp mode (input stage feedback resistor 1 GOhm), a pipette potential of 0 mV, and a gain of 100–200 mV/pA. For display, signals were low-pass filtered at 300 Hz.

RESULTS AND DISCUSSION

The folded edge of the mounted tissue offered a clear view of the mucociliary layer. Short and long cilia could be distinguished. The short cilia (20–50 μm long [9, 15]) were seen to beat spontaneously in a nonsynchronized way, while the long cilia (200–300 μm [9, 15]) did not show spontaneous movement, but bent in the tangential stream of mucosal perfusion, which removed part of the mucus. When perfusion was stopped, the long cilia assumed a more straight position, expanding the width of the mucociliary layer. The recording pipette was then positioned near the free ending of a long cilium, and gentle suction was applied under visual control to pull the free ending into the orifice of the pipette. When the pipette was slowly advanced, the distal part of the cilium seemed to progressively enter the orifice (Fig. 1 *B*), while fast biphasic current transients, familiar from extracellular recordings of action potentials (e.g., 5, 10), appeared on the current record (Fig. 2 *B*). The current transients

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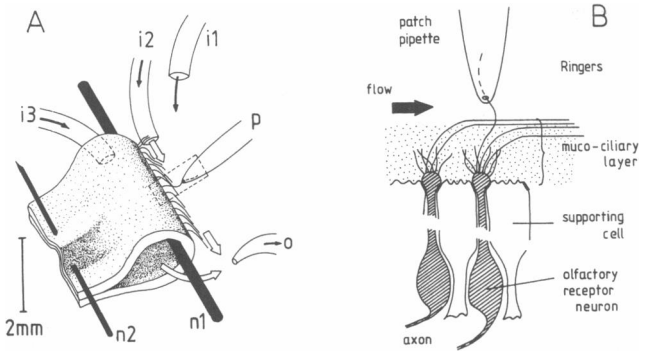

FIGURE 1 (*A*) Arrangement of isolated olfactory mucosa, with ciliated mucosal side facing outward, and flow system under the microscope. (*n1*, *n2*) Needles. (*i1*, *i2*, *i3*) Inlet capillaries. (*o*) Outlet suction capillary. (*p*) Patch pipette. (*B*) Blow-up of boxed-in area of *A*, with schematical cross-section through mucosa. The mucociliary layer of the frog has a width of 50–100 μm when the mucosa is covered with air. In our experiments the width depended on the rate of flow of the tangential stream of solution.

became larger as more of the cilium was pulled in. Pipette resistance did not increase significiantly. To obtain a favorable signal/noise ratio it was usually sufficient to pull in half the length of a cilium. Given 20 long cilia per neuron, this means that at least 95% of the total chemosensory area of the neuron remained accessible to odorants.

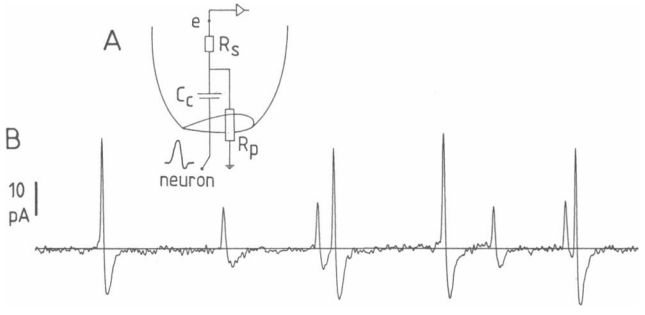

FIGURE 2 (*A*) Equivalent network of loose-patch ciliary recording. (C_c) Membrane capacitance (300–600 fF) of part of a sensory cilium which was pulled into the patch pipette. (R_p) Resistance of orifice of patch pipette (near 30 MOhm). (R_s) Resistance (near 1 MOhm) between electrode wire (*e*) and cilium, measured after breaking a patch pipette at a point 100 μm from the tip. *e* was kept at virtual ground. With R_p/R_s in the order of 30, the capacitive current transients were recorded with only 3–4% attentuation. (*B*) Recording of biphasic current transients from olfactory cilia. The pipette was held at 0 mV, the record low-pass filtered at 500 Hz, record length is 0.4 s. Two peak amplitudes of the action potential–driven capacitive currents were observed, indicating that cilia from two different neurons had been pulled into the pipette. The earlier, upward-directed halfwaves represent capacitive current directed out of the cilia. Integration of the current transients on the computer yielded waveforms which closely resembled the action potentials recorded previously from isolated olfactory cells under whole cell conditions (6). However, small contributions of non-capacitive current are not excluded.

The final position of the pipette orifice was 50–100 μm from the apical surface. When perfusion was resumed the long cilia were seen to bend again and, at the flow-rate used, the width of the mucociliary layer decreased to ~50 μm. With slight negative pressure maintained in the pipette, the recording of current transients continued during flow of solution. After some practice, it took <1 min to pull a cilium into the pipette. The success rate was ~60%. After an unsuccessful trial, it was necessary to take a fresh pipette. With pipettes of 25–30 MOhm resistance, transients of two or more amplitudes were often observed, indicating that cilia from more than one receptor neuron contributed to the signal (Fig. 2 *B*, Fig. 3 *A*). Thinner pipette openings (30–35 MOhm) allowed recording from "single units," as evidenced by current transients of uniform size (Fig. 3 *B*). Trials with pipettes of 35–40 MOhm resistance were unsuccessful, presumably because the rate of mucus flow through the orifice became too small to capture a cilium.

The origin of the current transients was localized by adding 1 μM of tetrodotoxin (TTX) to the mucosal

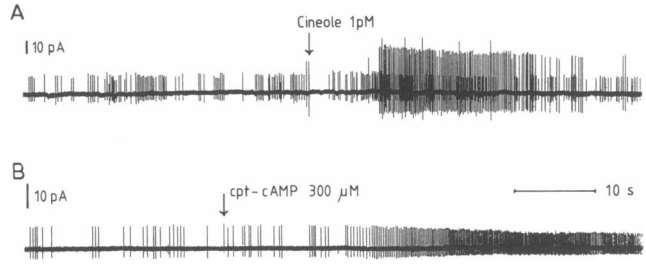

FIGURE 3 Chemo-responses of in situ olfactory neurons during ciliary recording. (*A*) Response to 1 pM cineole, added at arrow. The odorant reached the tissue with a delay of 8.5 s. Total length of record 77 s. Note that recording was from two neurons, which responded differently. The one with the smaller capacitive currents had a high background activity and a tendency to fire bursts. Both neurons showed desensitization. We are now using a computer program to separate responses from two neurons. (*B*) Response to 300 μM cpt-cAMP, added at arrow to the mucosal solution. Capacitive currents were of uniform size, indicating that recording was probably from one neuron only. Compared with *A*, the onset of the response to cAMP was more delayed and more gradual, most likely because the agent had to enter the cell before it became effective. Desensitization was weak, as was found in all responses to cAMP. Interestingly, in the recordings shown above there are no significant shifts in the dc level, which would be indicative of odorant-induced depolarizing currents gated by cAMP (e.g., 4, 11, 13, 14, 18, 21). The reason is probably that the ciliary recording segment was sufficiently well shielded from the bath, both chemically and electrically. Thus receptor currents may be expected from the ciliary segment *in the pipette*, when it is exposed to odorants or cAMP contained in the pipette solution. (In similar recordings from photoreceptors [3] and insect antennae [10], low-frequency receptor currents are well documented.)

solution, where it had no effect. However, when TTX was added to the interstitial washing solution, the generation of current transients stopped. This experiment shows (*a*) that the constant flow of interstitial washing solution prevented mucosal agents from reaching this compartment and (*b*) that spike generation occurred not at the ciliary membrane, but at a membrane facing the interstitial space, presumably somal membrane and/or axon hilloc. Indeed, previous binding studies failed to demonstrate the presence of voltage-dependent Na channels in the ciliary membranes (2).

It may be presumed, therefore, that the current transients are electrotonically conducted into the cilia, and are recorded by capacitive coupling through the ciliary membrane (circuit in Fig. 2 *A*). Note that, unlike in classical loose patch-clamping (19), the membrane patch from which capacitive current is recorded is positioned not before, but *in* the pipette, i.e., it has passed the resistance (R_p) of the pipette orifice. A similar geometry was previously used with photoreceptors (3). A cylindrical cilium of 0.1 μm radius, pulled into the pipette for a length of 50–100 μm, couples to the pipette interior with a capacitance (C_c) of 300–600 fF. Action potentials of frog olfactory cells (6) may be roughly modeled with a sine-wave of 100 Hz. At 100 Hz the 300–600 fF represent an impedance of 2.5–5 GOhm, which, for a cellular voltage excursion of 100 mV will allow a current flow of 20–40 pA. The current transients recorded (Fig. 2 *B*) were of this order of magnitude. The shunt resistance (R_p) of 30 MOhm between pipette and bath will cause little loss of current, because the series resistance (R_s) from cilium to the electrode wire (e) in the pipette is much smaller.

To investigate the response to odorants, cineole was added to the mucosal perfusate. Cineole is a strong excitatory stimulus for frog olfactory neurons (17). Of 78 cells tested by ciliary recording, 22 responded with an increase in the rate of firing. The responses were often phasic-tonic (Fig. 3 *A*). Of the 22 cells, 9 responded to 1 pM, 5 to 10 pM, 3 to 100 pM. Other concentrations were not tested in these cases. Eight cells, of the 56 which did not respond to picomolar concentrations, would not respond to higher concentrations either. The effective concentration of 1 pM is $5*10^5$-fold lower than the lowest effective concentration published for isolated olfactory cells (see Table 3 in reference 6), and >1,000-fold lower than the lowest effective concentration found in single-unit recordings by means of electrodes pushed into the mucosa (8). However, similarly low thresholds to odorants were previously found in on-cell patch clamp recordings from ciliary knobs of receptor cells in situ (7).

The response to cyclic adenosine monophosphate (cAMP), which is considered to be a second messenger in the reception mechanism (13, 14), is shown in Fig. 3 *B*. A concentration of 300 μM of chloro-phenyl-thio cAMP (cpt-cAMP), applied to the ciliated side of the tissue, increased the firing rate to five times the basal level. The effect was reversible. On washout of cpt-cAMP, the spike rate transiently dropped below the basal rate. Of 28 cells tested, all responded with an increase in spike rate. Where investigated, the response was dose-dependent. Similar responses were obtained with 0.3 μM forskolin and with 100 μM isobutyl methylxanthine. The results show that recording from olfactory cilia is suitable to study the signal chain of sensory transduction in these neurons.

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