METHODS

1 Animal and cell preparation

Newts (*Cynops pyrrhogaster*) were purchased from a commercial supplier, housed in aquaria in room temperature (20 to 25°C), and fed a commercially available dried tubifexes. Receptor neurons were dissociated enzymatically from the olfactory epithelium of the newt (Figure 5). The animals were anaesthetized by cooling on ice, decapitated, and pithed. Nasal cavities were opened and the olfactory mucosae were excised under a dissection microscope. The mucosae were incubated in a Ca^{2+} and Mg^{2+}-free solution (110 mM NaCl, 2.5 mM KCl, 5 mM HEPES, 5 mM glucose; pH 7.6) containing 0.2% collagenase (Wako Pure Chemical Industries, Osaka, Japan) for 10 min at 35 °C. The tissue was then rinsed with Ringer's solution (110 mM NaCl, 2.5 mM KCl, 1.0 mM CaCl, 1.6 mM MgCl, 5 mM HEPES, 5 mM glucose pH7.6) and kept at 4 °C for 1 hr. This step appeared to facilitate obtaining dissociated cells with intact cilia. The tissue was then teased with forceps and gently triturated in a fire-polished Pasteur pipette. The cell suspension was stored at 4 °C until use (up to 10 h later). This procedure was modified from Kurahashi (1989).

2 Whole cell patch clamp

Recording pipettes with a highly tapered shank were pulled from borosilicate glass capillaries of 1.2 mm outer diameter and 0.6 mm inner diameter (A-M Systems, Carlsborg, WA, USA) using a pipette puller (PP-83; Narishige, Tokyo, Japan). The pipette tip was trimmed with a microforge (Figure 6). Only pipettes that had smooth tips with inner diameter of 3 to 7 μm were used in experiments (Figure 7). The pipette resistance was 2 to 5 MΩ when filled with Ringer's solution. Before use, the pipette tip was dipped into a mixture of molten Parafilm (American National Can, Greenwich, CT,

USA), light mineral oil, and heavy mineral oil so that a high seal resistance (1 to10 GΩ) could be obtained. This step was modified from that used by Collins et al. (1992), who reported that high-resistance seal formation with large diameter pipettes is facilitated by hydrocarbon coating. Pipettes of large tip diameter (5-6 μm) have decreased access resistance and enhanced mechanical stability during recording.

Approximately 500 μl of suspension containing dissociated cells were transferred to a recording chamber. Experiments were performed under a phase contrast inverted microscope (IMT-2; Olympus, Tokyo, Japan). Solitary olfactory receptor neurons could easily be identified by their unique morphology (Kurahashi, 1989). Receptor neurons with intact cilia were chosen for experiments. Electrical recordings from olfactory receptor neurons were made with a patch clamp amplifier (EPC7; List, Darmstadt, Germany) in whole cell recording configuration (Hamill et al., 1981) (Figure 8). The data were sampled at 24 kHz by a 12-bit analog-to-digital converter (Digidata 1200; Axon Instruments, Foster City, CA, USA) linked to a PC, and stored in a DAT data recorder (RD-120TE; TEAC, Tokyo, Japan). Data analysis was performed on a PC using the 'pCLAMP 6.0' software (Axon Instruments) and 'Origin 4.0' software (Microcal Software, Northampton, MA, USA). Voltage ramps and voltage steps in the voltage-clamp mode were controlled with pCLAMP. Membrane potentials were corrected for the ~ -5 mV junction potential between internal and external solutions. All recordings were performed at room temperature (20 to 25°C). Data are shown by means ± standard errors.

3 Perfusion system

The perfusion system was modified from the design of Hodgkin et al.

(1984) (Figure 8). External solutions were fed by gravity into a four-way valve that was operated pneumatically by remote control from an electronic pulse generator or PC. The solutions then flowed through an application pipette into the recording chamber. After a patch pipette sealed to a cell, the cell was moved near the outlet of the application pipette. In this way, a complete solution change around the recorded cell could be achieved under 200 ms as judged from junction currents (Figure 9)(see Hodgkin et al., 1984). The timings of stimulation were estimated from the junction currents and indicated by a horizontal bar in the following figures.

Intracellular perfusion was carried out with a commercial pipette perfusion system (2PK+; Adams & List Associates, Westbury, NY, USA) with modification (see the upper part of Figure 8). In order to stabilize the cell, slight positive and negative pressures were applied until zero net pressure across the pipette tip was achieved by successive approximation.

4 EOG recordings

The newts were pithed and decapitated; the roof of a nasal cavity was removed, exposing olfactory epithelium. The EOG (Ottoson, 1956) was recorded with a glass pipette electrode contacted on the olfactory epithelium (Figure 10). The electrode was filled with normal saline, and connected through an Ag/AgCl electrode to a conventional amplifier. Olfactory stimulation was accomplished by an application of purified moist nitrogen gas that was in equilibrium with an odorant-water solution of known concentration by passing a nitrogen gas stream over evaporation tubes containing the appropriate odorant solution. The odorant concentrations are expressed as the concentrations of odorant solutions in the evaporation tubes. The flow rate was 10 ml/sec through 0.5 mm internal diameter Teflon tubing,

the end of which was located at ~10 mm from the preparation). Each odorant was presented for 2 s with about a 90 s interval between different stimuli. No difference in response amplitudes was observed when this interval was increased (data not shown). One record was obtained from one animal within 3 hours from the decapitation.

5 Solutions

Standard Ringer's solution contained 110 mM NaCl, 2.5 mM KCl, 1.0 mM $CaCl_2$, 1.6 mM $MgCl_2$, and 5 mM HEPES (pH 7.6). The standard pseudo-intracellular solution contained 100 mM KCl, 2 mM $MgCl_2$, 1.263 mM $CaCl_2$, 5 mM EGTA, 10 mM HEPES, and 0.05 mM Na_2-GTP (pH 7.6). The free-Ca^{2+} concentration in this solution was 10 nM, calculated with the 'BAD4' software (Brooks and Storey, 1992). In all whole-cell experiments except for that in Figure 13, I added 1 mM ATP to the standard pseudo-intracellular solution. All odorants (cineole, anisole, isoamyl acetate, (+) limonene, and isovaleric acid) were diluted in the standard Ringer's solution in whole cell recordings. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless noted otherwise.