Patch-Clamp Studies of Isolated Mouse Olfactory Receptor Neurons

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ABSTRACT Olfactory receptor neurons isolated from embryonic, neonatal, and adult mice were studied using the patch-clamp technique. Several distinct types of ion channels were characterized in patches of membrane from the neuronal soma and the dendritic knob of receptor neurons, including a 130-pS Ca^{++}-activated K^+ channel with voltage-dependent kinetics, an 80-pS Ca^{++}-activated K^+ channel with voltage-insensitive kinetics, a 25-pS K^+ channel with properties similar to inward rectifiers, and a 40-pS K^+ channel that was activated and then inactivated by rapid depolarization. Evidence of large-conductance (>200 pS) Cl^- channels, which were Ca^{++} insensitive and increasingly active at depolarizing membrane potentials, and voltage-activated Ca^{++} channels (16 pS) was also obtained. From K^+ channel activity recorded from cell-attached patches, the intracellular [Ca^{++}] was inferred to be below 0.1 μM, and the membrane potential was inferred to be approximately −50 mV. The receptor neurons had high input resistances, and action potentials could be elicited by picoampere amounts of depolarizing current. The receptor neurons responded to applied odorant molecules and to forskolin with increases in membrane conductance. These results provide a description of the membrane properties of olfactory receptor neurons and a basis for understanding their electrical activity and response to odorants.

INTRODUCTION

Olfactory receptor neurons are responsible for both the detection of odorant molecules and the initial discrimination among them. Because of this fundamental role in the olfactory process, an analysis of the physiology and membrane properties of these receptor cells is crucial to understanding the function of the olfactory system. A necessary prerequisite to elucidating the series of events leading from the detection of odorant molecules to the transmission of electrical impulses to the brain is a clear description of the specific membrane conductances present in the receptor neurons.

Despite the importance of olfactory receptor neurons, obtaining information about their electrical properties has proven a difficult task because of the

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limitations of conventional electrophysiological techniques and the morphology of the epithelium. Stable intracellular recordings are technically difficult to obtain because the neurons are small and their somata are located below the epithelial surface. Penetration with intracellular electrodes often damages the cells, even when using high-resistance electrodes, whose low current-passing capabilities limit the control over the membrane potential. The close apposition of the cells in the epithelium and the presence of tight junctions between support cells and receptor neurons precludes simple visual identification and prevents ready exchange of the ions bathing the neurons. This structural integrity also limits the use of pharmacological agents and hinders attempts to restrict odorant application to individual neurons.

Many of these problems can be circumvented by using the patch-clamp technique (Hamill et al., 1981) to study isolated receptor neurons. This technique allows both macroscopic currents and currents through individual ion channels to be measured with a high degree of temporal and spatial resolution. When applied to isolated receptor cells, it enables experiments to be carried out on identified cells under defined biochemical and pharmacological conditions, and allows localized regions of individual receptor cells, such as the dendritic ending, to be examined in detail. The patch-clamp technique is especially advantageous for recording from small cells, causing little damage to the cell and allowing stable recordings to be made for long periods of time.

We present here characteristics of ion channels found in the membranes of mouse olfactory receptor neurons. An examination of the single channel properties, the distribution of channels on the cell surface, and changes in channel characteristics that may occur during development was done in order to gain insight into their role in the activity and odorant response of these cells. The developmental aspect is relevant to mammalian olfactory receptor neurons, where changes in the response to odorants occur just before birth (Gesteland et al., 1980, 1982). In addition, the response of isolated receptor cells to odorants and the possible role of cAMP in this response (Koyama and Kurihara, 1972; Menevse et al., 1977; Pace et al., 1985) were investigated. Preliminary reports of some of the work presented here have been made (Maue and Dionne, 1984, 1986).

METHODS

Preparation of Isolated Olfactory Receptor Neurons

Our procedure for preparing isolated, viable olfactory receptor neurons from mouse nasal epithelia is described elsewhere (Maue and Dionne, 1987). In brief, 9–16-wk-old "nude" mouse heterozygotes (Balb/c/Nu) were obtained from the Athymic Mouse Facility, University of California, San Diego (supported by the National Cancer Institute) and 9-wk-old male Balb/c and timed pregnant Swiss mice (for embryonic tissues) were obtained from MTS Laboratories (San Diego, CA). The mice were killed by cervical dislocation and the nasal epithelia were quickly removed. Large numbers of olfactory receptor neurons were then isolated from the tissue using enzymatic treatment (0.025% trypsin type III, Sigma Chemical Co., St. Louis, MO), divalent-free Dulbecco's modified Eagle's medium, and mechanical disruption.

The isolated neurons retained their morphology and could be easily identified: somata were spherical or ovoid, 5–8 μm in diameter, with a dendrite ~1 μm in diameter extending from the soma and terminating in a swelling or "knob" bearing several fine cilia. Dendrites were generally 5–15 μm long and occasionally as long as 30–35 μm. 5–10 cilia were visible on most cells, with up to 15–20 visible in some cases; cilia were usually 10–45 μm long. As expected for mammalian olfactory neurons (Lidow and Menco, 1984), the cilia were not motile. After dissociation, a suspension of the isolated cells was applied to glass coverslips previously coated with concanavalin A and left undisturbed for 30–60 min at room temperature, allowing the cells to settle on and adhere to the coverslips before they were used in experiments.

Patch-Clamp Technique and Data Analysis

Now-standard methods (Hamill et al., 1984) were used to record and analyze single channel data from the receptor neurons. Patch-clamp electrodes were fabricated from flint glass (Kimble Co., Toledo, OH) or hematocrit capillary (VWR Scientific, Norwalk, CA) tubing using an electrode puller (700C, David Kopf Instruments, Tujunga, CA) and coated with Sylgard 182 (Dow Corning Corp., Midland, MI). Uncoated pipettes made with Corning 8161 or 7052 capillary glass (Corning Glass Works, Corning, NY) were also used successfully. After the tips were fire-polished and the electrodes were filled with saline, the resistances were generally between 3 and 5 MΩ.

The neurons were viewed at 500× magnification with differential interference contrast (Nomarski) optics. Patch electrodes were manually positioned against the cell surface with an E. Leitz, Inc. (Rockleigh, NJ) manipulator. High-resistance seals (1–10 GΩ) between the electrode and the cell surface were formed by applying gentle suction to the lumen of the pipette. Currents flowing through the small patch of membrane circumscribed by the electrode tip were measured at room temperature with a patch-clamp amplifier built in the laboratory. The signal was low-pass-filtered using an eight-pole Bessel filter (Frequency Devices Inc., Haverhill, MA) and digitally recorded with a PDP-11/23 laboratory computer system (Digital Equipment Corp., Marlboro, MA) equipped with a Cheshire Data interface and BASIC-23 software. Unless otherwise stated, the signal was prefiltered at 2 kHz (−3 dB) and digitally sampled at 100-μs intervals. The computer was also used to apply voltage pulses of specified duration and amplitude to the patch pipette via the patch-clamp electronics.

Digitized single channel current records were analyzed using an operator-assisted computer program (Leibowitz and Dionne, 1984) to measure the amplitude and duration of the single channel events, as well as the length of time between events. The distributions of the open and closed durations were determined by compiling histograms of many measurements, and exponential probability distribution functions fitted to the histograms were optimized using a maximum likelihood method. Since most of the data were recorded at 100-μs intervals and processed at a resolution time of 200 μs, brief exponential components with time constants of 200–300 μs (as observed in many of the closed-time distributions described below) may be artifactually enhanced and should be identified as "apparent" until greater experimental resolution confirms their accuracy. This problem is an unavoidable result of limited resolution and occurs in all single channel studies, although it has been identified as a problem only recently (Roux and Sauve, 1985; Blatz and Magleby, 1986). The values given for single channel conductance are slope conductances derived from linear regression analysis of the average single channel current amplitude recorded at different membrane potentials. Unless otherwise stated, the potentials given here are those of the recording pipette relative to the bath solution. All numerical values cited with uncertainties are means ± standard deviation.

Solutions

All of the salines used in these studies contained 5.0 mM of the Na salt of HEPES and had the pH adjusted to 7.4 with HCl or KOH. The "normal" saline consisted of (millimolar): 140 NaCl, 5.6 KCl, 2.0 $MgCl_2$, 2.0 $CaCl_2$, and 9.4 glucose. "Elevated-K^+ saline" was the same as above except 140 mM KCl was substituted for the NaCl. Salines with buffered [Ca^{++}] contained (millimolar): 145.6 KCl, 13.4 glucose, 2.0 EGTA, and adjusted amounts of $MgCl_2$ and $CaCl_2$, as calculated using the stability constants of Owen (1976) and Chaberek and Martell (1959). Salines containing K^+ channel blockers had 10 mM 4-aminopyridine (4-AP), 30 mM tetraethylammonium (TEA), or 50 mM CsCl substituted for an equivalent amount of KCl. In some experiments, 145.6 mM K^+-glutamate was substituted for the KCl in the elevated-K^+ saline or one of the salines with buffered [Ca^{++}]. The saline in the pipette for the Ca^{++} channel experiments contained (millimolar): 60.0 $BaCl_2$, 40.0 sucrose, 30.0 TEA, and 2.0 $MgCl_2$.

Heptane, isobutyl alcohol, ethyl acetate, and benzaldehyde (Mallinckrodt Co., Paris, KY) were chosen as standard odorants and applied together at concentrations of 10 or 100 μM. These compounds have been used as effective odorant stimuli for a number of species, including rats (Gesteland et al., 1982). Although the thresholds of individual cells appear to be in the nanomolar-to-micromolar range (Getchell, 1974), concentrations between 10 and 100 μM have often been found to be effective and were used here in order to maximize the number of cells that would respond and the magnitude of the responses. Mass spectrometry was used to verify the concentration of the odorants in the solutions.

Perfusion System and Odorant Application

For the patch-clamp recordings, fragments of coverslips containing the isolated receptor neurons were placed in a small drop of saline (~0.2 ml) between a 40× water immersion objective and a glass plate. This drop could be continuously perfused at ~1.5 ml/min via segments of 23-gauge stainless-steel tubing suspended from the microscope objective. Saline containing the mixture of odorants was pressure-applied to individual cells from a "puffer" pipette (tip opening, ~10 μm) in 1-s pulses at 1–2-min intervals. A second pipette delivered control applications of saline 6 s before each odorant application.

Forskolin, an activator of adenylate cyclase (Seamon and Daley, 1981), was applied to the cells as follows: ~10 s after the recording of the current signal had begun, a puffer pipette containing 50 μM forskolin, 50 μM of the phosphodiesterase inhibitor Ro20-1724, and 0.2% ethanol dissolved in saline was introduced into the bath, its tip was positioned near the cell, and its contents were ejected by pressure for the duration of the recordings (3–6 min). Control applications of 0.2% ethanol in saline were also performed.

RESULTS

Ca^{++}-activated K^+ Channels

130-pS Ca^{++}-activated K^+ channels. The most frequently observed channel type in these neurons was a large-conductance Ca^{++}-activated K^+ channel (Fig. 1). These channels were detected in excised (n = 19) and cell-attached patches of membrane (n = 5) from both the soma (n = 21) and dendritic knob (n = 3) of receptor neurons from mature (n = 23) and neonatal (n = 1) mice. With elevated-K^+ saline on both sides of the membrane, the single channel current-voltage relationship was linear and the estimated unitary conductance was 133 ± 14 pS (n = 14) (Fig. 2). The conductance was independent of whether the channel was

found in the soma or the dendritic ending, in excised or cell-attached patches, or in neurons from mature or neonatal mice; it was not altered by substitution of glutamate for Cl^- or by changes in intracellular [Ca^{++}], and was not significantly reduced (123 ± 9 pS, n = 4) with normal saline on either side of the membrane. On rare occasions (four patches), the channel exhibited apparent

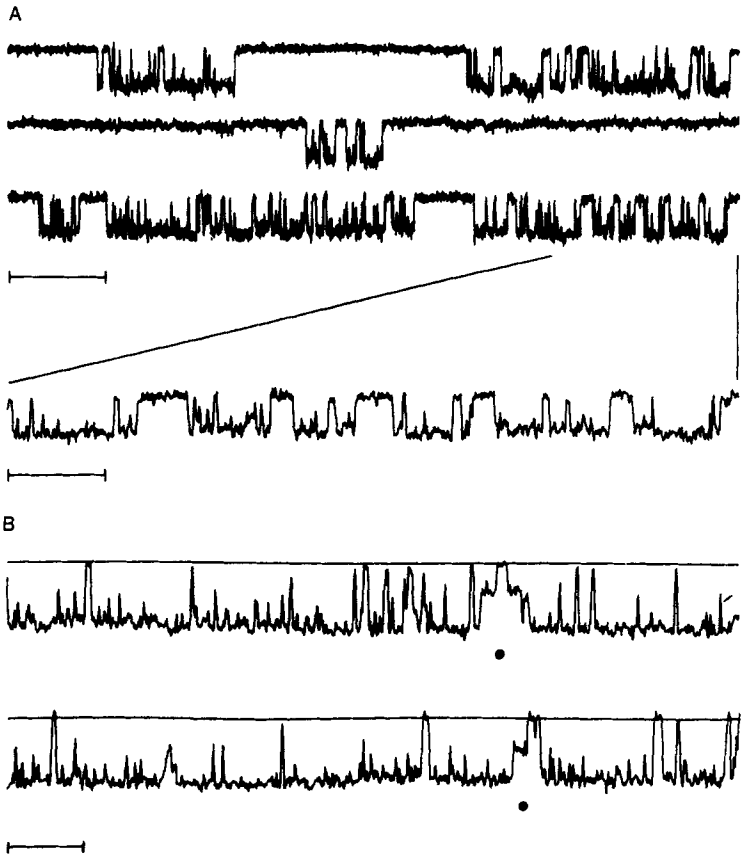

FIGURE 1. Activity of a 130-pS K^+ channel in excised patches. Downward deflections represent channel openings. (*A*) The top three traces of the current record illustrate the kinetic behavior of the channel. A portion has been expanded to show the brief closures between openings. Scale bars: upper record, 50 ms; lower record, 12.5 ms. Intracellular [Ca^{++}] = 0.5 μM. (*B*) Subconductance states of the 130-pS K^+ channel are indicated by the filled circles. These two records are from different patches. The baseline was drawn where all the channels are closed. Scale bar, 10 ms. Intracellular [Ca^{++}] = 1.0 μM.

subconductance states ~45% of the full conductance (Fig. 1*B*). As shown in the examples, the channel could enter the subconductance state from either a closed state or the higher conductance state and could exit from the subconductance state to either state. The infrequent occurrence of the subconductance state prevented more detailed characterization.

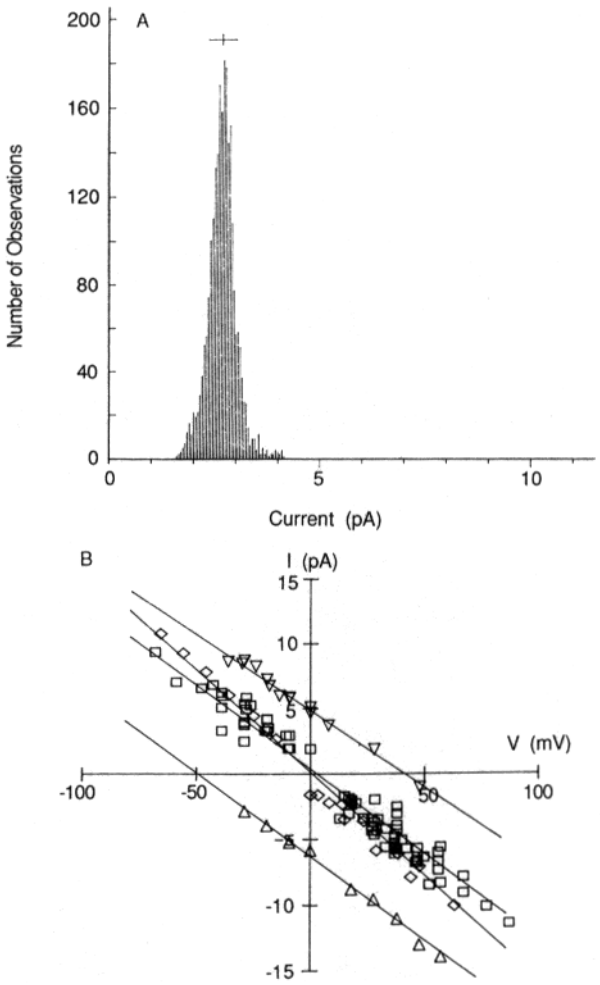

FIGURE 2. Measurement of single channel currents. (*A*) An example of the distribution of single channel current amplitudes (n = 2,356) measured at −15 mV from an excised, inside-out patch of somal membrane from an isolated olfactory receptor neuron. This patch appeared to contain only one type of channel. Estimates of mean single channel current such as this (2.67 ± 0.33 pA) were used in the plot shown in *B*. (*B*) The single channel current-voltage relationship for the 130-pS K^+ channel. Data were collected from 17 inside-out patches of membrane. Measurements were made under different ionic conditions (see Methods): (△) elevated-K^+ saline in pipette, normal saline in bath; (▽) normal saline in pipette, elevated-K^+ saline in bath; (□) elevated-K^+ saline in pipette and bath; (◇) K^+-glutamate on one side of the membrane, elevated-K^+ saline on the other.

The 130-pS channel was selective for K^+. When normal saline replaced elevated-K^+ saline on one side of the membrane, the measured reversal potential of the single channel currents shifted 43 ± 5.2 mV (n = 4), although the calculated shift of the K^+ equilibrium potential (E_K) was 82 mV (Fig. 2). The

reversal potential did not change in response to changes in [Ca^{++}] or [Cl^-]. If the discrepancy between the calculated and measured shifts in the reversal potential is indicative of an imperfect selectivity of the channel for K^+ over Na^+, then the estimated permeability ratio calculated using the Goldman-Hodgkin-Katz relationship (Goldman, 1943; Hodgkin and Katz, 1949) is ~6.72:1.

The 130-pS channel was sensitive to changes in intracellular [Ca^{++}] (Fig. 3). In six of eight patches, the fraction of time the channel was open increased dramatically when the [Ca^{++}] was increased from submicromolar to micromolar

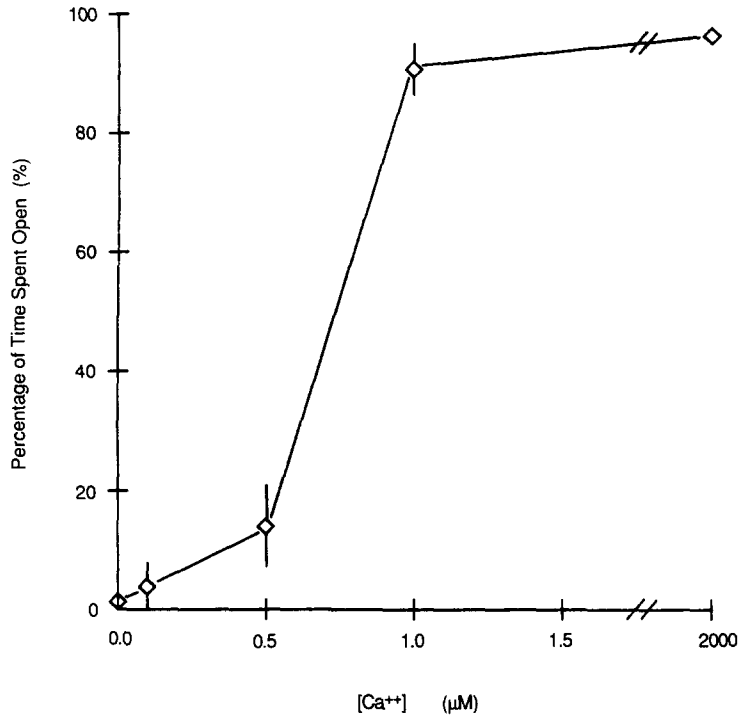

FIGURE 3. Ca^{++} sensitivity of the 130-pS K^+ channel. Data were collected from 13 patches of membrane at a pipette potential of 40 mV. The points shown are the means ± standard deviation of the measured percentage of time the channel was open at a given [Ca^{++}]. The line between the points was drawn by eye.

concentrations. In two of three cases, the activity of the channel also decreased when the [Ca^{++}] was lowered. As shown in Fig. 3, below 0.5 μM the channel was open very infrequently, whereas at 0.7 μM it was open ~50% of the time, and at 1.0 μM it was open >90% of the time.

The open-duration distribution of the 130-pS channel was well fitted by a single exponential (Fig. 4*A*), although on a few occasions there appeared to be an excess of brief openings (Fig. 4*B*). Because these were concentrated in the first two or three bins of the histogram and were a relatively small percentage of the total, the uncertainty in fitting this component with a second exponential

was great. Furthermore, the fit of the entire distribution was not substantially improved using two exponentials, and it was not clear whether these events represented a real population or were an artifact of the analysis. The mean channel open time was dependent on both the membrane potential and the

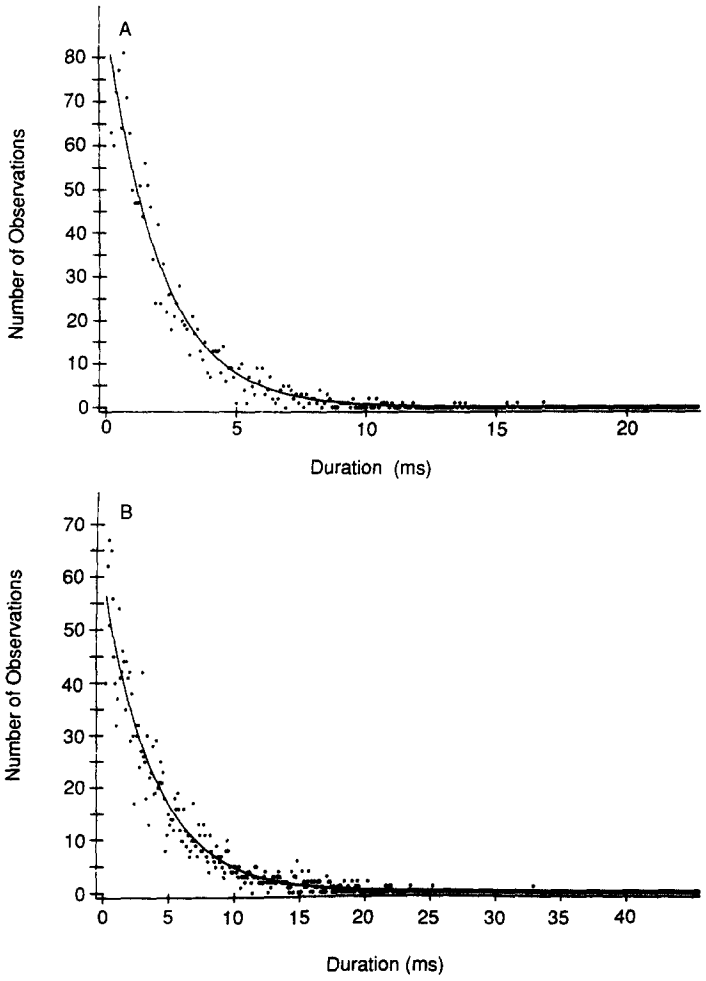

FIGURE 4. Open-duration distributions of the 130-pS channel. Both histograms were generated from data from the same patch of membrane under the same ionic conditions. Exponentials were fitted using maximum likelihood criteria. Minimum duration during the data analysis was 200 μs. (*A*) Pipette potential, 15 mV. τ = 2.06 ms (n = 1,661). (*B*) Pipette potential, −15 mV. τ = 3.97 ms (n = 2,250). This distribution shows an excess of brief-duration events, which was observed occasionally in the open-duration distributions of this channel type.

intracellular [Ca^{++}] (Fig. 5). At a low [Ca^{++}], the mean channel open time increased with depolarization about fivefold in 150 mV (Fig. 5*A*). Increasing the [Ca^{++}] increased the mean channel open time at a given potential, with little or no change in the voltage dependence of the open time (Fig. 5, *B–D*). The open-

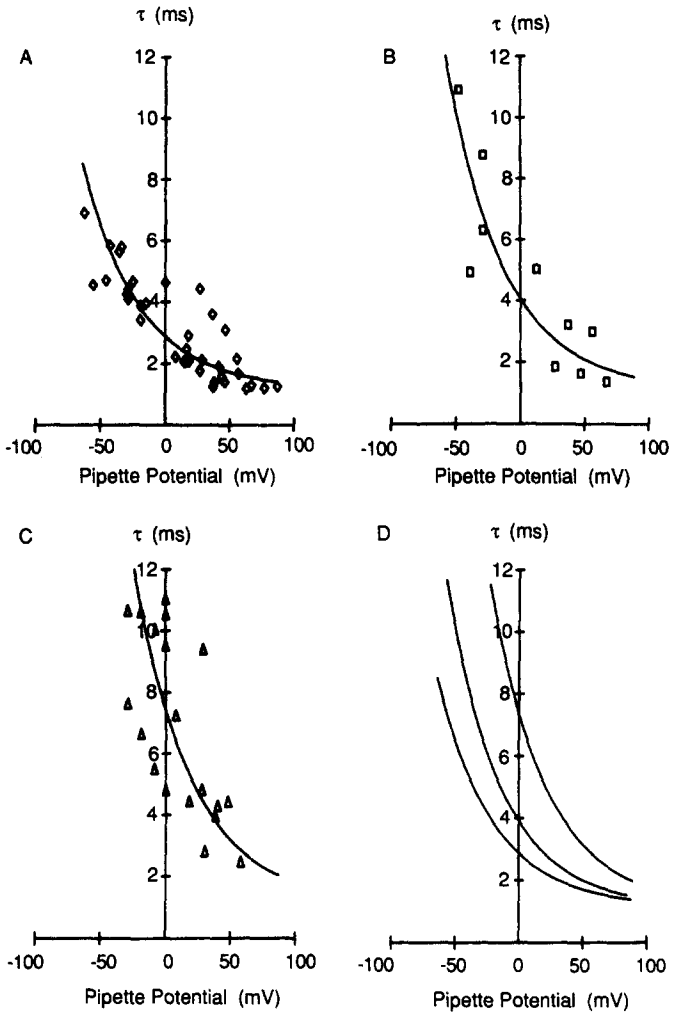

FIGURE 5. Mean channel open time as a function of [Ca^{++}] and voltage for the 130-pS K^+ channel. The data points represent estimates of mean channel open time derived from open-duration distributions from 10 patches of membrane. The data in *A–C* were fitted with single-exponential curves having the same rate constant and *x*-axis asymptote, but different *y*-axis intercepts. (*A*) [Ca^{++}] = 0.1 μM. (*B*) [Ca^{++}] = 0.5 μM. (*C*) [Ca^{++}] = 1.0 μM. (*D*) Curves fitted to the data in *A–C* drawn again to illustrate the increase in the mean channel open time that occurs with depolarization and elevation of intracellular [Ca^{++}].

time distribution was independent of where the channel was located (soma or dendritic knob), whether the patch was cell-attached or excised into solutions with low [Ca^{++}], and whether Cl^- or glutamate was the major intracellular anion. In addition, the voltage dependence of the open distributions was unaffected by the anions present. Finally, the mean open time of this channel from receptor neurons of neonatal mice appeared to be similar to and exhibit roughly the same dependence on [Ca^{++}] as that observed in neurons from adult mice.

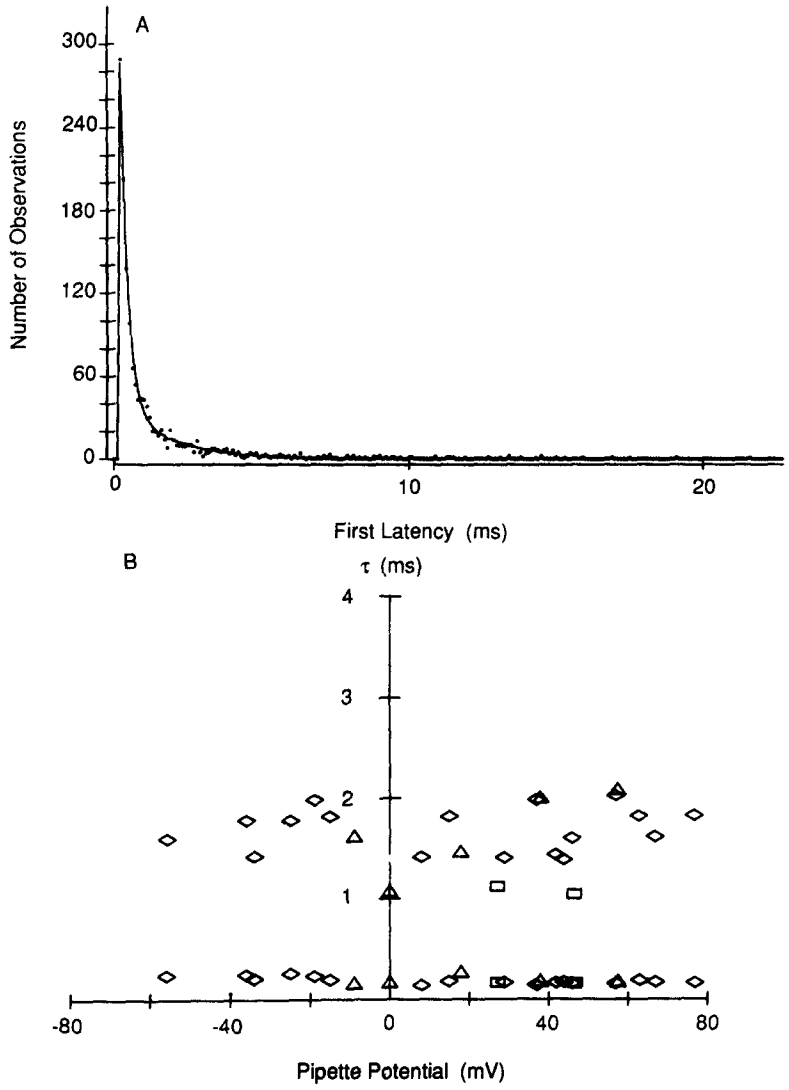

FIGURE 6. Distribution of closed intervals of the 130-pS K^+ channel and the insensitivity to [Ca^{++}] and voltage. (*A*) The distribution of closed intervals was well fitted by the sum of two exponential components with time constants of 0.248 and 1.776 ms (n = 1,494). Pipette potential, −36 mV. (*B*) The time constants of the two components were unaffected by either membrane potential or [Ca^{++}]: 0.1 (◇), 0.5 (□), and 1.0 (△) μM Ca^{++}. These data were collected from three patches of membrane.

The distribution of closed intervals recorded during periods of high activity was well described by the sum of two exponential curves (Fig. 6*A*). The time constants of these components differed by ~10-fold (0.186 ± 0.037 ms for the fast component and 1.626 ± 0.305 ms for the slower component; n = 24) and appeared to be independent of voltage, [Ca^{++}], and the intracellular anion (Fig.

6*B*). Examination of channel activity revealed periods of high activity separated by closed intervals up to several hundred milliseconds in duration (Fig. 1*A*). This suggests that the intervals plotted in Fig. 6*A* and the resulting time constants probably correspond to the short closed intervals within the periods of high activity of the channel (see the expanded portion of Fig. 1*A*).

The 130-pS channel was reversibly blocked in the presence of Cs ions in the intracellular solution. In seven excised patches, the activity of the channel was blocked or substantially reduced by the addition of Cs^+ to the intracellular solution. In cases where the activity was not completely blocked, the residual activity appeared to be very "noisy" and of reduced amplitude. In four of the cases, where the patch of membrane withstood the extensive wash apparently required to remove the Cs^+ solution, the activity returned. The reduction in channel activity occurred when the electrochemical potential drove the Cs ions to the channel, and could be partially overcome by elevated K^+ concentrations on the extracellular side of the membrane and ion flux in the opposite direction. The channel activity was not affected by intracellular TEA or 4-AP in the four patches where this was examined.

80-pS Ca^{++}-activated K^+ channels. A second type of Ca^{++}-activated K^+ channel was characterized by its rapid, "flickery" kinetic behavior (Fig. 7). This channel was identified in both excised (n = 13) and cell-attached patches (n = 5) from the soma (n = 17) and dendritic knob (n = 1) of receptor neurons from adult mice. Its single channel current-voltage relationship was linear over a range of ±80 mV (Fig. 8). With elevated-K^+ saline bathing both membrane surfaces, the single channel conductance was 92 ± 9 pS (n = 4). If normal saline replaced the KCl on one side of the membrane, the single channel conductance was 82 ± 10 pS (n = 7). The conductance of the channel was similar in patches of membrane from the soma (82.4 ± 13.3 pS, n = 17) and the dendritic knob (81.5 pS, n = 1), and in excised (83.3 ± 11.5 pS, n = 13) and cell-attached (79.8 ± 17.3 pS, n = 5) recordings.

The 80-pS channel was K^+ selective. In symmetrical K^+ salines, the reversal potential of the single channel currents was 0 mV. The reversal potential did not change when glutamate was substituted for Cl^- or with changes in $[Ca^{++}]$. However, when Na^+ was substituted for K^+, the reversal potential of the single channel currents shifted toward the new E_K (Fig. 8). The magnitude of the shift (50.2 ± 7.7 mV) was not accurately predicted by the shift in E_K (82 mV), which suggests that the relative permeability of this channel to K^+ was ~9.75 times greater than that for Na^+.

Like the 130-pS channel, the activity of the 80-pS channel depended upon the intracellular $[Ca^{++}]$ (Fig. 9); however, it was less sensitive to changes in $[Ca^{++}]$, and was open only 50–60% of the time in 1.0 μM Ca^{++}. By comparison, at 1.0 μM the 130-pS channel was open >90% of the time.

The kinetic behavior of the 80-pS channel was quantitatively distinct from that of the 130-pS channel. The open-duration distribution was well described by a single exponential (Fig. 10*A*), with a mean channel open time of ~1 ms (1.21 ± 0.34 ms, n = 39). In contrast to the 130-pS channel, the open time appeared to be independent of voltage and intracellular $[Ca^{++}]$ (Fig. 10*B*). At least two exponential components were required to adequately describe the

distribution of closed intervals within periods of high activity (Fig. 11*A*). The time constants of these components did not appear to depend on voltage or [Ca^{++}] (Fig. 11*B*) and had mean values of ~0.204 ± 0.035 and 1.928 ± 0.368 ms ($n = 29$). Neither the open-duration distribution nor the distribution of closed intervals was influenced by whether the patch was excised or cell-attached, or whether it was from the neuronal soma or the dendritic knob.

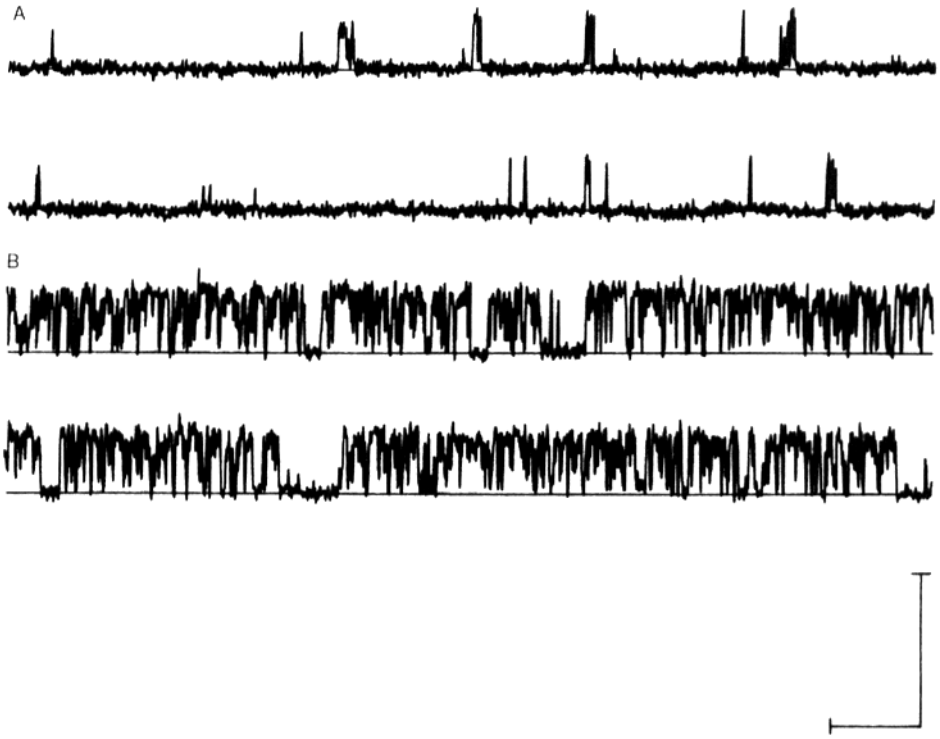

FIGURE 7. The kinetic behavior and Ca^{++} sensitivity of the 80-pS K^+ channel. Recordings were made at a pipette potential of 0 mV with normal saline in the pipette and elevated-K^+ saline in the bath. In all four traces, upward deflections represent channel openings. (*A*) Two contiguous traces illustrate the level of activity when the intracellular [Ca^{++}] was 0.1 μM. (*B*) Two contiguous traces recorded from the same patch of membrane when the intracellular [Ca^{++}] was changed to 1.0 μM. Scale bars, 10 pA, 20 ms.

Intracellular Cs^+ blocked the activity of the 80-pS channel when the electrochemical potential drove Cs^+ into the channel ($n = 4$ patches). The effect was reversible ($n = 2$) and did not occur when ion flux was in the opposite direction.

Voltage-activated Channels

Voltage-activated K^+ channels. A channel activated by rapid depolarizations (Figs. 12*A* and 13) was observed in cell-attached patches of olfactory receptor neurons from adult ($n = 6$) and embryonic (E19) ($n = 1$) mice. The single channel

conductance was 39.3 ± 8.3 pS (n = 3) with normal saline in the pipette and 41.3 ± 9.8 pS (n = 4) with elevated-K^+ saline. The channel appeared to be selective for K^+, as the single channel currents reversed near E_K and did not change when glutamate was partially substituted for Cl^- (n = 1). The channel was activated after sudden depolarizations of 30–40 mV or more (Figs. 12*A* and 13), but not by hyperpolarization. As the depolarizations were made larger, the latency between the initiation of the voltage step and the first opening of the channel decreased substantially, while the percentage of time the channel was

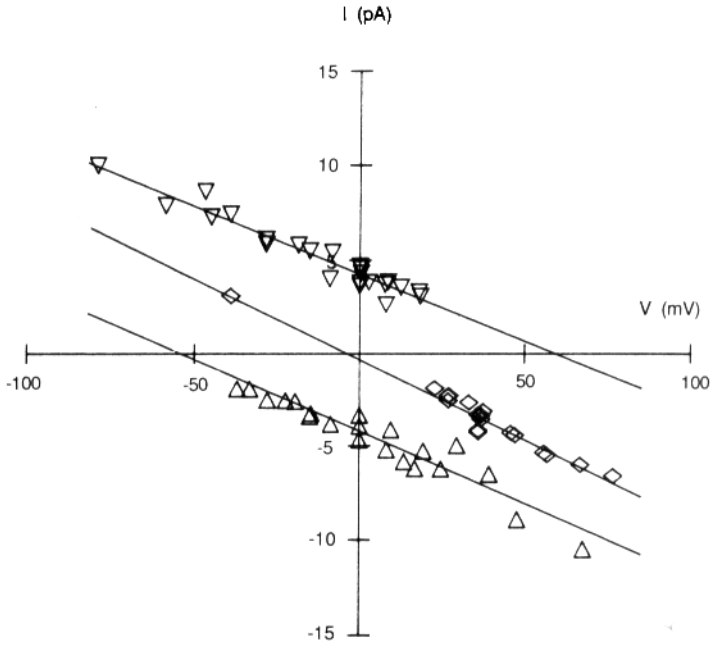

FIGURE 8. Single channel current-voltage relationship for the 80-pS K^+ channel. The data shown were collected from 11 excised, inside-out membrane patches. The data points represent mean single channel currents estimated from the amplitude distributions. Measurements were made under different ionic conditions (see Methods): (△) elevated-K^+ saline in the pipette, normal saline in the bath; (▽) normal saline in the pipette, elevated-K^+ saline in the bath; (◇) elevated-K^+ saline in both the pipette and bath.

open during the pulse increased rapidly (Fig. 12*B*). At all test voltages, the channel inactivated within 300–900 ms (Fig. 13). Kinetic analysis was done on the activity in two patches. The open-duration distribution was well fitted by a single exponential (Fig. 14*A*). The mean channel open time increased with depolarization and varied from 2.2 to 6.5 ms when channel activity was sampled at 0.2-ms intervals, and from 9.4 to 15.9 ms when sampled at 1.0-ms intervals (Fig. 14*B*), which suggests that there were brief, closed intervals that were poorly resolved at the slower sample rates. The distribution of closed intervals appeared

to have at least two exponential components with time constants of ~0.3 and 2.6 ms.

Voltage-activated Ca^{++} channels. In experiments designed to detect Ca^{++} channels, depolarizing voltage steps occasionally elicited small inward currents from patches on the neuronal soma and the dendritic knob when the patch pipettes contained saline with elevated $BaCl_2$. The activity was infrequent and short-lived in both cell-attached and excised patches. The single channel conductance, estimated from the activity in cell-attached patches, was ~16 pS.

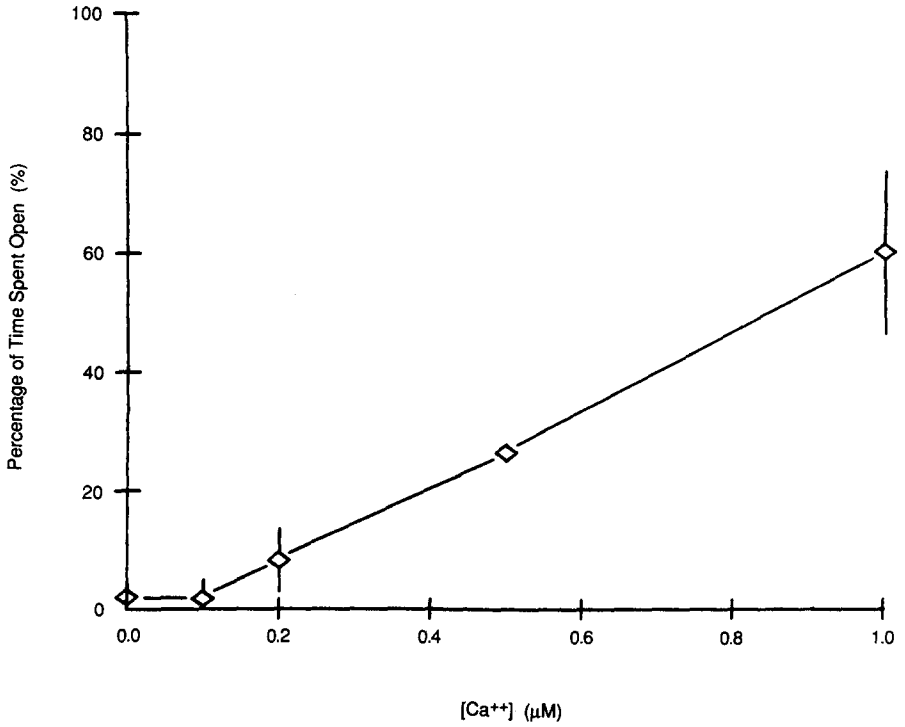

FIGURE 9. Sensitivity of the 80-pS K^+ channel to changes in intracellular $[Ca^{++}]$. Data were collected from six patches of membrane at a pipette potential of 40 mV. Points represent the mean and standard deviation of the percentage of time the channel was open at a given $[Ca^{++}]$. The line between the points was drawn by eye.

"Long-Duration" (LD) Channels

Openings of extremely long duration were characteristic of an ion channel observed in patches of membrane from the soma (n = 10) and the dendritic knob (n = 2) of olfactory receptor neurons (Fig. 15*A*). The activity of the LD channel was most often observed at hyperpolarized membrane potentials. It was not affected by changes in intracellular $[Ca^{++}]$, but compared with cell-attached recordings, the activity of the LD channels in excised patches was more labile and disappeared within minutes.

In both cell-attached and excised patches, the single channel current-voltage relationship for the LD channel appeared to be nonlinear, rectifying at depolarized potentials. This property, or an extremely low opening probability at depolarized potentials, may explain the inability to clearly reverse the direction

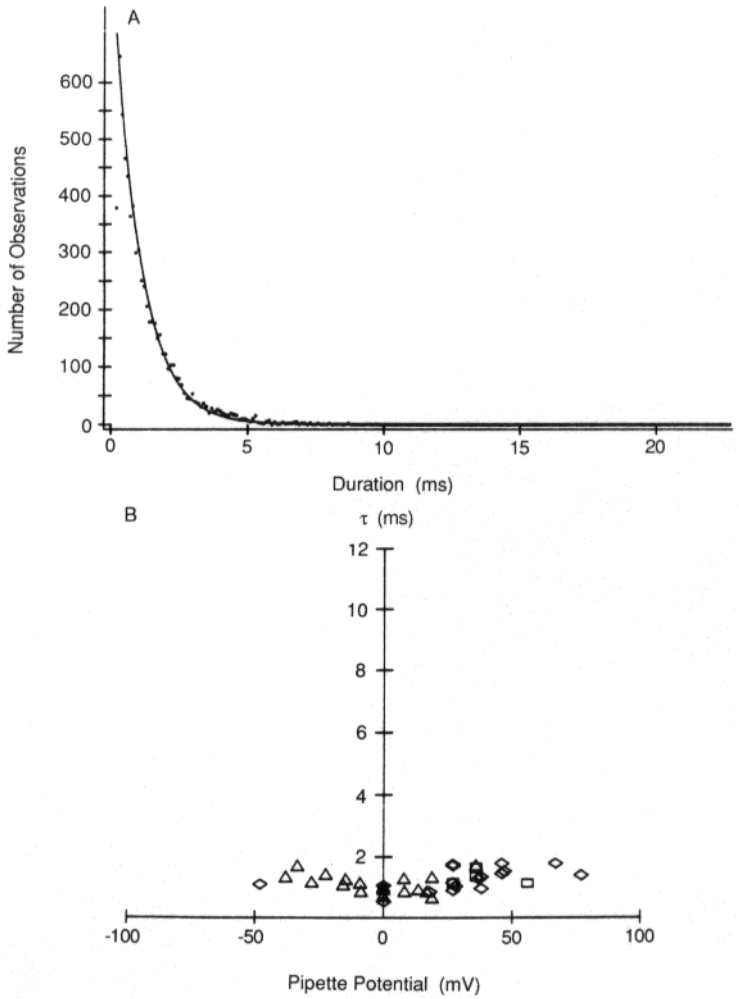

FIGURE 10. Kinetic behavior of the 80-pS channel. (*A*) Open-duration distribution for the 80-pS K^+ channel (n = 6,882). Data were collected from an inside-out patch of membrane with normal saline in the pipette and elevated-K^+ saline in the bath. Pipette potential, 0 mV. (*B*) Data from eight patches of membrane demonstrating the insensitivity of the mean channel open time to either membrane potential or to intracellular [Ca^{++}]: 0.1 (◇), 0.5 (□), and 1.0 (△) μM Ca^{++}.

of current flow through this channel. The conductance of the channel in cell-attached patches with elevated-K^+ saline in the pipette was 20.6 ± 9.4 pS (n = 5), similar to that in excised patches bathed in symmetrical elevated K^+ (29.4 ± 11.4 pS, n = 5) or with normal saline on one side of the membrane (24.9 ± 12.8

pS, $n = 5$). The conductance was similar in patches from the dendritic knob and the soma. Estimates of the reversal potential of the single channel currents were near E_K regardless of the anions present, and shifted with E_K when the $[K^+]$ on one side of the patch was changed ($n = 2$).

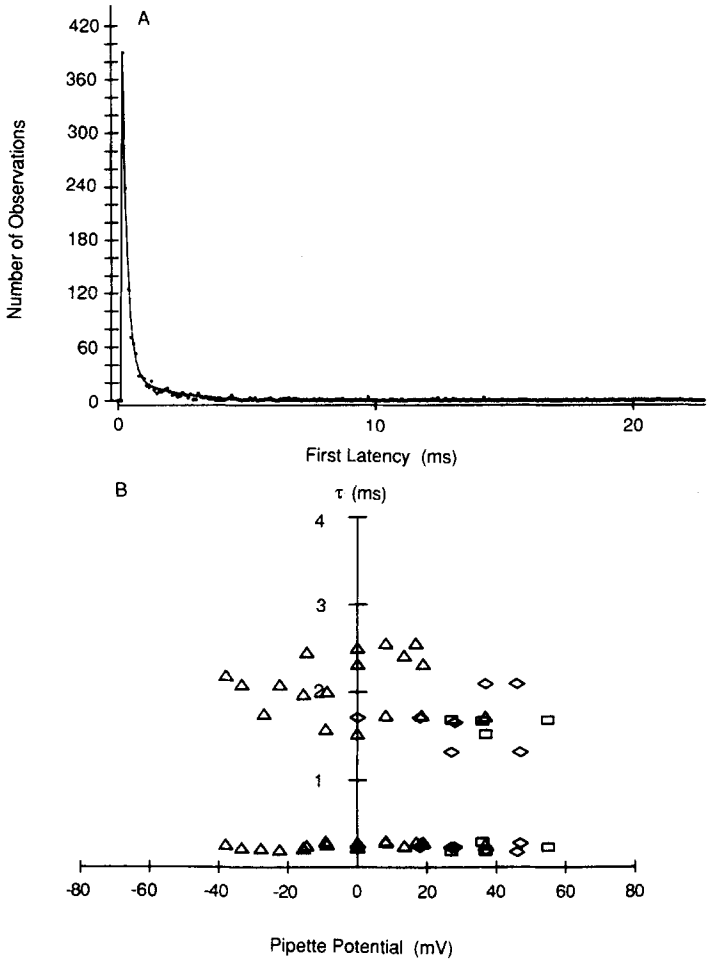

FIGURE 11. Distribution of closed intervals of the 80-pS channel. (*A*) The distribution of closed intervals was well fitted by the sum of two exponentials, with time constants of 0.185 and 1.686 ms ($n = 1,446$). Pipette potential, 27 mV. (*B*) The time constants of the two components were unaffected by changes in either membrane potential or intracellular $[Ca^{++}]$: 0.1 (◇), 0.5 (□), and 1.0 (△) μM Ca^{++}. The data were collected from seven patches of membrane.

The kinetic behavior of the LD channel was its most characteristic feature. It displayed long open durations, often exceeding 100 ms, that were only occasionally interrupted by brief closures (Fig. 15*A*). Estimates of the mean channel open time were 35–50 ms, and exceeded 100 ms at slower sample rates (Fig. 15*B*).

The open time was voltage dependent, increasing e-fold with ~33 mV hyperpolarization (Fig. 15*B*). With an elevated-K^+ saline containing Cs^+ (see Methods) bathing the extracellular surface of the membrane (n = 1), the channel was still active at hyperpolarized potentials, although its conductance was slightly reduced and the openings were 1–10 ms in duration.

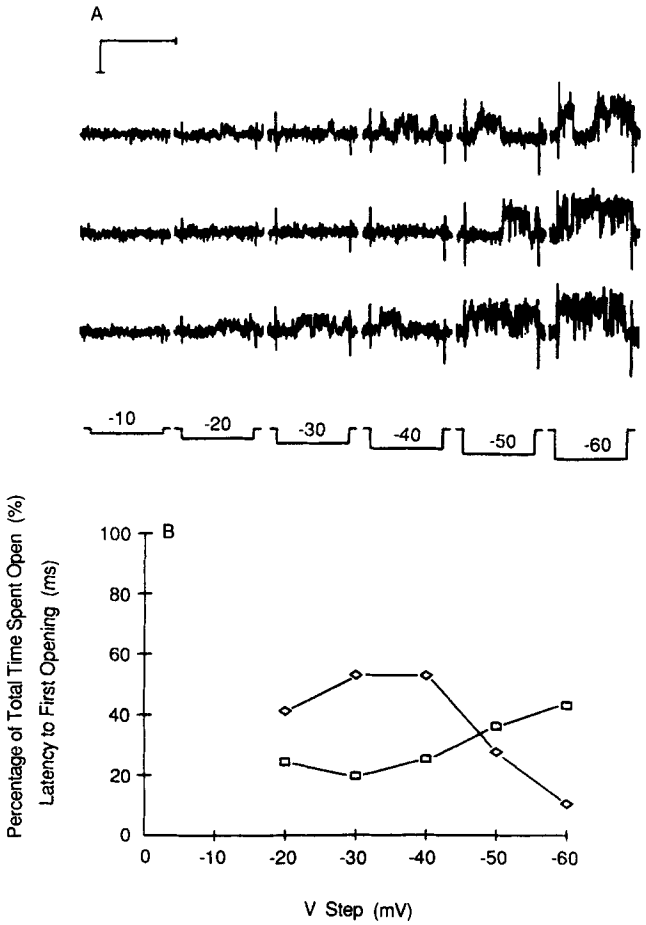

FIGURE 12. Activation of the voltage-activated K^+ channel. (*A*) Recordings from a cell-attached patch stimulated with 200-ms voltage pulses at 1 Hz. The magnitude of the depolarizing steps is indicated below the series of pulses. Records were leak-subtracted using an average response induced by a small, hyperpolarizing voltage pulse. Scale bar, 2 pA, 200 ms. (*B*) As the magnitude of the depolarization was increased, the percentage of time the channel was open (□) increased and the latency to the first opening (◇) decreased.

Cl^- Channels

Large-conductance Cl^--selective channels (210.8 ± 20.5 pS, n = 7) were observed in patches of membrane from the neuronal soma and dendritic knob. With KCl

on both sides of the membrane, the single channel currents reversed near 0 mV. The reversal potential remained unchanged when Na^+ or Cs^+ replaced K^+ on one side of the membrane, but shifted toward E_{Cl} when glutamate was substituted for Cl^- (Fig. 16). The activity of these channels in excised patches was not altered by changes in intracellular [Ca^{++}] (n = 2). The percentage of time the channel was open was greater at more depolarized membrane potentials (e.g., 13% at a pipette potential of −37 mV compared with 61% at −17 mV). The distribution of open durations appeared to be well described by one exponential component,

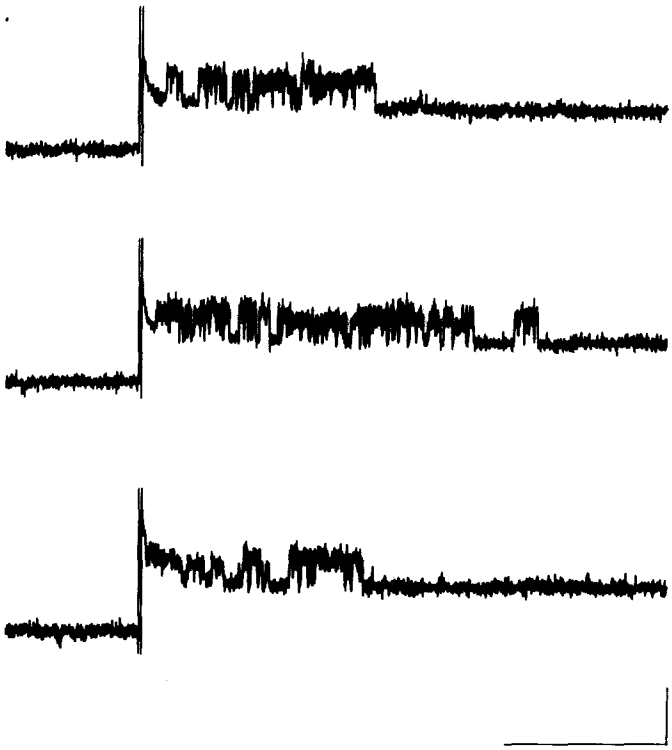

FIGURE 13. Inactivation of the voltage-activated K^+ channel. Current recordings were made in the cell-attached configuration in response to a maintained depolarization (the pipette potential was changed from 56 to −56 mV). The high level of channel activity ceased within ~500 ms. Scale bars, 5 pA, 200 ms.

with a mean channel open time of 3.5–4 ms, varying weakly with voltage. The distribution of closed intervals had at least two components, with time constants of ~0.25 and 2–4 ms.

Receptor Cell Properties

Response to odorant molecules and forskolin. Measurements of single channel activity and holding current in cell-attached patches suggested that the response of receptor neurons to odorants included increases in membrane conductance. Although a more direct assay of the responses would have been to examine the

specific whole-cell currents, difficulty in obtaining stable whole-cell recordings led us to adopt this alternative approach. One advantage of this approach was that the cell remained intact and the cytoplasmic constituents were left undisturbed. Moreover, since factors influencing the activity of a variety of ion

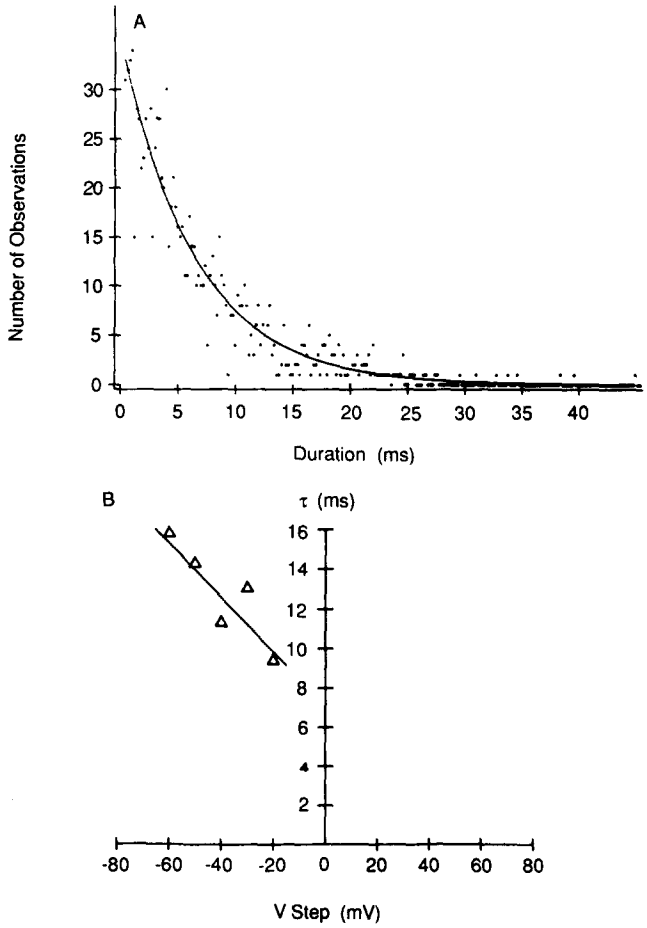

FIGURE 14. Kinetic behavior of the voltage-activated K^+ channel. (*A*) An open-duration distribution from activity recorded at 200-μs intervals (n = 1,067). The recording was made in the cell-attached configuration with the pipette containing normal saline. Pipette potential, −57 mV. Estimated time constant, 6.44 ms. (*B*) When sampled at 1-ms intervals, the apparent mean channel open time increased twofold as the depolarizing voltage step was increased from −20 to −60 mV. With the channel activity sampled at such slow rates, the apparent open times would be described more accurately as the duration of bursts containing very brief (<1 ms) closed gaps.

channels in these cells were known, changes in their activity could provide an indication of changes occurring in the cell.

Application of the standard mixture of odorants to isolated neurons elicited changes in membrane conductance in 15 of 60 cell-attached patches. Odorant

concentrations of 1–50 μM in the puffer pipette induced responses in only 1 of 17 cells, while 100 μM odorants induced responses in 14 of 43 cells. Responses were never observed in excised patches (n = 13), in any of the non-neuronal cells also isolated during the dissociation (n = 5), or with control applications of saline (53 cells).

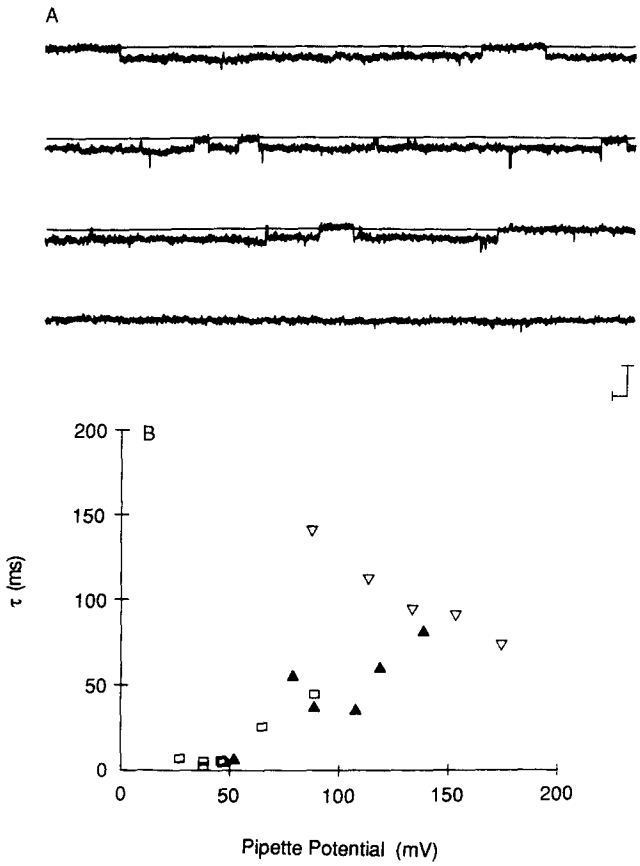

FIGURE 15. Kinetic behavior of the LD channel. (*A*) Four contiguous current records illustrate the long open durations of this channel while recorded in the cell-attached configuration with elevated-K^+ saline in the pipette. Channel openings are indicated by downward deflections in the current traces, and a baseline has been drawn through the data where the channel is closed. Each trace is 860 ms long. Pipette potential, 57 mV. Scale bars, 10 pA, 20 ms. (*B*) Mean channel open time as a function of membrane potential. The data were collected from six patches of membrane. The symbols represent values obtained from recordings digitized at different sample intervals: 100 (□), 200 (▲), and 400 (▽) μs.

The changes that occurred in the monitored patches of membrane when the cells were exposed to odorants included the following. (*a*) There was an inward current of 5–50 pA that began 1–2 s after the application of the odorants and lasted 1–15 s. This current was observed while patches on the soma and the dendritic knob of neurons from adult and from 2-d-old mice were monitored.

Inward currents were observed with either elevated-K^+ saline or normal saline in the pipette. (*b*) There was an increase in the peak-to-peak noise of the holding current. This induced noise had a time course similar to that of the inward current described above. (*c*) There was an increase in the frequency of single channel activity. With elevated-K^+ saline in the recording pipette, the single channel currents were inward. Although these responses were repeatable in a given cell, they usually could be elicited only two to three times before the cell became unresponsive or the recording configuration was lost.

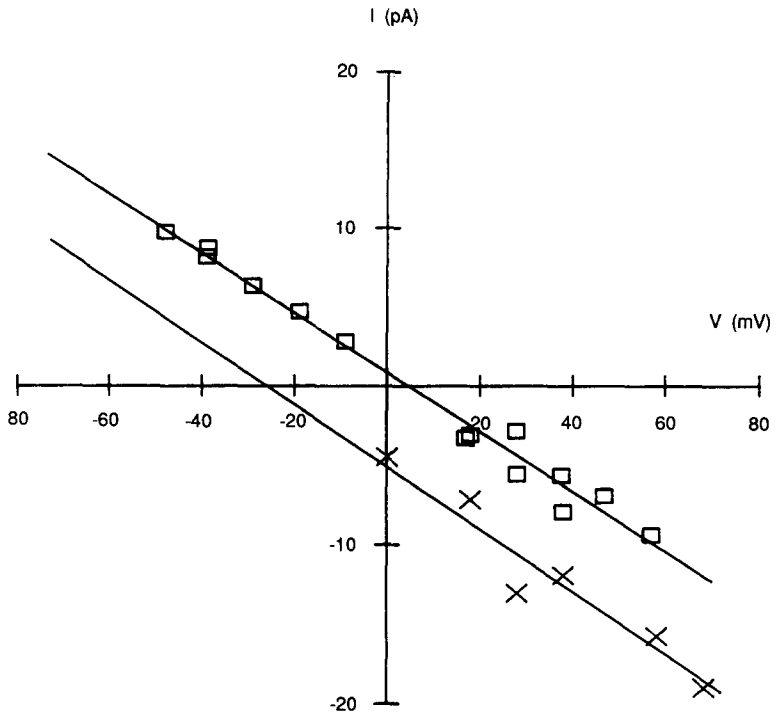

FIGURE 16. Current-voltage relationship for the 200-pS Cl^- channel; data are from five patches. The single channel conductance appeared to be a linear function of membrane potential, and the reversal potential for the currents corresponded to E_{Cl} in either symmetrical or asymmetrical Cl^- solutions. (□) Elevated-K^+ saline in both the pipette and bath. (×) 75% K^+-glutamate plus 25% KCl in the pipette, normal saline in the bath.

To determine whether the odorant-induced responses involved membrane depolarization, 145 mM KCl was substituted for the mixture of odorants in the puffer pipette. In contrast to the odorants, the application of KCl induced an outward current of 2–10 pA and a concomitant decrease in the peak-to-peak noise of the holding current in 9 of 15 patches. The other patches showed no detectable changes. The changes were observed with either elevated-K^+ saline or normal saline in the recording pipette, and could be detected in patches on the soma when KCl was applied to the dendritic knob.

When forskolin and Ro20-1724 were applied to the cells, changes in current were recorded in 2 of 10 cell-attached patches. Although the number of applications that resulted in a change was not dramatic, the changes were similar to those observed in response to odorants: an inward current of 5–30 pA developed, with a latency of 2–3 s and a duration of 25–50 s, and was accompanied by an increase in the peak-to-peak noise. These responses were not observed when saline containing 0.2% ethanol was applied to the neurons ($n = 4$) or forskolin was applied to excised patches of membrane ($n = 2$) or non-neuronal cells ($n = 3$).

Indications of intracellular [Ca^{++}]. The levels of activity of the 80-pS and the 130-pS Ca^{++}-activated K^+ channels in cell-attached patches of membrane suggested that the intracellular [Ca^{++}] in the mouse receptor neurons was below 0.1 μM. This estimate is based upon the percentage of time the 80-pS channel (0.67 ± 1.0%, $n = 3$) and the 130-pS channel (0.68 ± 0.66%, $n = 2$) were open in cell-attached patches and the assumption that the relationship between the percentage of time the channels were open and the [Ca^{++}] was the same for both excised and cell-attached patches.

Estimates of membrane potential. The reversal potential of K^+ channels recorded in the cell-attached configuration with 145 mM K^+ in the pipette suggested that the membrane potential of the mouse receptor neurons was −52 ± 17 mV ($n = 10$), with a range of −30 to −80 mV. The variation in these estimates could be due to an actual variability in the membrane potential of the cells, slight inequalities in the [K^+] on both sides of the membrane during the measurements, and inaccuracies in the estimates of the single channel reversal potential.

Action potentials and single channel activity. Current waveforms associated with action potentials in the receptor neurons were recorded from cell-attached patches of membrane ($n = 6$ cells) (Fig. 17). Similar currents have been recorded from other cell types during a cell-attached recording (Fenwick et al., 1982). The waveforms recorded here were biphasic, with an initial outward component of 1–5 pA lasting 2–5 ms, followed by an inward component of approximately the same amplitude lasting 3.5–8.5 ms (Fig. 17). They occurred spontaneously at infrequent and irregular intervals, although occasionally they occurred in "bursts" of two or three or more. In some cases, their appearance was elicited by rapid hyperpolarizations of the membrane patch.

In some instances, it appeared that the opening of a single ion channel could trigger an action potential in the receptor cell (Fig. 17). Although the channels were open only 1–2% of the total recording time, 78–94% of the action potentials occurred during a channel opening. Furthermore, if the pipette potential was changed to make the inward single channel currents larger, the latency between the opening of the channel and the first action potential–associated currents decreased dramatically, e.g., from ~82 ms when single channel currents were 1.67 pA to ~12 ms when single channel currents were 2.71 pA. As the calculations of Fenwick et al. (1982) suggest, this can occur when the input resistance of the cell is very high.

In other instances, the action potentials also appeared to trigger the opening of single channels in the cell-attached patch. Superimposed upon the latter phases

of the action potential–associated currents were single channel currents (Fig. 17) whose shape suggested they originated in the patch (Fenwick et al., 1982). The opening of these channels appeared to be correlated with the appearance of the action potential–associated waveforms. They did not occur spontaneously or in response to rapid membrane depolarizations with any regularity. Their amplitude and polarity were consistent with those of the Ca^{++}-activated K^+ channels characterized in these cells, and subsequent analysis of the channels in the patches after they were excised confirmed the presence of one of the Ca^{++}-activated channels.

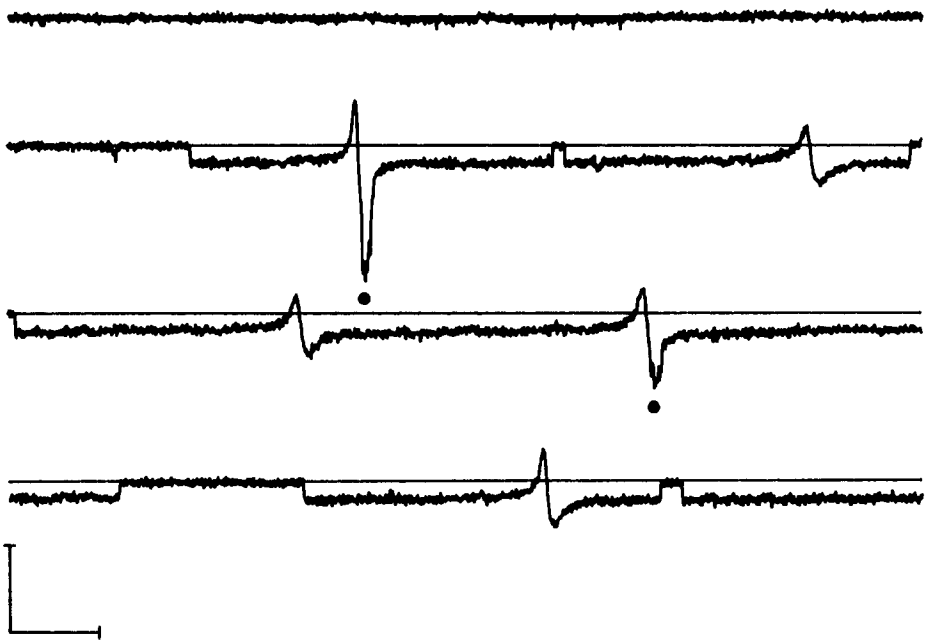

FIGURE 17. Interaction between action potentials and single channel activity. Four contiguous current traces are shown with a baseline drawn where the channels are closed. The openings of the channels (and, in this case, inward currents) are represented by downward deflections from the baseline. Currents associated with action potentials in the receptor cells appear as biphasic waveforms. As described in the text, it appeared that action potentials could be triggered by currents flowing through single channels. In addition, there appeared to be channels in the patch whose openings were temporally associated with the late phase of the action potential (indicated by the filled circles). Elevated-K^+ saline in the pipette. Pipette potential, +37 mV. Scale bars, 10 pA, 20 ms.

DISCUSSION

Specific Types of Ion Channels

The ion channels found in the membranes of mouse olfactory receptor neurons and described here are similar to those found in other excitable cells, and may account for the specific membrane conductances that have been described recently in olfactory neurons of other species.

The 130-pS Ca^{++}-activated K^+ channel has the properties of large-conductance Ca^{++}-activated K^+ channels reported in a variety of cell types (Marty, 1981; Pallotta et al., 1981; Wong et al., 1982; Gallin, 1984; Blair and Dionne, 1985; Findlay et al., 1985; Gardner, 1986), although its single channel conductance is somewhat lower than other values cited (150–240 pS). The infrequent occurrence of sublevels ~40% of the full conductance has been observed in many of these large Ca^{++}-activated channels (Barrett et al., 1982; Gallin, 1984). The kinetic behavior of the 130-pS channel, including both the closed and open intervals and the influence of voltage and $[Ca^{++}]$ upon them, is very similar to that of the channel in cultured skeletal muscle (Magleby and Pallotta, 1983) and chick ciliary neurons (Gardner, 1986). The range of activation of the channel by Ca^{++} is comparable to that described for channels in rat skeletal muscle (Magleby and Pallotta, 1983) and anterior pituitary cells (Wong et al., 1982).

There are few reports of other cells with ion channels that have properties similar to the 80-pS Ca^{++}-activated K^+ channel. The conductance of this channel and its estimated selectivity for K^+ over Na^+ resemble those of channels in kidney cortical tubules (Hunter et al., 1984). Its lower sensitivity to intracellular $[Ca^{++}]$ (when compared with the 130-pS channel) is comparable to that of Ca^{++}-sensitive channels in *Xenopus* neurons (Blair and Dionne, 1985) and rat lacrimal cells (Marty et al., 1984). In addition, the voltage insensitivity of its kinetic behavior is similar to that reported for a Ca^{++}-sensitive K^+ channel in smooth muscle (Benham et al., 1984).

Ca^{++}-dependent K^+ currents have been reported for salamander olfactory receptor neurons (Firestein and Werblin, 1985). In addition, prolonged afterhyperpolarizations, attributed to Ca^{++}-dependent K^+ currents, have also been observed in olfactory neurons from the lobster (Anderson and Ache, 1985). The 130-pS channel or the 80-pS channel or both may represent the unitary conductance mechanisms underlying these currents.

The correlation between openings of Ca^{++}-dependent K^+ channels in cell-attached membrane patches and the "repolarizing phase" of action potential–associated currents (Fig. 17) suggests that repolarization may be an important role of these channels in mouse olfactory receptor neurons. In many excitable cells, this is one role postulated for Ca^{++}-dependent K^+ currents following membrane depolarization and Ca^{++} influx, as may occur during an action potential (Hille, 1984). In bursting neurons, Ca^{++}-activated K^+ channels have been implicated in the control of interspike interval and firing frequency (Hille, 1984). The presence of more than one type of Ca^{++}-sensitive channel in the same cell has been reported in several types of cells (Marty et al., 1984; Peterson and Maruyama, 1984), including sensory cells (Ashmore and Meech, 1986). In most of these cases, the channels often have different Ca^{++} sensitivities and are thought to provide a method of "fine-tuning" the activity of the cell (Peterson and Maruyama, 1984).

Voltage-activated K^+ channels found in the mouse receptor neurons resemble delayed rectifier K^+ channels described in other cell types. Their conductance is similar to that of the delayed rectifier in skeletal muscle (Standen et al., 1985) and, given our estimates of membrane potential, their range of activation is similar to that of the delayed rectifier in NIE-115 cells (Quandt and Narahashi,

1982) and in PCC4 cells (Ebihara and Speers, 1984). The kinetic behavior of the 40-pS channel, including the decrease in the latency of opening and the increase in the time spent open with increasing depolarization, the slow time course of inactivation with prolonged depolarization, and the voltage dependence of the open- and closed-duration distributions, are similar to delayed rectifier channels in frog skeletal muscle (Standen et al., 1985), PCC4 cells (Ebihara and Speers, 1984), and NIE-115 neuroblastoma (Quandt and Narahashi, 1982). At higher rates of stimulation, the activation of the delayed rectifier in frog skeletal muscle became less regular, a phenomenon attributed to a slowly developing inactivation process (Standen et al., 1985); the 40-pS K^+ channel in mouse receptor neurons exhibited similar behavior. The 40-pS K^+ channel may underlie the voltage-activated K^+ current in salamander receptor neurons (Firestein and Werblin, 1985). As in other excitable cells, these channels may act in concert with the Ca^{++}-sensitive K^+ channels to repolarize the cell after an action potential.

We found modest evidence for the existence of Ca^{++} channels in the mouse olfactory neurons. Their small single channel conductance is similar to the 6–7-pS value obtained for Ca^{++} channels in snail neurons using elevated $BaCl_2$ solutions (Brown et al., 1982). The channels described in the present study may be the underlying mechanism of the Co^{++}-sensitive, transient inward current in salamander receptor neurons (Firestein and Werblin, 1985). Although its role in olfactory neurons has yet to be determined, Ca^{++} channel activity appears to be fundamental in many biological responses, including electrical excitability, contraction, and secretion (Hille, 1984).

Mouse olfactory receptor neurons also contained another type of K^+ channel with characteristically long open durations, the LD channel, which was most often observed at hyperpolarized membrane potentials. The single channel conductance of the LD channel (30 pS), the absence of a detectable outward current, the long open durations, and the voltage dependence are similar to features reported for inwardly rectifying K^+ channels in cardiac tissue (Bechem et al., 1983; Sakmann and Trube, 1984; Trube and Heschler, 1984). The labile nature of LD channel activity, especially in excised patches, also appears to be a characteristic property of the inwardly rectifying K^+ channels of cardiac tissue (Bechem et al., 1983; Sakmann and Trube, 1984). This is the first observation of a conductance mechanism active at hyperpolarized membrane potentials in olfactory receptor neurons, and the role these channels have in the receptor cells is unclear. In *Aplysia* neurons (Kandel and Tauc, 1966) and cat spinal cord motor neurons (Nelson and Frank, 1967), inwardly rectifying K^+ conductances have been implicated in the maintenance of resting potential.

Cl^- currents have not been previously described in olfactory receptor neurons. The channel described here does share some of the characteristics of the large-conductance Cl^- channels (200–400 pS) in mouse macrophages, chick myotubes, and cultured rat skeletal muscle (Blatz and Magleby, 1983; Schwarz and Kolb, 1984). It may also resemble Cl^- channels with lower conductances (45–65 pS), since their conductance was found to be a steep function of $[Cl^-]$ and the estimates of single channel conductance were obtained at slightly lower $[Cl^-]$ than used here (Blatz and Magleby, 1985).

Cl^- fluxes have been measured across intact nasal epithelia after their exposure

to particular odorants (Takagi et al., 1966; DeSimone et al., 1985), and it has been suggested that this may be a mechanism for the inhibitory responses of receptor cells (Gesteland et al., 1965). However, the indirect nature of these measurements has made it difficult to identify the cell type in the mucosa responsible for the odorant-induced fluxes, and the role of the Cl^- channels described in these patch-clamp studies remains unclear. Cl^- channels in skeletal muscle have been proposed to stabilize the membrane potential.

Voltage-activated Na^+ channels were not detected in membrane patches of receptor neurons, although there was evidence that action potentials occurred in the isolated cells of the preparation (Fig. 17). Previous studies (Getchell, 1973; Masukawa et al., 1983; Rafols and Getchell, 1983) suggest that Na^+ channels are located primarily in the initial axonal segment at the base of the soma, a region that may be lost upon dissociation or not discernible in many of the isolated mouse neurons. In the studies described here, any focus on specific regions of the cells was directed toward the distal dendritic elements of the receptor cells, a region that prior work has discounted as a possible spike-initiating zone (Getchell, 1973).

Physiological Responses from Isolated Cells

Exposure of the olfactory mucosa in vivo to odorants induces an altered frequency of action potentials in the olfactory neurons sensitive to those particular odorants (Gesteland et al., 1965). Similar results were obtained in the studies of isolated mouse olfactory neurons presented here, as only a portion of the cells tested responded to the odorants. The significance of the exact proportion that responded is unclear, however, since several factors may have influenced the frequency of success in eliciting responses. (*a*) The stimulation cocktail containing four compounds may not have contained an odorant to which many cells respond. (*b*) The cell isolation procedure may have caused damage to receptor proteins. (*c*) The concanavalin A used to anchor the isolated cells to the coverslip may have interfered with some of the responses. Concanavalin A has been shown to interfere with the ability of certain odorants to evoke potential changes across intact epithelia (Shirley et al., 1983; Wood et al., 1983). Although in the studies presented here, most odorant applications were made to receptor cells whose dendritic ending and cilia extended away from the glass and up into the bath solution, it is conceivable the number of cells responding to the odorants was reduced. (*d*) Chemical sensitivity may depend upon the separation of the ionic milieu surrounding the apical portion of the cell from that surrounding the basal part of the receptor neurons. (*e*) A portion of the chemical response might be generated in neighboring non-neuronal cells. Whether or not any of these explanations has merit, the problem of stimulating isolated olfactory neurons is not unique to these studies or to this preparation (Anderson and Ache, 1985; Firestein and Werblin, 1985; Trotier, 1986; Dionne, 1987).

In the isolated receptor neurons that did respond, increases in neuronal membrane conductance were associated with the application of odorants. This is consistent with measurements of impedance changes at the mucosal surface (Gesteland et al., 1965) and intracellular recordings (Trotier and MacLeod, 1983) after exposure of the mucosa to odorants.

The response of the mouse olfactory neurons to odorants also appeared to include membrane hyperpolarization. Although in these studies the cell-attached recording configuration did not allow us to determine whether the odorant-evoked changes were elicited secondarily or were a component of the initial response, odorant-induced increases in the activity of a K^+ channel reconstituted into artificial bilayers has been reported (Vodyanoy and Murphy, 1983). In addition, increased permeability to K^+ and hyperpolarization have been proposed as a mechanism for the inhibitory responses that have been observed in response to odorants (Gesteland et al., 1965).

The specific roles that the channels described in this study have in the odorant response of the receptor neurons remains unknown. The small proportion of cells responsive to the applied odorants, combined with the probability of one of the channels described here being in the patch of membrane being monitored, did not allow us to definitively ascribe a role for these channels in the odorant response. There were no major differences between the spectrum of channels found in somatic membrane and in the dendritic knob. For the 130-pS Ca^{++}-activated K^+ channel and the voltage-activated K^+ channel, at least, there were no differences between the behavior of the channels in neurons from adult mice and those in neurons from embryonic mice; such a difference, if it existed, could explain the prenatal change in the response of mammalian receptor neurons to odorants. It did appear that small depolarizing currents flowing through ion channels were sufficient to elicit action potentials in the mouse receptor cells. This is consistent with intracellular experiments, where injecting picoampere amounts of current could elicit action potentials in frog (Trotier and MacLeod, 1983) and salamander (Masukawa et al., 1985) receptor neurons, and may be a general property of the cells related to their odorant response, since odorant-evoked currents reported in lobster neurons were 5–6 pA (Anderson and Ache, 1985).

Proposals that odorant responses involve intracellular second messengers have been supported by biochemical evidence that odorants increase the activity of adenylate cyclase in olfactory preparations (Pace et al., 1985; Sklar et al., 1986). In the studies presented here, there were indications that the application of forskolin, an activator of adenylate cyclase, mimicked the effects of odorant application. Both responses had roughly the same time course and involved membrane hyperpolarization and increases in membrane conductance. Increases in membrane conductance in response to increased levels of cAMP at the intracellular surface of the membrane have been recorded in isolated patches of membrane from the soma, dendrite, and cilia of bullfrog receptor neurons (Nakamura and Gold, 1987). Since receptor neurons often exhibit low levels of spontaneous activity (Getchell, 1974; Trotier and MacLeod, 1983), which become more regular in response to odorants (Getchell, 1974), perhaps odorants modulate the activity of ion channels via cAMP in a manner comparable to the regulation of activity in other spontaneously active or rhythmically active cells by cAMP (Siegelbaum and Tsien, 1983; Levitan, 1985). K^+ channels are often the point at which cellular activity and responsiveness are modulated (Siegelbaum and Tsien, 1983; Levitan, 1985), and if the response to odorants involves a second messenger, such as cAMP, it would not be unreasonable to expect K^+

channels to be among a spectrum of targets in the cell. The effects that intracellular second messengers such as cAMP, cGMP, ATP, and pH have on K^+ channels include changes in the affinity for Ca^{++}, opening probability, and inactivation (Strong, 1984; Trube and Heschler, 1984; Ewald et al., 1985). The results presented here should provide a useful foundation for future investigations of the membrane conductances of these cells, including the effects of intracellular second messengers on them, and ultimately contribute to an understanding of electrical excitability in these neurons.

This study presents research performed in partial fulfillment of the requirements for the Ph.D. degree in Physiology and Pharmacology at the University of California, San Diego, by R.A.M. The research was supported by the Office of Naval Research, The University of California, and the National Institutes of Health.

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