Nonselective Suppression of Voltage-gated Currents by Odorants in the Newt Olfactory Receptor Cells

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abstract Effects of odorants on voltage-gated ionic channels were investigated in isolated newt olfactory receptor cells by using the whole cell version of the patch–clamp technique. Under voltage clamp, membrane depolarization to voltages between −90 mV and +40 mV from a holding potential (V_h) of −100 mV generated time- and voltage-dependent current responses; a rapidly (< 15 ms) decaying initial inward current and a late outward current. When odorants (1 mM amyl acetate, 1 mM acetophenone, and 1 mM limonene) were applied to the recorded cell, the voltage-gated currents were significantly reduced. The dose-suppression relations of amyl acetate for individual current components (Na^+ current: I_{Na}, T-type Ca^{2+} current: $I_{Ca,T}$, L-type Ca^{2+} current: $I_{Ca,L}$, delayed rectifier K^+ current: I_{Kv} and Ca^{2+}-activated K^+ current: $I_{K(Ca)}$) could be fitted by the Hill equation. Half-blocking concentrations for each current were 0.11 mM (I_{Na}), 0.15 mM ($I_{Ca,T}$), 0.14 mM ($I_{Ca,L}$), 1.7 mM (I_{Kv}), and 0.17 mM ($I_{K(Ca)}$), and Hill coefficient was 1.4 (I_{Na}), 1.0 ($I_{Ca,T}$), 1.1 ($I_{Ca,L}$), 1.0 (I_{Kv}), and 1.1 ($I_{K(Ca)}$), suggesting that the inward current is affected more strongly than the outward current. The activation curve of I_{Na} was not changed significantly by amyl acetate, while the inactivation curve was shifted to negative voltages; half-activation voltages were −53 mV at control, −66 mV at 0.01 mM, and −84 mV at 0.1 mM. These phenomena are similar to the suppressive effects of local anesthetics (lidocaine and benzocaine) on I_{Na} in various preparations, suggesting that both types of suppression are caused by the same mechanism. The nonselective blockage of ionic channels observed here is consistent with the previous notion that the suppression of the transduction current by odorants is due to the direst blockage of transduction channels.

key words: olfactory receptor cell • suppression • odorant • action potential • newt

INTRODUCTION

Odorant binding to receptor proteins at the ciliary surface of olfactory receptor cells activates enzymatic cascades (for review see Bakalyar and Read, 1991; Breer and Boekhoff, 1992; Ronnett and Snyder, 1992) causing the opening of two types of ionic channels; cAMP-gated cationic channels and Ca^{2+}-gated Cl^- channels (for review see Gold and Nakamura, 1987; Firestein, 1992; Kurahashi and Yau, 1994; Reed, 1992; Restrepo et al., 1996). This initial excitation causes a slow and graded voltage change; its amplitude is dependent on stimulus concentration (Trotier and MacLeod, 1983; Kurahashi, 1989a; Firestein et al., 1993). A graded receptor potential is then encoded into spike trains that transmit olfactory information to the brain.

Recently, Kurahashi et al. (1994) have shown that the transduction current induced by odorant stimuli is suppressed by the odorants themselves. Although the mechanism of this suppression was not revealed by their experiments, they speculated, based on the rapid kinetics, that suppression would be due to a direct effect of odorants on ionic channels rather than to an effect on olfactory receptor proteins or on second messenger metabolism.

It has been reported that newt olfactory receptor cells express various kinds of ionic currents: a Na^+ current (I_{Na}), a T-type Ca^{2+} current ($I_{Ca,T}$), an L-type Ca^{2+}current ($I_{Ca,L}$), a delayed rectifier K^+ current (I_{Kv}) and a Ca^{2+}-activated K^+ current ($I_{K(Ca)}$) (Kurahashi, 1989*a*; Kawai et al., 1996). Similar currents have been observed in olfactory receptor cells from several species (catfish: Miyamoto et al., 1992; coho salmon: Nevitt and Moody, 1992; xenopus: Schild, 1989; tiger salamander: Firestein and Werblin, 1987; Dubin and Dionne, 1994; mouse: Maue and Dionne, 1987). In this study, we report that voltage-gated currents in newt olfactory receptor cells were suppressed nonselectively by odorants such as amyl acetate, acetophenone, and limonene.

MATERIALS AND METHODS

Preparation

Receptor cells were dissociated enzymatically from the olfactory epithelium of the newt, *Cynops pyrrhogaster*. Dissociation protocols were similar to those reported previously (Kurahashi, 1989*b*). In short, the animal was anaesthetized by cooling on ice, decapitated, and then pithed. The mucosae excised from the olfactory cavity were incubated for 5 min at 30°C in a Ringer solution containing 0.1% collagenase (Sigma Chem. Co., St Louis, MO) with no added Ca^{2+}. The tissue was then rinsed twice and triturated with a normal Ringer solution (in mM): 110 Na^+, 3.7 K^+, 3 Ca^{2+},

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2 HEPES, 15 glucose, 10 ppm phenol red (pH adjusted to 7.4 with NaOH). Isolated cells were plated on the concanavalin A-coated glass coverslip. Cells were maintained at 4°C (up to 10 h) before use. In the present experiment, we selected receptor cells which had lost their cilia to study the ionic currents of the somatic membrane.

Recording Procedures

Membrane currents were recorded in the whole cell-recording configuration (Hamill et al., 1981). Pyrex tubing (1.2 mm o.d.) was pulled in two steps on a pipette puller (Narishige Scientific Instruments, Tokyo, PP-83). To minimize stray capacitance, the external wall of the pipette was coated with an insulating resin (Apiezon wax; Apiezon Products Ltd, London) up to ~100 μm from the tip. Residual capacitance was compensated electrically. The recording pipette was filled with pseudo-intracellular (K^+) solution (in mM): 119 KCl, 1 $CaCl_2$, 5 EGTA, 10 HEPES (pH adjusted to 7.4 with KOH), or Cs^+ solution: 119 CsCl, 1 $CaCl_2$, 5 EGTA, 10 HEPES (pH adjusted to 7.4 with CsOH). The resistance of the pipette was ~20 MΩ.

For recording, the culture dish was mounted on the stage of an inverted microscope with phase contrast optics (Diaphot TMD-2; Nikon, Tokyo). A stainless steel ring was put into the dish to reduce the dead space of the recording chamber to ~0.15 ml. The indifferent electrode was an Ag-AgCl wire connected to the culture dish via an agarose bridge. A patch–clamp amplifier (Axopatch-1D; Axon Instruments Inc., Burlingame, CA) linked to an IBM-compatible PC, was used to measure membrane current and voltage. The voltage- and current-clamp procedures were controlled by using the software pCLAMP (Axon Instruments Inc.). Current and voltage signals from the amplifier were monitored on an oscilloscope (Tektronix, Beaverton, OR) and a thermal array recorder (Graphtec, Fujisawa, Japan, WR7900). Data were low-pass filtered (4-pole Bessel type) with a cut-off frequency of 5 kHz and then digitized at 10 kHz by an analog-to-digital interface (Lab Master DMA; Scientific Solutions Inc., Solon, OH). All experiments were performed at room temperature (23–25°C).

Application of Drugs and Odorants

Several pharmacological agents were used for isolating each component of ionic currents: choline chloride to substitute for NaCl, CsCl to block K^+ current, $CoCl_2$, $NiCl_2$ and $CdCl_2$ to block Ca^{2+} current. These agents were dissolved in the superfusate, and were applied to the cell from the U-tube system (at a rate of 0.9 ml/min). Odorants (amyl acetate, acetophenone or limonene) were dissolved in solutions also containing 5 mM DMSO, dimethylsulfoxide, and were applied to the cell from the U-tube system by pressure ejection (0.5 kgw/cm^2) or by internal perfusion of the recording pipette. The stream of superfusate was directed at the cell by placing the tip of the U-tube outlet ~1 mm from the cell. The puffer pipettes (tip diameter ~5 μm) were placed ~30 μm from the cell. The time resolution of the onset and offset of the pressure ejection system was estimated by using puff application of 3 M KCl solution; the recording pipette was placed in a steady stream of normal Ringer's solution from the nozzle of the U-tube, and the time course of the junction current change induced by pressure ejection of 3 M KCl solution from the puffer pipette placed ~30 μm from the recording pipette was measured. The onset and offset of the junction current reached a steady level within 20 ms, indicating that the time resolution of the pressure ejection system was ~20 ms. A special pipette holder was designed to enable exchange of the pipette solution while recording whole cell currents. The pipette, filled with the standard solution at the beginning of recording, was connected to a 1-ml syringe filled with the solution containing an odorant by a thin polyethylene tube. Final outer diameter of the polyethylene tube was ~0.2 mm. To exchange the solution of the recording pipette, positive pressure was applied to the syringe. Choline chloride, $CoCl_2$, $NiCl_2$, and $CdCl_2$ were purchased from Nacalai Tesque Inc. (Kyoto, Japan), amyl acetate, acetophenone, and limonene from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

Data Analysis

The dose-suppression curves of odorants (inhibition curves) were described by the Hill equation

$$R/R_0 = (1 - C^n/(C^n + IC_{50}{}^n)), \quad (1)$$

where R_0 is the peak amplitude of membrane current without odorant, R is that in the presence of odorant, C is the concentration of the odorant, n is Hill coefficient, and IC_{50} is the concentration of the odorant at which the membrane current becomes half of the maximum. The experimental data were least square-fitted to the Hill equation by a commercial software program, *KaleidaGraph* (Synergy Software, PCS Inc., Reading, PA).

RESULTS

Suppression of Total Currents by Odorants

Under voltage clamp (holding potential, $V_h = -100$ mV), depolarizing step pulses induced time- and voltage-dependent currents (Fig. 1 *A*). At step voltages between −90 mV and +40 mV, current responses consisted of a transient (< 15 ms) inward current and a delayed outward current. The initial transient inward current was completely blocked by bath application of 1 mM amyl acetate and the late sustained outward current was reduced (Fig. 1 *B*). Similar phenomena were observed in the all cells recorded (N = 17). Current reduction was also observed by the intrapipette perfusion of 1 mM amyl acetate (N = 3, Fig. 1 *D*) and by bath application of 1 mM acetophenone or 1 mM limonene (Fig. 2).

Dose-suppression Relation of Amyl Acetate on the Na^+ Current (I_{Na})

To determine the effective concentration of odorants on each ionic current, we isolated ionic currents by pharmacological agents, and the recorded cells were exposed to several concentrations of amyl acetate. In the somatic membrane of the newt olfactory receptor cell, five ionic currents have been identified; a Na^+ current (I_{Na}), a T-type Ca^{2+} current ($I_{Ca,T}$), an L-type Ca^{2+}current ($I_{Ca,L}$), a delayed rectifier K^+ current (I_{Kv}) and a Ca^{2+}-activated K^+ current ($I_{K(Ca)}$) (Kurahashi, 1989*a*; Kawai et al., 1996). Among these ionic currents, it has been reported that transient inward currents such as I_{Na} are essential to generating action potentials in olfactory receptor cells (catfish: Miyamoto et al., 1992; xenopus: Schild, 1989; tiger salamander: Firestein and Werblin, 1987; Dubin and Dionne, 1994; cultured rat cells: Trombley and Westbrook, 1991; dissociated adult rat cells: Rajendra et al., 1992). Therefore, we first studied the effects of amyl acetate on I_{Na}. In the experiment of

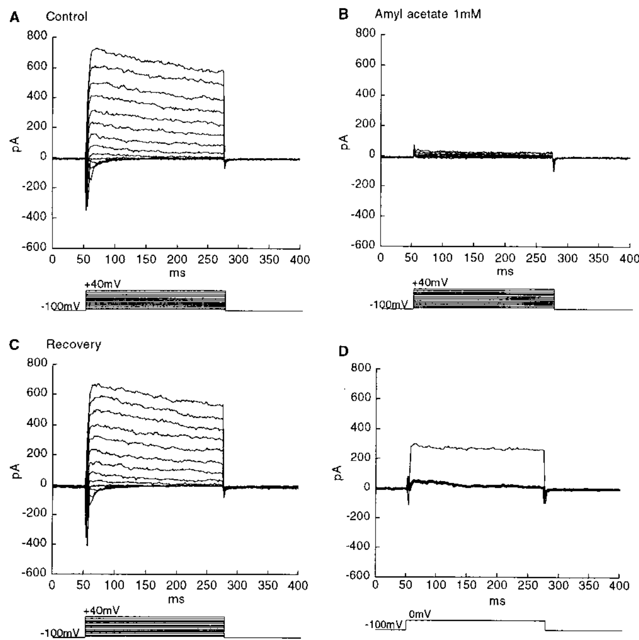

FIGURE 1. Suppression by amyl acetate of membrane currents of an isolated newt olfactory receptor cell. (*A*) membrane currents were induced by depolarization from V_h of -100 mV. Command voltages were increased in 10 mV step from -90 mV to $+40$ mV. The cell was bathed in the normal Ringer solution and the recording pipette was filled with K^+ solution. (*B*) membrane currents induced by the same depolarization as in *A* in the presence of 1 mM amyl acetate in the bath. (*C*) membrane currents measured after the wash out of amyl acetate. (*D*) membrane currents evoked by depolarization to 0 mV ($V_h = -100$ mV) in the normal Ringer solution. The pipette solution was exchanged from the standard (*thin line*) to the 1 mM amyl acetate-containing solution (*thick line*) by an intrapipette perfusion technique.

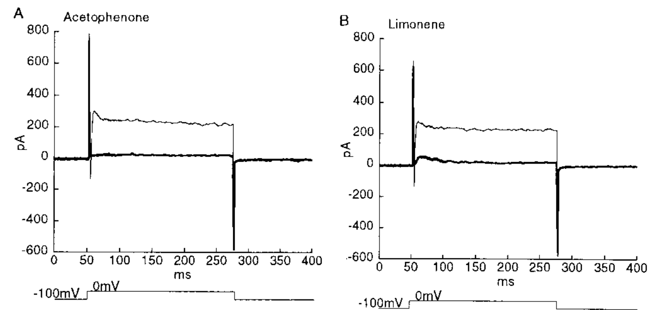

FIGURE 2. Effects of acetophenone and limonene on membrane currents of an isolated newt olfactory receptor cell. (*A*) membrane currents evoked by depolarization to 0 mV ($V_h = -100$ mV) in the normal Ringer solution (*thin line*) and in the Ringer solution containing 1 mM acetophenone (*thick line*). (*B*) membrane currents evoked by depolarization to 0 mV ($V_h = -100$ mV) in the normal Ringer solution (*thin line*) and in the Ringer solution containing 1 mM limonene (*thick line*). The effect of each odorant was examined in the same cell.

Fig. 3 *A*, a depolarizing step to -40 mV ($V_h = -100$ mV) induced I_{Na} of about 180 pA in the odorant-free solution. As the concentration of amyl acetate was increased, the peak amplitude of I_{Na} was reduced and the inactivation kinetics were accelerated slightly. Fig. 3 *B* shows the inhibition curve for amyl acetate (N = 11). The data could be fitted by the Hill equation (Eq. 1 of MATERIALS AND METHODS) with 0.11 mM of half-blocking concentration (IC_{50}) and a Hill coefficient (n) of 1.4.

Dose-suppression Relation of Amyl Acetate on Various Ionic Currents

Inward currents such as I_{Na}, $I_{Ca,T}$ and $I_{Ca,L}$ were more sensitive to amyl acetate than the outward current such as I_{Kv}. Fig. 4 shows the inhibition curves of amyl acetate on $I_{Ca,T}$, $I_{Ca,L}$, I_{Kv}, and $I_{K(Ca)}$ in the olfactory receptor cells. Each current was isolated as described previously (Kawai et al., 1996; Kurahashi, 1989*a*). The inhibition curve of $I_{Ca,T}$ was fitted by the Hill equation with IC_{50} = 0.15 mM and Hill coefficient, $n = 1.0$. Inhibition curves of $I_{Ca,L}$, I_{Kv} and $I_{K(Ca)}$ were also fitted by the Hill equation. The IC_{50} for each current was 0.14 mM ($I_{Ca,L}$), 1.7 mM (I_{Kv}), and 0.17 mM ($I_{K(Ca)}$), and Hill coefficients (n) were 1.1 ($I_{Ca,L}$), 1.0 (I_{Kv}) and 1.1 ($I_{K(Ca)}$). These results are consistent with the observation on the total current; the transient inward current was almost completely eliminated by 1 mM amyl acetate, while outward current was only partially suppressed (Fig. 1 *B*). Judging from similar IC_{50} and Hill coefficient values, it is likely that the suppression of $I_{K(Ca)}$ is secondary to the suppression of $I_{Ca,L}$. It remains open whether the Ca^{2+}-

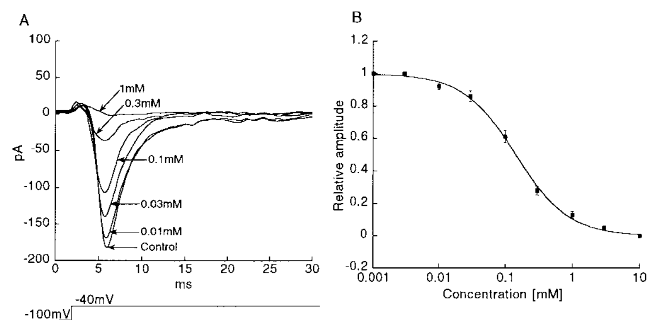

FIGURE 3. Effects of various concentrations of amyl acetate on a Na^+ current (I_{Na}) of an isolated newt olfactory receptor cell. (*A*) I_{Na} evoked by depolarization to -40 mV ($V_h = -100$ mV) in a Ca^{2+}-free solution containing 110 mM Na^+ and amyl acetate of various concentration (0, 0.01, 0.03, 0.1, 0.3, 1 mM) recorded by using the pipette filled with Cs^+ solution. (*B*) relation between the amount of suppression of I_{Na} and amyl acetate concentration (N = 11). Replotted from *A*. The continuous line is a mathematical fit of the data points to Eq. 1. Short vertical bars represent SEM (the standard error of the mean).

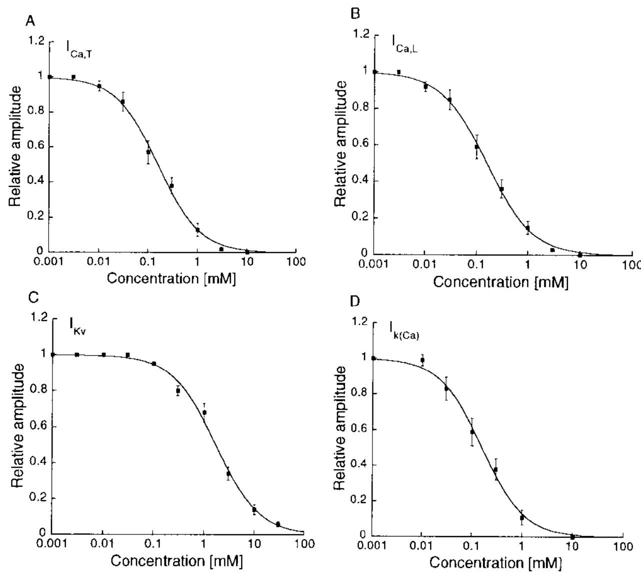

FIGURE 4. Effects of amyl acetate on a T-type Ca^{2+} current ($I_{Ca,T}$), an L-type Ca^{2+} current ($I_{Ca,L}$), a delayed rectifier K^+ current (I_{Kv}) and a Ca^{2+}-activated K^+ current ($I_{K(Ca)}$) of an isolated newt olfactory receptor cell. (*A*) inhibition of $I_{Ca,T}$ evoked by depolarization to −40 mV (V_h = −100 mV) in Na^+-free solution containing 10 mM Ca^{2+} and 0.1 mM Cd^{2+} recorded by using pipettes filled with Cs^+ solution (N = 6). Cd^{2+} was added to the solution to block $I_{Ca,L}$ selectively. The continuous line shows a least squares fit of the data points to Eq. 1. Short vertical bars represent SEM. (*B*) inhibition of $I_{Ca,L}$ evoked by depolarization to −10 mV (V_h = −100 mV) in Na^+-free solution containing 10 mM Ba^{2+}. To amplify the Ca^{2+} current, Ba^{2+} was substituted for Ca^{2+}. 0.1 mM Ni^{2+} was added to the solution to block $I_{Ca,T}$ selectively. Pipette was filled with Cs^+ solution (N = 7). (*C*) inhibition of I_{Kv} evoked by depolarization to +50 mV (V_h = −100 mV) in Na^+, Ca^{2+}-free solution recorded by using pipettes filled with K^+ solution (N = 5). (*D*) inhibition of $I_{K(Ca)}$ evoked by depolarization to 0 mV (V_h = −100 mV). $I_{K(Ca)}$ was obtained by subtracting the record in Na^+, Ca^{2+}-free solution containing 3 mM Co^{2+} from that in Na^+-free solution containing 3 mM Ca^{2+} and 0.1 mM Ni^{2+}. Recording pipette was filled with K^+ solution (N = 6).

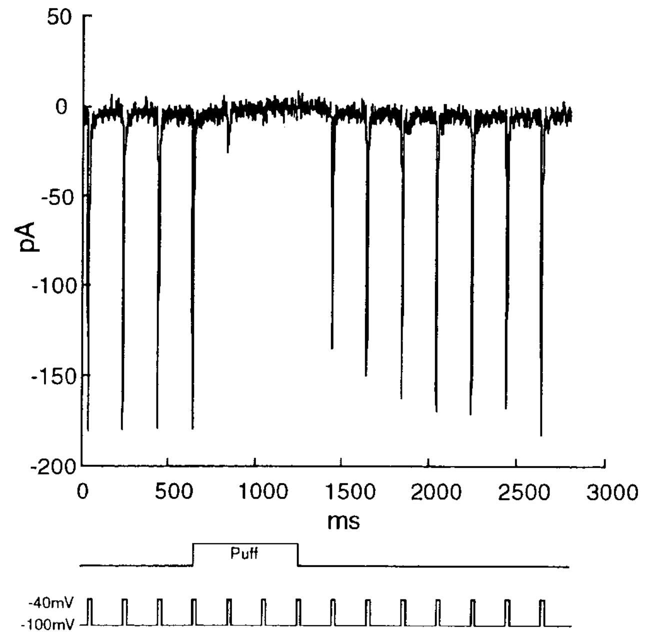

FIGURE 5. Time course of suppression of I_{Na} in an isolated newt olfactory receptor cell by amyl acetate. I_{Na} was evoked by repetitive depolarization to −40 mV (V_h = −100 mV) in Ca^{2+}-free solution containing 110 mM Na^+ and was recorded by using pipette filled with Cs^+ solution. Voltage steps were applied to the cell for 20 ms every 200 ms. 1 mM amyl acetate was applied by pressure ejection for 600 ms between the onset of the fourth voltage pulse and onset of the seventh pulse.

activated K^+ channel is also affected by odorants directly.

Time Course of Suppression of I_{Na} by Amyl Acetate

To elucidate the blocking mechanisms by odorants, we studied the time course of the suppressive effect of amyl acetate on I_{Na}. Fig. 5 shows the effects of pressure ejection of 1 mM amyl acetate on I_{Na}, evoked by repetitive depolarizing pulses. In the absence of amyl acetate, the peak amplitude of I_{Na} was ∼180 pA. After the onset of puff application of amyl acetate, the current responses started to decay immediately, and disappeared completely in 400 ms. After cessation of the puff, the response amplitude recovered rapidly. The time course of the recovery phase could be fitted by a single exponential function with a time constant of 130 ms, which is significantly slower than the time resolution (∼20 ms) for our U-tube system.

Effects of Amyl Acetate on Activation and Inactivation Curves of I_{Na}

Nonselective blockage of ionic currents has also been demonstrated for local anesthetics (LAs: benzocaine, lidocaine, procaine and tetracaine) in various preparations (I_{Na}: Courtney, 1975; Hille 1977*a,b*; I_K: Almers, 1976; Andreasen and Hablitz, 1993; I_{Ca}: Akaike et al., 1982; Sugiyama and Muteki, 1994). It has been shown that the LAs modify the inactivation kinetics of I_{Na}, without causing a detectable change in activation kinetics (Courtney, 1975; Hille, 1977*b*). To verify the possibility that the nonselective suppression by odorants is responsible for the same mechanism as the LA effect, we measured activation and inactivation curves of I_{Na} in the presence and absence of amyl acetate (Fig. 6).

The activation curves were not changed significantly by the application of amyl acetate (Fig. 6 *A*). In the control solution, the relation between the relative conduc-

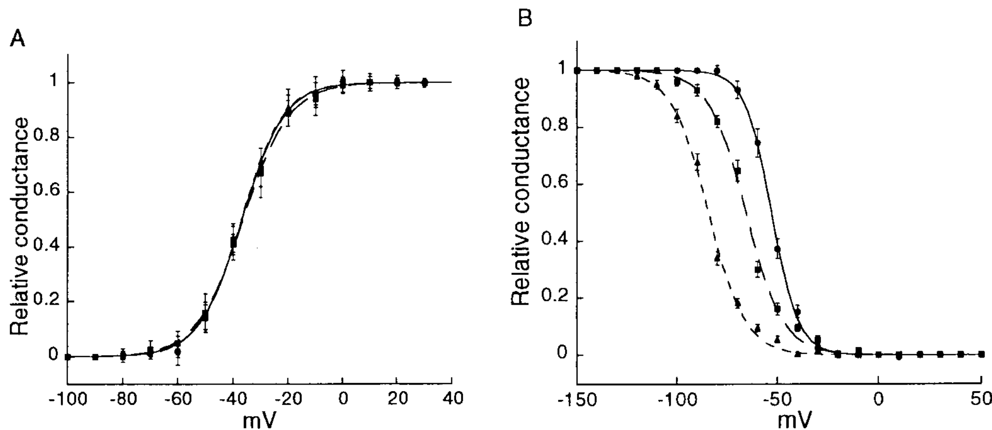

FIGURE 6. Effects of amyl acetate on activation and inactivation curves of I_{Na}. I_{Na} was recorded in Ca^{2+}-free solution containing 110 mM Na^{+}. Recording pipette was filled with Cs^{+} solution. V_h was −100 mV in all recordings. (*A*) activation curves in the control solution (●) and in solutions containing amyl acetate (■, 0.01 mM; ▲ 0.1 mM). Relative conductance at a specified membrane voltage was estimated as a ratio of the recorded current amplitude to that expected from the maximum conductance (the linear part of individual I-V curves near the reversal potential). Symbols represent mean of seven cells and vertical bars SEM. Lines represent a single Boltzmann function obtained by the least-squares nonlinear fit to the data. (*B*) inactivation curves in the control solution (●) and in the solution containing amyl acetate (■, 0.01 mM; ▲ 0.1mM). Relative conductance was estimated as a ratio of the current amplitude induced by depolarization to −40 mV after a 1-s conditioning pulse of various voltages (−150 to + 50 mV) to that induced by the same depolarization without conditioning pulses. The relative conductance is plotted against the conditioning voltage. Each symbol represents mean of seven cells, and short vertical bars SEM. Lines represent a single Boltzmann function.

tance (g_{Na}) and the membrane voltage was fitted by a single Boltzman function with a half-activation voltage of −34 mV. In the presence of amyl acetate, g_{Na} was also fitted by the single Boltzman function with a half-activation voltage of −33 mV at 0.01 mM (*filled squares*) and −34 mV at 0.1 mM (*filled triangles*).

However, inactivation curves were shifted significantly by the presence of amyl acetate (Fig. 6 *B*). g_{Na} showed a strong reduction by a conditioning polarization (duration = 1 s) more positive than −70 mV and became almost zero at −20 mV. The relation was fitted by the Boltzman function with a half-inactivation voltage of −53 mV. g_{Na} in the solution containing 0.01 mM amyl acetate began to inactivate by at a conditioning polarization more positive than −100 mV. The best fit of data points by the Boltzman function could be obtained with a half-inactivation voltage of −66 mV. This value is 13 mV more negative than that of g_{Na} in the control solution. Furthermore, the higher concentration of amyl acetate (0.1 mM) shifted the inactivation curve to −84 mV. These results are very similar to the effects of LAs, and therefore strongly suggest that suppression of ionic currents by odorants and LAs are both caused by the same mechanism.

Suppression of Action Potential Generation by Odorants

As shown above, various odorants suppressed voltage-gated currents in newt olfactory receptor cells. These results raise the possibility that action potential generation may be modulated by odorants due to the suppression of the voltage-gated currents. Most odorants are membrane permeable, so it is likely that they can penetrate into the olfactory epithelium and may affect the ionic channels present in the somatic membrane (Lowe and Gold, 1991).

In the present study, we recorded action potentials induced by current injections of various intensities (2, 4, 6, 8, and 10 pA) in the control Ringer solution and in the solution containing amyl acetate (0.1 and 1 mM). Under both conditions the resting potential did not change, perhaps because more than 60% of K^{+} channels remained unaffected even by 1 mM amyl acetate (Fig. 4 *C*). An example is shown in Fig. 7. When a cell was depolarized by current injection of more than 6 pA in the control medium, a single action potential was generated (Fig. 7 *A*). Application of 0.1 mM amyl acetate suppressed the generation of the action potential induced by current injection of 6 pA (Fig. 7 *B*). At 8 and 10 pA current injection, an action potential was also generated, but the peak amplitude was reduced (Fig. 7 *B*). At 1 mM, action potentials were completely abolished (Fig. 7 *C*). Similar results were obtained from seven other cells. The action potentials evoked by current injection were also blocked by the application of 1 mM acetophenone (N = 3) and 1 mM limonene (N = 3) (not shown). These results show that action potentials are suppressed by odorants due to blocking the voltage-gated currents in the somatic membrane of the isolated newt olfactory receptor cells.

DISCUSSION

In the present study we studied the effects of odorants on the voltage-gated currents in the somatic membrane

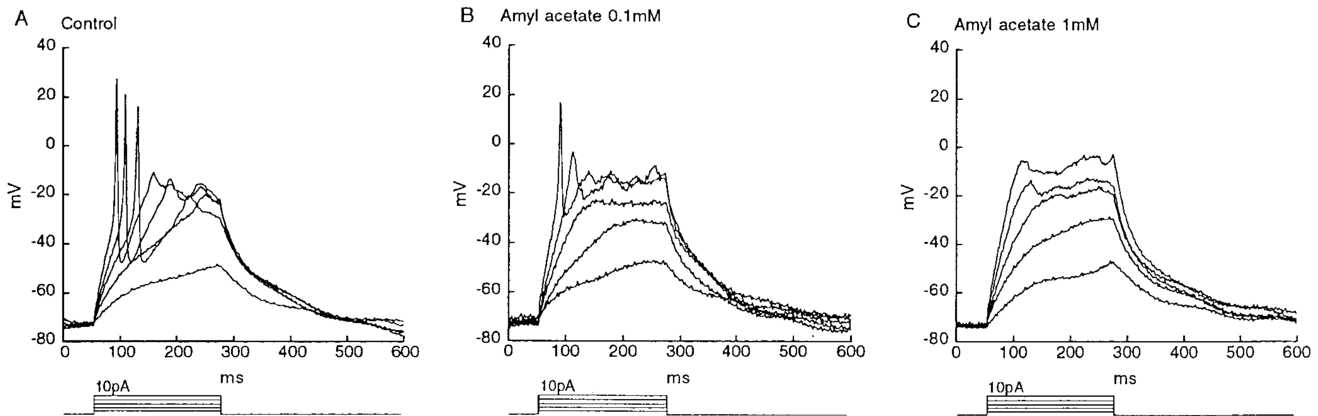

FIGURE 7. Effects of odorants on the action potentials of an isolated newt olfactory cell evoked by current injections of various intensities (2, 4, 6, 8, 10 pA). Recording were made under the current clamp condition by using pipette filled with K^+ solution. (*A*) responses recorded in the normal Ringer solution. (*B*) responses in the Ringer solution containing 0.1 mM amyl acetate. (*C*) responses in the Ringer solution containing 1 mM amyl acetate.

of isolated newt olfactory receptor cells by using whole cell recording. We found that the voltage-gated currents were suppressed nonselectively by odorants such as amyl acetate, acetophenone, and limonene.

Comparison with Other Na^+ Channel Blockers

TTX is well known as a Na^+ channels blocker. Schwarz et al. (1973) have shown that the half-blocking concentration (IC_{50}) of TTX on an axonal Na^+ channel was 3 nM, and the time constant (τ) of recovery was 70 s at 20°C. These values were quite different from those of amyl acetate recorded in our experiments ($IC_{50} = 0.11$ mM, $\tau = 130$ ms). The time constant for amyl acetate ($\tau = 130$ ms) was significantly slower than the time resolution of washout (< 20 ms) by the stream from our U-tube system, suggesting that the time constant of recovery is determined not by time resolution of washout but by the unbinding process of the odorant from the Na^+ channel or time to remove accumulated odorant from cell membranes.

Local anesthetics (LAs) are also known as Na^+ channel blockers. Hille (1977*a,b*) has shown that I_{Na} in the single myelinated nerve fiber is blocked by various kinds of LAs. He reported that the half-blocking concentration for LAs such as neutral, tertiary amine and quaternary types was all in the range of 0.1–1.0 mM (Hille, 1977*a*), which is similar to that for amyl acetate in the present experiment. He also studied the kinetics of I_{Na} blocked by LAs of different lipid solubility and of different charge type. The time constant of recovery from a neutral LA (benzocaine) blockage was faster than 0.2 s (Hille, 1977*b*). In his perfusion systems, the solution was exchanged in a small fraction of a second, therefore, this time constant should contain a contribution from the speed of solution change. The rates of tertiary amine LAs such as lidocaine, procaine and tetracaine were faster than 1 s at pH 8.3, and that of a quaternary LA (QX-572) was more than 200 s (Hille, 1977*b*). Time constant of recovery in our experiment (130 ms) was similar to that of neutral LA, while it was much faster than that of the quaternary LA.

It has been shown that the voltage dependence of the inactivation curve of I_{Na} is shifted to more negative voltages by the application of various LAs (Courtney, 1975; Hille, 1977*b*). In this study, the inactivation curve of I_{Na} was also shifted to negative voltages by amyl acetate (Fig. 6 *B*), and the inactivation kinetics were accelerated (Fig. 3 *A*). Furthermore, LAs nonselectively suppress not only I_{Na} but also various kinds of voltage-gated currents such as I_K (Almers, 1976; Andreasen and Hablitz, 1993) and I_{Ca} (Akaike et al., 1982; Sugiyama and Muteki, 1994). These observations are similar to our present results, suggesting that the blocking mechanisms of voltage-gated currents by odorants may be the same as those by LAs. However, it is still unclear how the odorant molecules alter the ionic permeation of voltage-gated channels in the olfactory receptor cells. It may be worthwhile to investigate the gating kinetics with single channel recording.

Comparison with the Transduction Current

Kurahashi et al. (1994) have shown that the odorant-induced transduction current is also suppressed by odorants. In the present experiment, we have shown that odorants suppress the voltage-gated currents. One remarkable difference from their results is the effect of

limonene. They reported that limonene did not affect the transduction current, while in this study 1 mM limonene suppressed the voltage-gated currents significantly. This discrepancy would be explained by the difference of concentration used in these experiments. They dissolved limonene directly into the Ringer's solution. Since limonene is extremely hydrophobic, the actual concentration must have been lower than in the present experiments, in which limonene was first dissolved in DMSO, which was then mixed with Ringer's solution. The real concentration in the present experimental solution may be close to 1 mM.

Kurahashi et al. (1994) have also studied the blocking kinetics by analyzing the effects of a brief odorant pulse on the transduction current induced by the previous application of odorant. In their experiment, the second odorant pulse caused an immediate (<20 ms) decrease in the transduction current evoked by the first pulse, and the transduction current suppressed by the second odorant pulse was released rapidly after the end of the second pulse. The mechanisms of suppression of the transduction current were not revealed by their experiments. However, because of the rapid kinetics of suppression they suggested that it might be due to a direct effect of odorants on the transduction channels rather than to an effect on olfactory receptor proteins or on second messenger metabolism.

In the present experiment, we studied the effects of a puff application of amyl acetate on I_{Na} evoked by repetitive depolarizing pulses in order to analyze the blocking kinetics by odorants. The onset of puff application reduced the amplitude of I_{Na} immediately (too fast to measure), and at the end of the application the amplitude of I_{Na} recovered rapidly with a time constant of 130 ms. These rapid kinetics of suppression are similar to that reported by Kurahashi et al. (1994), suggesting that the blocking mechanism of the transduction current by odorants may be similar to that for voltage-gated currents, as well. Furthermore, the nonselective blockage of ionic channels observed here is consistent with their proposal that the suppression of the transduction current by odorants is due to the direct blockage of transduction channels.

Effects of Odorants on Action Potential Generation in the Olfactory Receptor Cells

Under current clamp, action potentials generated by current injection of various intensities were completely blocked by amyl acetate, acetophenone, and limonene (1 mM for each). Action potentials in newt olfactory receptor cells are evoked by the activation of transient inward currents, I_{Na} and $I_{Ca,T}$ (Kawai et al., 1996). Therefore, the suppression of ionic channels by odorants would cause some modification of spike generation. As shown in the inhibition curves of amyl acetate (Figs. 3 and 4), the inward currents (I_{Na} and $I_{Ca,T}$) were more sensitive to amyl acetate than the outward current (I_{Kv}); 96% of I_{Na} and 87% of $I_{Ca,T}$ were suppressed by application of 1 mM amyl acetate, whereas, only 47% of I_{Kv} was suppressed by 1 mM amyl acetate (from the Eq. 1). Therefore, the suppression of action potentials by odorants is consistent with the inhibition curves of each ionic current.

In the present experiments, the odorant was directly applied to the somatic membrane of isolated cells. It is asked whether odorants can similarly affect the somatic membrane in vivo, since it is known that the interstitial environment is sealed from the olfactory mucus layer by tight junctions (Usukura and Yamada, 1978; Graziadei and Graziadei, 1979). The only possible access is that the odorant given to the dendritic tip diffuses to the soma membrane. In fact, membrane permeation has been shown by Lowe and Gold (1991) who showed that an odorant induced a current in the cilia and dendrite enclosed by a suction electrode, while applying the odorant (2-hexylpyridine) to the outside of the electrode. In such a situation the odorant would have only limited access via the exposed plasma membrane. It may be worth testing how effective such permeation to the soma would be via the cilia and dendritic tip in vivo.

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