

Voltage-dependent and Odorant-regulated Currents in Isolated Olfactory Receptor Neurons of the Channel Catfish

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ABSTRACT The electrical properties of olfactory receptor neurons, enzymatically dissociated from the channel catfish (*Ictalurus punctatus*), were studied using the whole-cell patch-clamp technique. Six voltage-dependent ionic currents were isolated. Transient inward currents (0.1–1.7 nA) were observed in response to depolarizing voltage steps from a holding potential of -80 mV in all neurons examined. They activated between -70 and -50 mV and were blocked by addition of $1 \mu\text{M}$ tetrodotoxin (TTX) to the bath or by replacing Na^+ in the bath with *N*-methyl-D-glucamine and were classified as Na^+ currents. Sustained inward currents, observed in most neurons examined when Na^+ inward currents were blocked with TTX and outward currents were blocked by replacing K^+ in the pipette solution with Cs^+ and by addition of 10 mM Ba^{2+} to the bath, activated between -40 and -30 mV, reached a peak at 0 mV, and were blocked by $5 \mu\text{M}$ nimodipine. These currents were classified as L-type Ca^{2+} currents. Large, slowly activating outward currents that were blocked by simultaneous replacement of K^+ in the pipette with Cs^+ and addition of Ba^{2+} to the bath were observed in all olfactory neurons examined. The outward K^+ currents activated over approximately the same range as the Na^+ currents (-60 to -50 mV), but the Na^+ currents were larger at the normal resting potential of the neurons (-45 ± 11 mV, mean \pm SD, $n = 52$). Four different types of K^+ currents could be differentiated: a Ca^{2+} -activated K^+ current, a transient K^+ current, a delayed rectifier K^+ current, and an inward rectifier K^+ current. Spontaneous action potentials of varying amplitude were sometimes observed in the cell-attached recording configuration. Action potentials were not observed in whole-cell recordings with normal internal solution ($\text{K}^+ = 100 \text{ mM}$) in the pipette, but frequently appeared when K^+ was reduced to 85 mM . These observations suggest that the membrane potential and action potential amplitude of catfish

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olfactory neurons are significantly affected by the activity of single channels due to the high input resistance ($6.6 \pm 5.2 \text{ G}\Omega$, $n = 20$) and low membrane capacitance ($2.1 \pm 1.1 \text{ pF}$, $n = 46$) of the cells. Stimulation of voltage-clamped receptor neurons with a mixture of amino acids ($100 \mu\text{M}$ each of L-arginine, L-alanine, and L-norleucine with or without L-glutamate), which act at independent classes of receptor sites, elicited receptor currents that were heterogeneous in terms of voltage and ion dependence, time course, reversal potential, and sensitivity to drugs, and appeared to result from at least two different processes. These different types of receptor cell responses to odorant amino acids may reflect activation of different transduction pathways.

INTRODUCTION

Olfactory neurons transduce chemical information into electrical signals (action potentials) which convey information to the olfactory bulb (Getchell, 1986; Lancet, 1986). It is believed that binding of chemical stimuli to specific olfactory receptor proteins located on the plasma membranes of the cilia protruding from the apical ends of the olfactory neurons leads to stimulation of at least two different G-protein-linked second messenger pathways: the formation of cAMP (Pace, Hanski, Salomon, and Lancet, 1985; Sklar, Anholt, and Snyder, 1986; Boekhoff, Tarelius, Strotmann, and Breer, 1990; Breer, Boekhoff, and Tarelius, 1990; Bruch and Teeter, 1990) and the breakdown of phosphatidylinositol-4,5-bisphosphate to inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (Huque and Bruch, 1986; Boekhoff et al., 1990). The concomitant increase in intraciliary cAMP or IP_3 concentrations has been postulated to lead to opening of cAMP (Nakamura and Gold, 1987; Suzuki, 1989, 1990; Frings and Lindemann, 1990; Firestein, Zufall, and Shepherd, 1991; Firestein, Darrow, and Shepherd, 1991) or IP_3 -gated cation channels (Restrepo, Miyamoto, Bryant, and Teeter, 1990; Suzuki, 1992), causing cell depolarization and triggering the discharge of action potentials.

Biochemical studies by Boekhoff and co-workers (1990) suggest that, in the rat, individual chemical stimuli activate either the cAMP or the IP_3 second messenger pathway exclusively. If this premise is true, and since these two second messenger pathways (IP_3 and cAMP) appear to gate different ion channels, stimulation of olfactory neurons with different chemical stimuli should lead to activation of currents with different characteristics. An appropriate model system for the study of the heterogeneity of the response of olfactory receptor neurons to stimulation by diverse stimuli is the channel catfish (*Ictalurus punctatus*) which can detect L-amino acids at nanomolar concentrations (Caprio, 1978) through the use of olfactory receptor proteins specific for amino acids with different chemical structures (Caprio and Byrd, 1984; Bruch and Rulli, 1988).

In this paper we describe the voltage-gated currents in isolated olfactory neurons from the channel catfish using the whole-cell patch-clamp technique (Hamill, Marty, Neher, Sakmann, and Sigworth, 1981) and we present an initial characterization of the properties of the ionic currents triggered by stimulation with L-amino acid olfactory stimuli. We find that catfish olfactory neurons possess a variety of voltage-dependent currents including a tetrodotoxin (TTX)-sensitive Na^+ current, a slowly inactivating Ca^{2+} current, a delayed rectifier K^+ current, a transient K^+ current, a

Ca²⁺ activated K⁺ current, and an inward rectifier K⁺ current. In addition, we find that stimulation with a mixture of L-amino acids leads, in different cells, to the activation of currents which can be distinguished in terms of their voltage dependence.

A preliminary report of some of this work has been published elsewhere (Restrepo et al., 1990).

METHODS

Channel catfish, *Ictalurus punctatus*, were obtained from commercial suppliers and maintained in 250-gallon aquaria under dim artificial light (12 L:12 D light cycle) at 17°C. Fish were fed commercial pellets and maintained for up to 2 mo. Catfish were humanely killed, the nasal cavities were opened, and the olfactory epithelia were quickly removed. The epithelium were cut into ~2 × 2-mm pieces and incubated for 15 min at room temperature in divalent cation-free Ringer solution containing L-cysteine-activated papain (15 U/ml) and 2 mM EDTA. The tissue was rinsed three times with normal Ringer solution and gently triturated with a pasteur pipette. A 0.2-ml aliquot of the cell suspension was placed on a glass coverslip which formed the bottom of the experimental chamber. The chamber was attached to the fixed stage of an inverted microscope (model IM; Carl Zeiss, Inc., Thornwood, NY) equipped with phase contrast optics. Once cells had settled on the bottom of the chamber and a tight seal had been established on a cell, the chamber was continuously perfused with normal Ringer solution. Isolated olfactory neurons were readily distinguished from other types of cells, e.g., respiratory and basal cells, on the basis of their characteristic morphology (Canalón, 1978; Restrepo and Teeter, 1990). Catfish olfactory neurons can be classified into two types according to the processes extending from the olfactory knob (ciliated and stellate microvillous cells) (Erickson and Caprio, 1984). Because these features are difficult to distinguish at the light microscopic level, we did not attempt to differentiate between the two cell types. The cell suspension was stored on ice for up to 5 h before use.

Patch-Clamp Recordings

Membrane current and voltage were measured in isolated cells using the whole-cell configuration of the patch-clamp technique (Hamill et al., 1981) with an Axopatch 1B amplifier (Axon Instruments, Inc., Burlingame, CA). The current signal was filtered at 1 kHz, digitized at 20–40 kHz, and stored on an AT-compatible microcomputer running pCLAMP software (Axon Instruments, Inc.), which was also used to control the D/A converter for generation of clamp protocols. No further processing was done on the current traces shown in the figures. However, in some cases when the records were noisy, i.e., Fig. 12A, several time points were averaged or the currents were subjected to additional filtering using pCLAMP software to determine peak current magnitudes for the *I-V* relationships. Care was taken to choose a time interval brief enough so that variations of current with time did not alter the calculated current value by a significant amount.

Patch electrodes with resistances of 7–20 MΩ were fabricated from borosilicate glass capillaries with a Sutter P-80/PC electrode puller (Sutter Instrument Co., San Raphael, CA). Gigaohm seals were readily obtained with most cells by applying weak negative pressure (10–30 cm H₂O). The whole-cell configuration was attained by application of brief voltage pulses and additional negative pressure. The duration and strength of the negative pressure pulses were controlled by electromagnetic valves and a vacuum-aspirator. Leakage currents, generated by applying 1/10 amplitude hyperpolarizing voltage steps which did not activate voltage-dependent currents, were multiplied by 10 and subtracted from the current records generated

by depolarizing voltage steps. Pipette and membrane capacitance and series resistance were compensated electronically. Typical series resistance values after compensation were 10 M Ω . Cell capacitance was read directly from the amplifier after cancellation of the capacitive transients induced by 10-mV hyperpolarizing voltage pulses from a holding potential of -60 mV. Input resistance was calculated from the slope conductance of the current generated by a 0.18 V/s voltage ramp from -60 to -80 mV.

Solutions and Stimulus Application

Normal Ringer solution contained (mM): 100 NaCl, 3.5 KCl, 1.0 CaCl₂, 1.6 MgCl₂, and 10 HEPES-NaOH (pH 7.5). Ba²⁺, Co²⁺, tetraethylammonium⁺ (TEA⁺), and *N*-methyl-D-glucamine⁺ (NMDG⁺) were substituted for Na⁺ in normal Ringer solution, while TTX, apamin, charybdotoxin (CTX), and 4-aminopyridine (4AP) were simply added. The Ca²⁺-free Ringer solution contained 1 mM EGTA and no added Ca²⁺.

Patch pipettes were usually filled with a K⁺ internal solution containing (mM): 100 KCl, 0.1 CaCl₂, 2.0 MgCl₂, 2.0 Na₂ adenosine triphosphate (ATP), 1.1 EGTA, and 10 HEPES-KOH (pH 7.5), or a Cs⁺ internal solution that had the same composition as the K⁺ solution except that Cs⁺ was substituted for K⁺. Occasionally, 5–30 mM KCl or CsCl in the pipette solution was replaced with equimolar amounts of KF or CsF (Matteson and Armstrong, 1986; Hernandez-Cruz and Pape, 1989) and/or 200 μ M leupeptin was added (Huang, 1989) in an effort to minimize "run down" of currents.

The amino acid stimulus consisted of a mixture of 100 μ M each of L-alanine (L-Ala), L-arginine (L-Arg), and L-norleucine (L-Nle), with or without L-glutamate (L-Glu) in normal Ringer solution. The stimulus was applied at 20-s intervals by pressurizing a pipette containing the amino acid mixture and positioned close to the isolated neuron for 200 ms. Pressure pulses were generated using a computer-controlled Picospritzer (General Valve Corp., East Hanover, NJ) and were in the range from 2 to 5 psi. The tips of the stimulus pipettes ranged from 2 to 5 μ m. The latency of odorant-induced responses, although variable from cell to cell, was quite constant for a particular neuron and stimulus pipette placement.

RESULTS

Passive Membrane Properties

Gigaohm seals were easily obtained by applying weak negative pressure to the recording pipette. The probability of gigaseal formation was nearly 100%. Rupture of the membrane to attain the whole-cell configuration required not only additional suction (up to 2 m H₂O), but brief voltage pulses. Stable whole-cell recordings were obtained in ~50% of the attempts. Once the whole-cell configuration was obtained, a hyperpolarizing DC potential was applied until no net current was observed. This potential, relative to the bath, was taken as the resting membrane potential of the cell and varied from -25 to -80 mV (-45 \pm 11 mV, mean \pm SD, n = 52). The input resistance, which was estimated from the slope conductance induced by a 0.18 V/s voltage ramp from -60 to -80 mV, ranged from 1 to 14 G Ω (6.6 \pm 5.2 G Ω , n = 20). This value can be considered a lower limit for the membrane resistance assuming a large pipette-membrane seal resistance. The membrane capacitance averaged 2.1 \pm 1.1 pF (n = 46).

Isolated catfish olfactory neurons have a characteristic bottle shape and are 7–25 μ m long from the vesicle knob on the dendritic end to the base of soma (Cancalon, 1978). Approximation of the soma as a 6- μ m-diam sphere and the dendrite as a

5- μm -long, 2.8- μm -diam cylinder yields an approximate surface area of 194 μm^2 . In addition, catfish olfactory receptor neurons possess either 4–8 cilia (3–8 μm long and 0.3 μm in diameter) or 15–25 microvilli (2–6 μm long and 0.1 μm in diameter) extending from the apical end of the dendrite (Cancalon, 1978; Erickson and Caprio, 1984). These processes add $\sim 25 \mu\text{m}^2$ to the estimate of the area, yielding a total estimated surface area of 219 μm^2 . Assuming a specific membrane capacitance of 1 $\mu\text{F}/\text{cm}^2$ (Hille, 1984), the measured value of whole-cell capacitance (2.1 pF) is consistent with the estimate of cell surface area. These results indicate that catfish olfactory receptor neurons are small, electrically compact cells. Consequently, small, brief changes in membrane conductance would result in rapid changes in membrane potential.

Voltage Responses under Current Clamp

Spontaneous spike-like currents were frequently observed in cell-attached patches (Fig. 1 A). The amplitude of these currents varied markedly from cell to cell and over

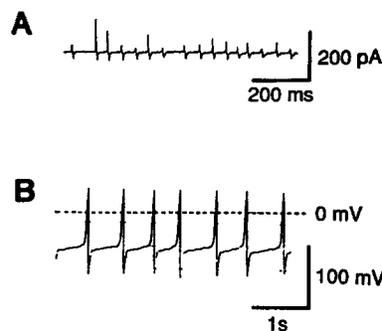


FIGURE 1. Spontaneous action potentials. (A) Spike-like currents recorded from a cell-attached patch. The holding potential was 0 mV. Pipette: Cs^+ internal solution. Bath: normal Ringer solution. Note that current amplitude displayed large variation. (B) Membrane potential recorded under whole-cell current clamp. In this cell, the resting potential was -52 mV immediately after rupture of the membrane. Pipette: 85 mM K^+ internal solution. Bath: normal Ringer solution.

time in the same cell. Spontaneous action potentials were not observed under whole-cell current clamp when the pipette contained the standard solution with 100 mM K^+ . However, spontaneous action potentials were often recorded from cells with resting potentials more negative than -40 mV when the K^+ concentration in the pipette was reduced to 85 mM (Fig. 1 B), resulting in a slight positive shift of E_{K} . The membrane potential frequently showed marked fluctuations resulting in continuously changing spike amplitudes. These fluctuations in membrane potential were sometimes suppressed by Ca^{2+} -free bath solution. The spontaneous discharge of action potentials typically ceased within a few minutes of establishing the whole-cell recording configuration. The neurons were depolarized by addition of 10 mM Ba^{2+} , 10 mM TEA^+ , or 10 mM 4AP to the bath, with Ba^{2+} having the largest effect. Neither apamin (50–250 nM) nor CTX (20–200 nM) altered the membrane potential.

Whole-Cell Currents

All voltage-clamped olfactory receptor neurons displayed time- and voltage-dependent inward and outward membrane currents in response to depolarizing voltage

steps (Fig. 2A). Small, hyperpolarizing voltage steps elicited only capacitive currents and small, sustained leakage currents, which were subtracted from the records unless otherwise noted. The transient inward currents activated between -70 and -50 mV, reached a peak at about -20 mV, and rapidly inactivated. Delayed outward currents were activated at slightly more positive potentials (-60 to -50 mV) and displayed little inactivation during 50-ms depolarizing steps. Although both

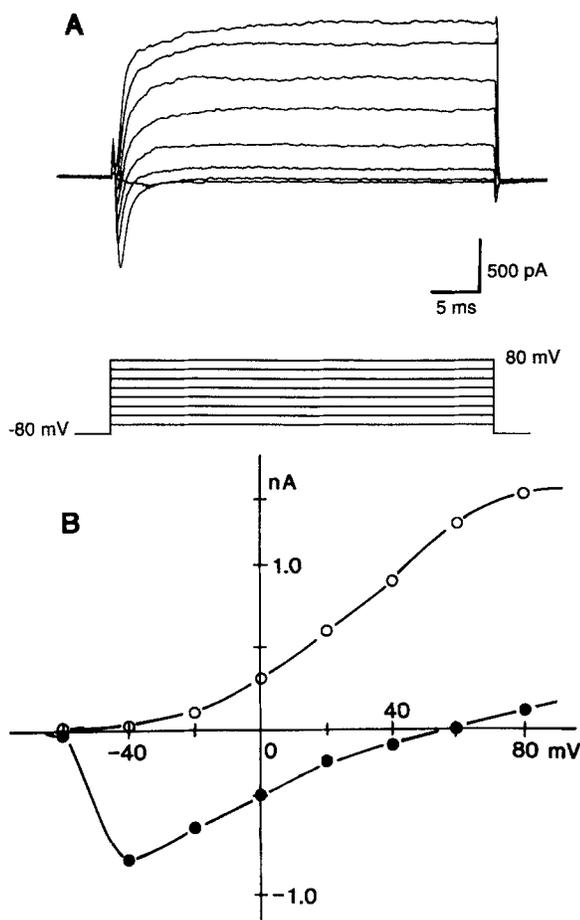


FIGURE 2. Typical whole-cell currents in an isolated catfish olfactory receptor neuron. (A) Transient inward and delayed outward currents in an olfactory receptor neuron in response to 40-ms voltage steps between -60 and $+80$ mV in 20-mV increments from a holding potential of -80 mV. Records are uncorrected for leakage and capacitive currents. (B) I - V relationships of peak inward current (filled circles) and steady-state outward current (open circles) from the records in A. Pipette: K^+ internal solution. Bath: normal Ringer solution.

inward and outward currents were activated at similar potentials, the amplitude of the inward current was always considerably larger than that of the outward current between -50 and -40 mV, the normal range of resting potential for these cells (Fig. 2B). Specific blocking agents, ion substitution, and various pulse protocols were used to isolate and partially characterize six components from the total voltage-dependent membrane currents.

Na⁺ Currents

Sodium currents were examined in the absence of large outward currents by substituting Cs⁺ for K⁺ in the pipette solution and addition of 10 mM Ba²⁺ to the bath. Inward Ca²⁺ currents were blocked by 2 mM Co²⁺ in the bath. Under these conditions, only transient inward currents were observed in response to depolarizing voltage steps (Fig. 3 *A*). The transient inward currents activated between -70 and

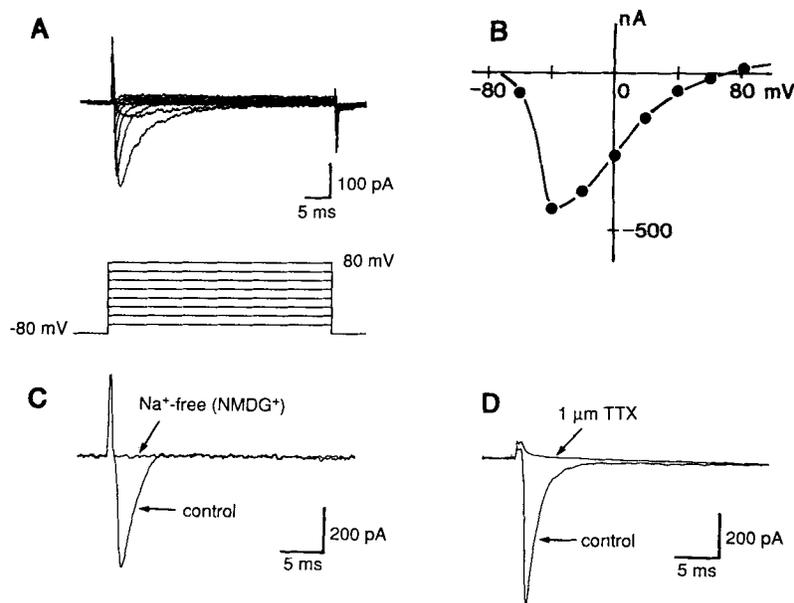


FIGURE 3. Na⁺ currents. (*A*) Transient inward currents elicited by 40-ms depolarizing voltage steps between -60 and +80 mV in 20-mV increments from a holding potential of -80 mV. Outward currents were blocked by substitution of Cs⁺ for K⁺ in the pipette. (*B*) *I-V* relationship for peak inward currents shown in *A*. Note that activation began at -60 mV and the current reached a maximum at -40 mV. Pipette: Cs⁺ internal solution. Bath: normal Ringer solution. (*C*) Transient inward currents are carried by Na⁺. Replacement of Na⁺ in the bath solution with NMDG⁺ blocked transient inward currents. Superimposed records of currents responses to 40-ms depolarizing voltage steps to -40 mV from a holding potential of -80 mV in normal and Na⁺-free Ringer solutions. Na⁺ was replaced with NMDG⁺. Pipette: Cs⁺ internal solution. (*D*) Effect of 1 μM TTX. Superimposed recording of responses to 40-ms depolarizing voltage steps to -40 mV from a holding potential of -80 mV before and after addition of 1 μM TTX to the bath. Pipette: Cs⁺ internal solution.

-50 mV and reached maximum amplitude, ranging from 0.1 to 1.7 nA, at -30 to -10 mV (Fig. 3 *B*). These currents were completely blocked when Na⁺ in the bath was replaced with NMDG⁺ (Fig. 3 *C*) or by addition of 1 μM TTX to the bath (Fig. 3 *D*).

The onset of the Na⁺ current, particularly with large voltage steps, was sometimes masked by the residual capacitive transient, precluding a detailed study of activa-

tion kinetics. However, the voltage dependence of inactivation and the time course of recovery from inactivation were examined. Steady-state inactivation curves were constructed from plots of normalized peak inward currents, generated by 40-ms depolarizing pulses to -20 mV from various holding potentials, as a function of holding potential (Fig. 4A). Inactivation was half-maximal at -62 ± 3 mV ($n = 6$) and complete at about -30 mV. This indicates that at the average resting potential of -45 mV, $< 10\%$ of the sodium channels in isolated catfish olfactory receptor neurons (which are axotomized during isolation) are available to activate and contribute to the generation of action potentials. Although the measured membrane potential is

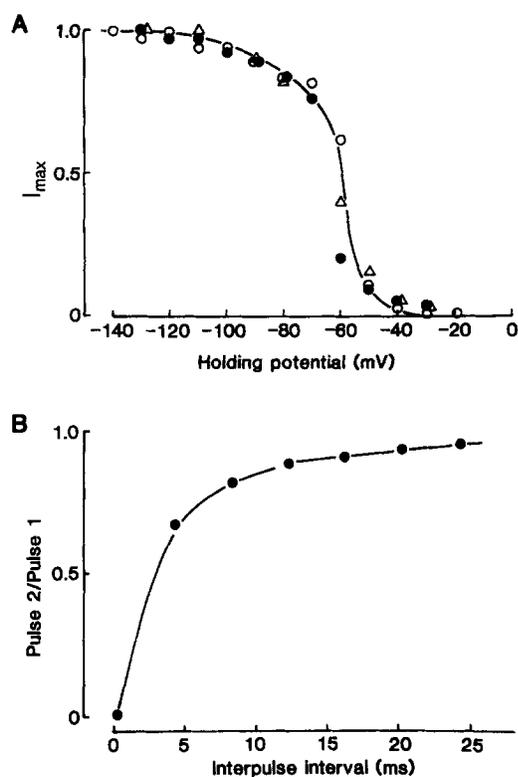


FIGURE 4. Properties of inactivation of the Na^+ current. (A) Voltage dependence of Na^+ current inactivation. The steady-state inactivation curve was obtained by plotting the normalized peak inward currents (I/I_{max}) generated by 40-ms depolarizing voltage steps to -20 mV from various holding potentials as a function of holding potential. Pipette: Cs^+ internal solution. Bath: normal Ringer solution. (B) Recovery from inactivation of Na^+ current in a different cell. Pairs of 4-ms voltage pulses from -80 to 0 mV were applied at different intervals. The time constant of recovery was 4 ms in this cell. Pipette: K^+ internal solution. Bath: normal Ringer solution.

probably a low estimate of the actual membrane potential because of the relatively low resistance of the seal (see Discussion), this suggests that the spontaneous action currents recorded extracellularly in the cell-attached configuration and the spontaneous action potentials sometimes observed under whole-cell current clamp do not depend entirely on Na^+ currents (Fig. 1).

Recovery of the Na^+ currents from inactivation was examined using a double-pulse protocol where brief pulses to 0 mV from a holding potential of -80 mV were separated by intervals of varying duration. The time constant of recovery was estimated from plots of the ratio of amplitudes of pulse 2 to pulse 1 as a function of interpulse interval (Fig. 4B). Recovery from inactivation was rapid, with a time

constant averaging 2.6 ± 0.9 ms in four cells (4 ms in the example in Fig. 4 B). Sodium currents typically decreased in amplitude within 30 min of establishing the whole-cell configuration, even when 2 mM ATP, 200 μ M leupeptin, and/or 5–30 mM F^- were included in the pipette solution.

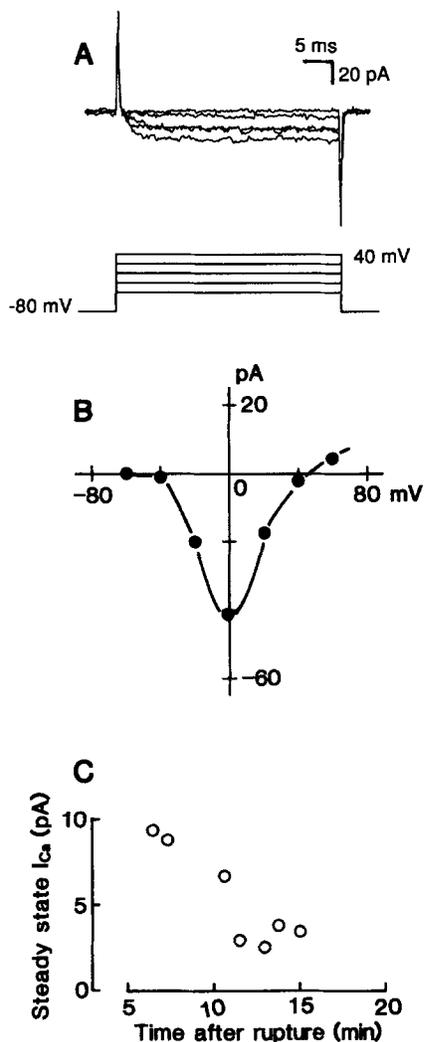


FIGURE 5. Ca^{2+} currents. (A) Sustained inward current elicited by 40-ms depolarizing voltage steps between -40 and $+40$ mV in 20-mV increments from a holding potential of -80 mV. Outward currents were blocked by replacing K^+ in the pipette with Cs^+ and addition of 10 mM Ba^{2+} to the bath. Sodium currents were blocked by replacing Na^+ in the bath with NMDG $^+$. (B) $I-V$ relationship for sustained inward currents shown in A. (C) Run down of Ca^{2+} current over time in another cell. Ca^{2+} current was evoked by a voltage ramp from -60 to $+60$ mV (0.18 V/s). The current amplitude recorded 15 min after rupture of the patch membrane was about one-third of that at 5 min after rupture. Pipette: Cs^+ internal solution. Bath: normal Ringer solution containing 10 mM Ba^{2+} , 10 mM TEA $^+$, and 2 μ M TTX. The holding potential was -60 mV.

Ca^{2+} Currents

When K^+ was replaced with Cs^+ in the pipette, and NMDG $^+$ and 10 mM Ba^{2+} were substituted for Na^+ in the bathing solution, small sustained inward currents were observed in response to depolarizing voltage steps (Fig. 5). The current was blocked by addition of 2 mM Co^{2+} or 5 μ M nimodipine to the bath solution (not shown). The maximum amplitude of the current was -50 ± 31 pA ($n = 4$) with 10 mM Ba^{2+}

present in the bath. The time constant of decay for these currents was >2 s and current amplitude was independent of holding potential in Ringer solution containing 10 mM Ba^{2+} . These characteristics are consistent with the sustained inward current being mediated by L-type Ca^{2+} channels. Ca^{2+} currents activated between -40 and -30 mV and reached a maximum at 0 mV (Fig. 5 B). The currents frequently became smaller over time and often completely washed out within 10 min after rupture of the patch membrane, even when the pipette contained 2 mM ATP, 200 μM leupeptin, and/or 5–30 mM F^- . A typical example of the time course of wash out is shown in Fig. 5 C. In this case, the steady-state current amplitude recorded 15 min after attaining the whole-cell configuration was one-third of that observed 5 min after rupture of the cell membrane. Consequently, experiments to examine Ca^{2+} currents were completed within 10 min of rupturing the patch membrane.

K⁺ Currents

Four K^+ currents were distinguished on the basis of their time, voltage, and ion dependence and pharmacological profiles; a Ca^{2+} -activated K^+ current, a transient K^+ current, a delayed rectifier K^+ current, and an inward rectifier K^+ current.

Ca²⁺-activated K⁺ Current

Voltage-dependent outward currents were usually reduced when Ca^{2+} was removed from or 2 mM Co^{2+} was added to the bath solution (Fig. 6). Sustained outward currents recorded in normal Ringer and Ringer containing 2 mM Co^{2+} and with Na^+ replaced by NMDG⁺ are shown in Fig. 6 A. The differences between the currents before and after exchange of the bath solutions are displayed in Fig. 6 A, 3 and plotted as a function of pulse potential in Fig. 6 B. A clear region of negative slope is apparent in the I - V relationship, suggesting activation of part of the outward current by Ca^{2+} . Unlike outward currents in some cells, which activated between -40 and -30 mV and continued to increase with increasing depolarization (Fig. 2), Ca^{2+} -dependent outward currents (i.e., those blocked by Ca^{2+} -free bath solution or addition of Co^{2+}) reached a clear maximum at $+20$ to $+30$ mV and displayed a negative slope region at voltages greater than $+30$ mV (Fig. 6 B). In some cells, a clear decrease in the amplitude of the outward currents produced by depolarization >40 mV could be observed directly (Fig. 6, C and D). The Ca^{2+} -activated K^+ current was, like the sustained inward Ca^{2+} current, quite susceptible to wash out over time and the peak outward current in these cells also decreased markedly within minutes of establishing the whole-cell configuration. The Ca^{2+} -activated outward currents were suppressed by addition of 20 nM CTX or 250 nM apamin, but not by 50 nM apamin (not shown). Ca^{2+} -activated K^+ currents were observed more frequently in the summer. The significance of this seasonal variation remains uncertain. Taken together, these results indicate the presence of large or intermediate conductance Ca^{2+} -activated K^+ channels (Castle, Haylett, and Jenkinson, 1989) in catfish olfactory receptor neurons.

Transient K⁺ Current

When voltage-dependent inward currents and Ca^{2+} -activated K^+ currents were blocked with Ca^{2+} -free bath solution containing 2 mM Co^{2+} and 1 μM TTX,

depolarizing pulses elicited outward K^+ currents consisting of both transient and sustained components. In almost all of the neurons examined, transient outward currents were observed that activated quickly and declined rapidly in amplitude to a nonzero steady-state level during a 2-s voltage pulse (Fig. 7, *A* and *B*). The half-time ($t_{1/2}$) for the decay was independent of pulse potential and ranged from 350 to 450

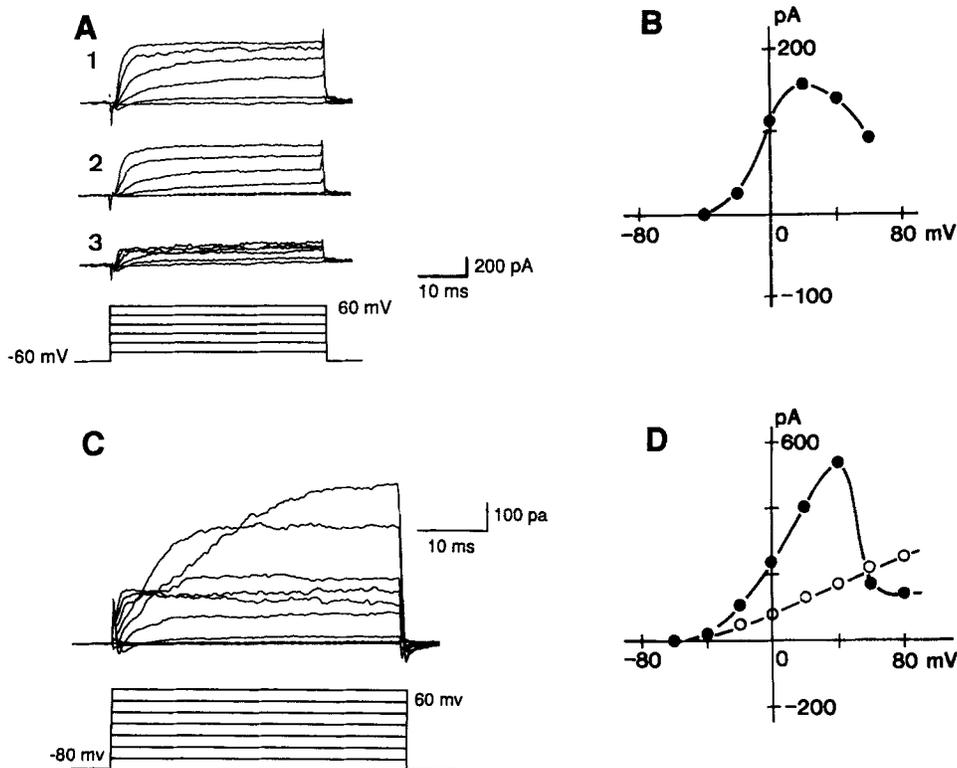


FIGURE 6. Ca^{2+} -activated K^+ currents. (*A*) Currents elicited by 40-ms depolarizing voltage steps between -40 and $+60$ mV in 20-mV increments from a holding potential of -60 mV in normal Ringer (1), in 2 mM Co^{2+} , Na^+ -free solution (2), and the difference between currents 1 and 2 (3). (*B*) $I-V$ relationship obtained from the records in *A*, 3. Outward current displayed a clear peak at 20 mV. Pipette for *A* and *B*: K^+ internal solution. (*C*) Outward currents elicited by 40-ms depolarizing voltage steps between -60 and $+80$ mV in 20-mV increments from a holding potential of -80 mV in a different cell (4 GΩ input resistance). (*D*) $I-V$ relationship for the same cell as in *C*. Current was measured at the end of the voltage pulses. Pipette for *C* and *D*: K^+ internal solution. Bath for *C* and *D*: normal Ringer solution (filled circles) and Ca^{2+} -free Ringer containing 2 mM Co^{2+} (open circles).

ms. Both the transient and sustained components of the outward currents recorded in Ca^{2+} -free Ringer containing 2 mM Co^{2+} were blocked by addition of 10 mM 4AP to the bath (not shown).

The transient component of outward current was inactivated by conditioning prepulses to voltages more positive than -40 mV. This voltage-dependent inactiva-

tion of the transient component was examined using the protocol illustrated in Fig. 8 *A*. Inactivation at holding potentials more positive than -40 mV was steep (Fig. 8 *B*), suggesting a similar voltage dependence to that of the inactivation of inward Na^+ current (Hille, 1984). On the basis of their voltage- and time-dependent inactivation and sensitivity to 4AP, these currents were classified as a type of A current (Hille, 1984).

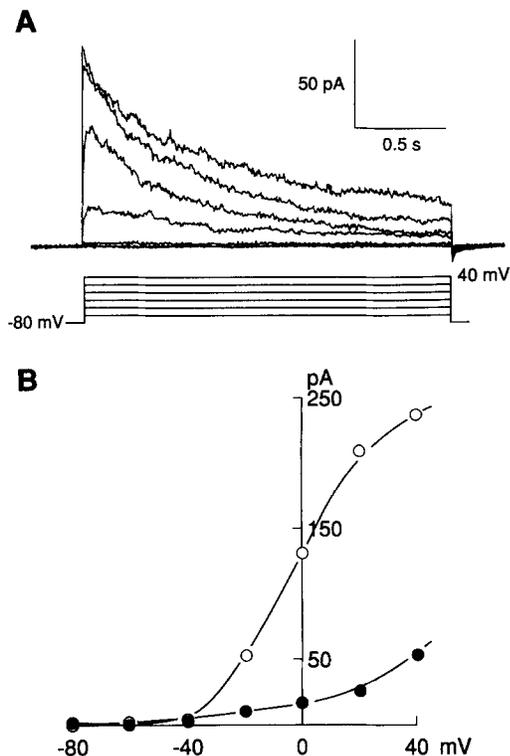


FIGURE 7. Transient K^+ current. (*A*) Outward currents elicited by 2-s depolarizing voltage steps between -60 and $+40$ mV in 20-mV increments from a holding potential of -80 mV. Bath: Ca^{2+} -free solution containing 2 mM Co^{2+} and 1 μM TTX. Pipette: K^+ internal solution. (*B*) I - V relationship of the Ca^{2+} -independent component of outward currents shown in *A*, measured at the peak (*open circles*) and just before the end of the voltage steps (*filled circles*).

Delayed Rectifier K^+ Current

When transient K^+ currents were blocked by holding the cells at -40 mV, outward currents that continued to rise during the voltage step were observed (Fig. 9, *A* and *B*). These currents were blocked by substitution of Cs^+ for K^+ in the pipette solution and by addition of 10 mM Ba^{2+} , 20 mM TEA^+ , or 10 mM 4AP to the bath. They resembled the delayed rectifier K^+ currents observed in a variety of cells (Hille, 1984).

Inward Rectifier K^+ Current

When cells were hyperpolarized from -60 to -120 mV and back to -60 mV with a voltage ramp (0.18 V/s), the membrane resistance usually decreased at potentials more negative than -80 mV (Fig. 10 *A*). The peak current evoked by a ramp from -60 to -120 mV was -10.4 ± 5.7 pA ($n = 4$). Voltage pulses from -50 to -100 mV from a holding potential of -60 mV elicited sustained inward currents that did not

reverse (Fig. 10 B). This current was not suppressed by substitution of Cs^+ for K^+ in the pipette solution, but was blocked by addition of 10 mM Ba^{2+} to the bath (Fig. 10 C, representative of four cells). This current was classified as an inward rectifier K^+ current.

Responses to Odorant Amino Acid Stimuli

Receptor currents elicited by mixtures of three or four amino acids (100 μM each of L-arginine, L-alanine, L-norleucine, and/or L-glutamate), which on the basis of neural cross-adaptation (Caprio and Byrd, 1984) and radioligand binding (Bruch and Rulli, 1988) act at independent receptor sites, were recorded from 13 of 64 receptor neurons. The amino acid mixtures produced inward currents at the resting potential

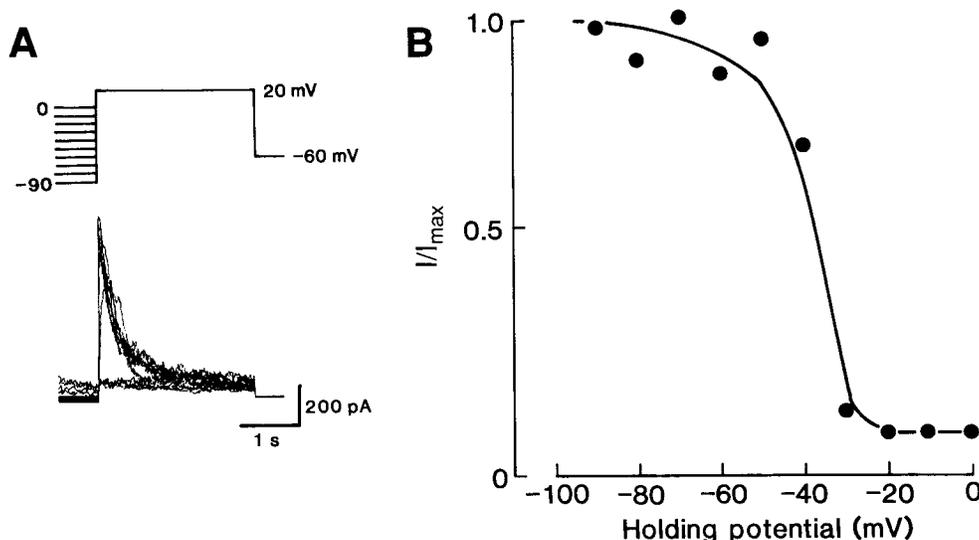


FIGURE 8. Inactivation of transient component of outward currents. (A) Transient and sustained outward currents evoked by 2.5-s depolarizing steps to +20 mV from various holding potentials. Pipette: K^+ internal solution. Bath: Na^+ -free Ringer solution. (B) Steady-state inactivation curve obtained from the records in A. Peak outward currents were plotted as a function of holding potential. Note incomplete inactivation of outward currents.

in all 13 neurons. The amino acid-induced currents were heterogeneous in terms of voltage and ion dependence, time course, reversal potential, and sensitivity to block by amiloride, and appeared to result from at least two different processes.

In 5 of the 13 cells responding to amino acids, stimuli evoked currents that displayed a region of negative slope conductance over which the current decreased in amplitude when the cell was progressively hyperpolarized below -60 mV (Fig. 11, A and C). The current responses appeared with a brief (~ 50 ms) latency after the onset of stimulation. The odorant-induced currents were diminished in Ca^{2+} -free bath solution containing 1 mM EGTA (Fig. 11 B and open circles in Fig. 11 C) and this effect was completely reversible.

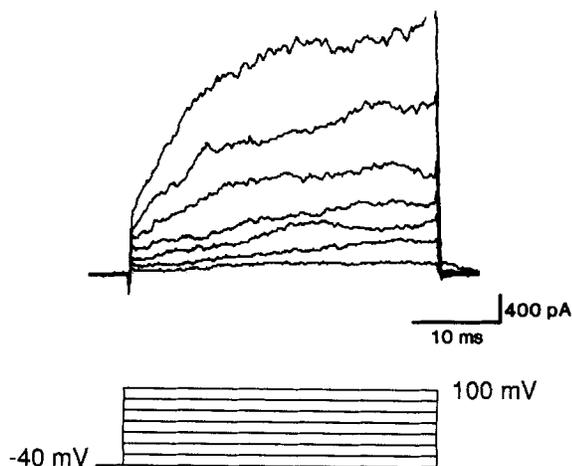
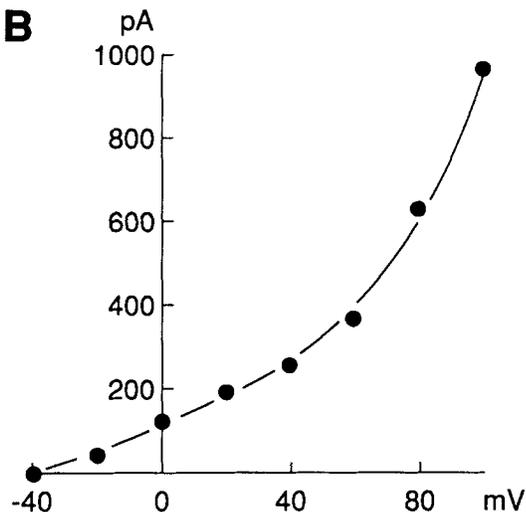
A

FIGURE 9. Delayed rectifier K^+ current. (A) Delayed outward current elicited by 40-ms depolarizing steps between -20 and $+100$ mV in 20-mV increments from a holding potential of -40 mV. Pipette: K^+ internal solution. Bath: normal Ringer solution. (B) I - V relationship of currents in A. Currents were measured at the end of the voltage pulse.

B

The reversal potentials of these responses averaged $+23.3 \pm 4.7$ mV ($n = 3$) with normal Ringer in the bath and K^+ internal solution without ATP in the pipette. When K^+ in the pipette was replaced with Cs^+ , the reversal potential was $+12.5 \pm 7.5$ mV ($n = 2$). These responses were observed only in neurons displaying rapidly inactivating outward currents (Fig. 7) and they were not dependent on the presence of ATP in the pipette solution. The former observation would explain the relatively small differences in the steady-state currents (before stimulus application) observed over the large range of holding potentials, -80 to $+20$ mV, even with high K^+ in the pipette (Fig. 11, A and B).

In four other receptor neurons, the responses to the amino acid mixture were characterized by marked outward rectification at positive potentials (Fig. 12). These currents reversed near 0 mV ($+1.1 \pm 1.9$ mV, $n = 4$) with normal Ringer in the bath and Cs⁺ internal solution in the pipette (Fig. 12, *B* and *D*). Addition of 100 μ M amiloride to the bath had no effect on the odorant-induced currents in these cells (not shown), while 10 mM extracellular Ba²⁺ blocked the inward currents elicited at

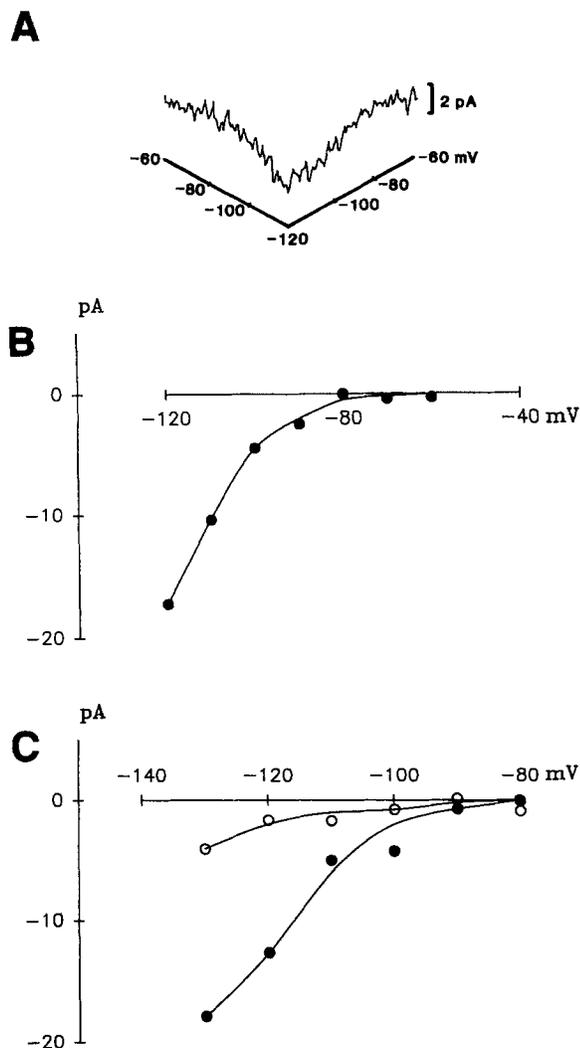


FIGURE 10. Inwardly rectifying K⁺ currents. (*A*) Currents evoked by a voltage ramp from -60 to -120 mV and back to -60 mV at a rate of 0.18 V/s. (*B*) *I-V* relationship for currents evoked by 60-ms hyperpolarizing voltage steps between -120 and -60 mV in 10-mV increments from a holding potential of -60 mV. Same cell as in *A*. Pipette: K⁺ internal solution. Bath: normal Ringer solution. (*C*) *I-V* relationship of inwardly rectifying currents in a different cell in the presence of internal Cs⁺ and in the presence (*open circles*) or absence (*filled circles*) of 10 mM extracellular Ba²⁺. Currents were evoked by hyperpolarizing steps from a holding potential of -60 mV. Pipette: Cs⁺ internal solution. Bath: normal Ringer solution.

negative potentials (Fig. 12 *D*). Furthermore, adaptation of the cell to 10 μ M forskolin, which stimulates cAMP formation, abolished the responses to odorant amino acids in these cells (Fig. 12 *D*).

In the remaining four neurons, amino acid mixtures elicited inward currents at negative holding potentials that were blocked by addition of 100–500 μ M amiloride to the bath (Fig. 13, *B* and *C*). Inhibition by amiloride was fully reversible. The

relationship between the amplitudes of the amino acid-induced currents and holding potential in these cells was linear, with a mean reversal potential of -14.8 ± 13.4 mV ($n = 4$) with Ringer in the bath and Cs^+ solution in the pipette. Even with Cs^+ in the pipette, these cells displayed a marked variation in baseline current at different holding potentials before the application of stimulus (Fig. 13, *A* and *B*). This may have resulted from activation of the Cs^+ -resistant component of outward current, since both internal Cs^+ and external Ba^{2+} were required to completely block K^+ currents in all receptor neurons.

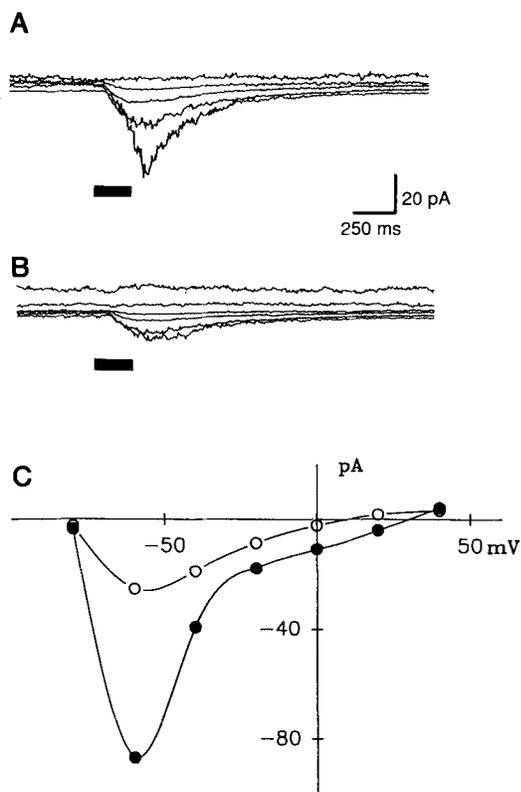


FIGURE 11. Responses to odorant amino acids. (*A*) Currents elicited by repeated application of a mixture of 100 μM each of four amino acids (L-Arg, L-Ala, L-Nle, and L-Glu) in Ringer at five different holding potentials between -60 and $+20$ mV. (*B*) Currents induced in the same cell by repeated applications of the four amino acid mixture in Ca^{2+} -free Ringer solution at six different holding potentials between -60 and $+40$ mV. The amino acid mixture was applied by pressurizing a "puffer" pipette during the period indicated by the solid bar. Inward currents displayed downward. (*C*) $I-V$ relationships obtained from a different cell in normal Ringer (filled circles) and Ca^{2+} -free Ringer (open circles). Pipette: K^+ internal solution without ATP.

DISCUSSION

Voltage-dependent Currents

In this study we demonstrated that olfactory receptor neurons, enzymatically dissociated from the olfactory mucosa of the channel catfish, display a variety of voltage- and ion-dependent conductances similar to those described in olfactory neurons from amphibians (Trotier, 1986; Firestein and Werblin, 1987; Schild, 1989; Suzuki, 1989; Trotier, Rosin, and MacLeod, 1989) and mammals (Pixley and Pun, 1990; Lynch and Barry, 1991*a, b, c*; Trombley and Westbrook, 1991). These included a transient Na^+ current, a sustained Ca^{2+} current, and four types of K^+ currents.

Sodium currents activated between -70 and -50 mV, which is somewhat more negative than the range (-50 to -30 mV) reported for olfactory receptor neurons from salamanders (Trotier, 1986; Firestein and Werblin, 1987), frogs (Suzuki, 1989; Trotier et al., 1989), lobsters (McClintock and Ache, 1989), and rats (Trombley and Westbrook, 1991) but similar to that reported for *Xenopus* receptor cells (Schild, 1989). Unlike Na^+ currents in salamander olfactory receptor cells, which are not

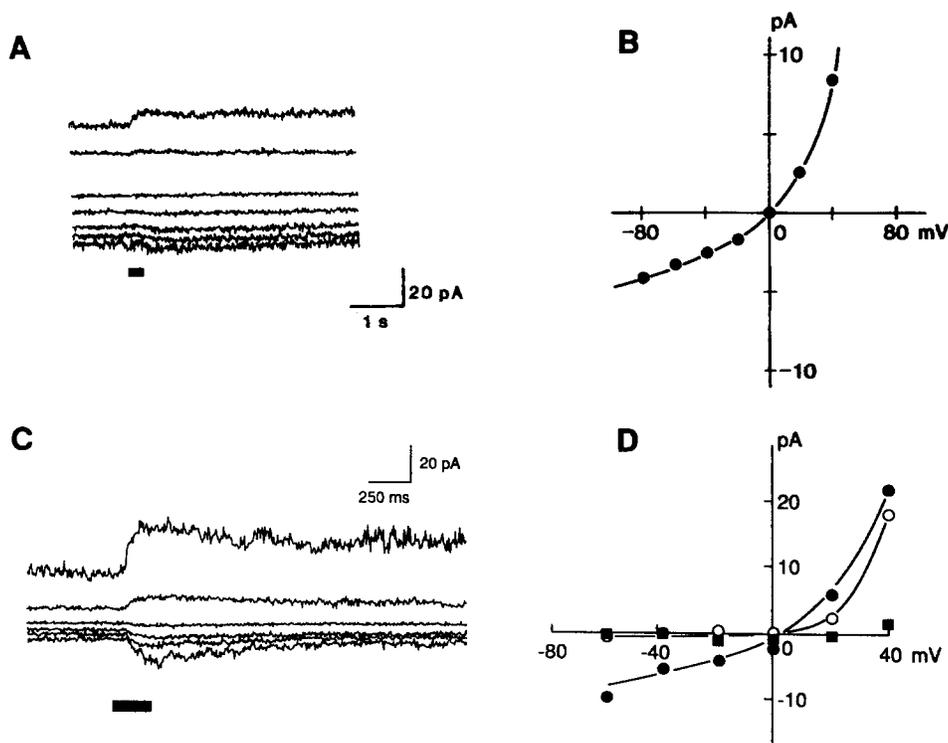


FIGURE 12. Two examples of outwardly rectifying response. (A) Currents induced by repeated applications of a mixture of $100 \mu\text{M}$ each of L-Arg, L-Ala, L-Nle, and L-Glu in normal Ringer at seven holding potentials between -80 and $+40$ mV. (B) I - V relationship obtained from the cell in A. (C) Currents induced by application of a mixture of $100 \mu\text{M}$ L-Ala and L-Arg in normal Ringer at six holding potentials between -60 and $+40$ mV. (D) Filled circles, I - V relationship obtained from the records in C; open circles, I - V relationship for responses in the same cell when bathed in Ringer with 10 mM Ba^{2+} ; squares, I - V relationship in the same cell after adaptation to forskolin ($10 \mu\text{M}$ added to normal Ringer) for 5 min. Pipette: for A and B, Cs^+ internal solution with 5 mM CsF and 0.5 mM ATP ; for C and D, Cs^+ internal solution with 30 mM CsF and 2 mM ATP .

sensitive to TTX (Trotier, 1986; Firestein and Werblin, 1987), those in catfish olfactory neurons were completely abolished by $1 \mu\text{M}$ TTX, as were those in rat olfactory receptor neurons (Trombley and Westbrook, 1991). Both TTX-sensitive and TTX-insensitive components of Na^+ current have been reported in frog olfactory neurons (Suzuki, 1989; Trotier et al., 1989).

The average resting potential of isolated catfish olfactory receptor neurons was -45 ± 11 mV ($n = 52$), which is similar to the values obtained by whole-cell recording from isolated receptor neurons from amphibians (Trotier, 1986; Firestein and Werblin, 1987; Schild, 1989; Suzuki, 1989; Trotier et al., 1989), but somewhat less negative than recently reported in cultured rat olfactory neurons (Trombley and Westbrook, 1991). Although these values are more negative than those commonly obtained using intracellular microelectrodes (Getchell, 1977; Trotier and MacLeod,

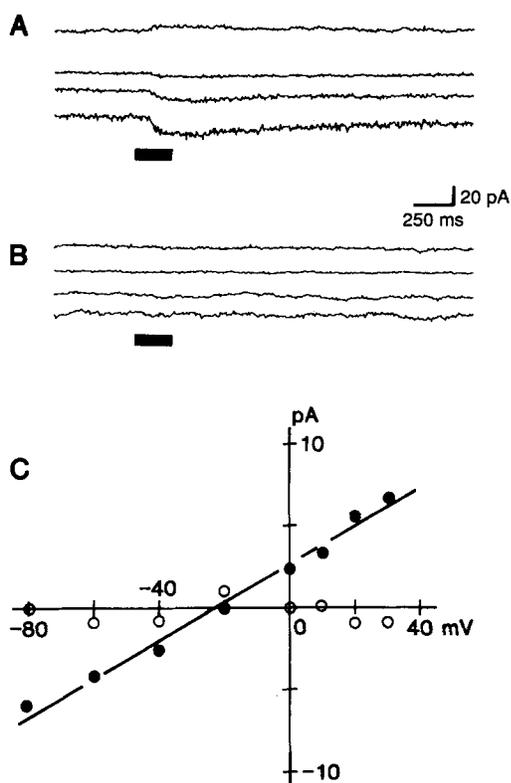


FIGURE 13. Odorant-induced responses. (A) Currents induced by repeated applications of a mixture of 100 μ M each of L-Arg, L-Ala, and L-Nle in normal Ringer at holding potentials of -60 , -40 , -10 , and $+10$ mV. (B) Currents induced by application of a mixture of the same three amino acids in Ringer containing 500 μ M amiloride. Same holding potentials as in A. (C) I - V relationships obtained from a different neuron in normal Ringer (filled circles) and Ringer solution containing 500 μ M amiloride (open circles). Pipette: Cs^+ internal solution containing 10 mM CsF substituted for 10 mM CsCl.

1983; Masukawa, Hedlund, and Shepherd, 1985; Anderson and Hamilton, 1987), the Na^+ current would be $>90\%$ inactivated at -50 mV in catfish olfactory receptor neurons. Spontaneous action potentials were often observed in the cell-attached configuration, suggesting that the actual resting potential was somewhat more negative than the zero-current potential measured in the whole-cell configuration. The slow time course of the action potentials recorded under current clamp (duration at half-maximal amplitude >35 ms) suggests that Ca^{2+} currents may have contributed to the responses. Schild (1989) suggested that the maximum (most negative) value observed is the best estimate of the resting potential because seal resistance is in parallel with the membrane resistance. Low and variable seal resistance, variable lengths of axon present in each neuron, and the presence of neurons at different stages of differentiation (Masukawa et al., 1985) may also account for some of the

variability in measured input resistance and resting membrane potential. The maximum resting potential observed in catfish olfactory receptor neurons was -80 mV and consequently the actual value of the average resting membrane potential in mature catfish olfactory neurons in situ may be more negative than -46 mV. Alternatively, the short time constant for recovery from inactivation of Na^+ current (<5 ms) observed in catfish olfactory neurons may make it possible for the Na^+ channels inactivated at -50 mV to recover during the brief hyperpolarizing potentials often observed in these cells which presumably result from spontaneous opening of individual K^+ channels.

Ca^{2+} currents were observed in almost all of the olfactory neurons examined, but they were small, never exceeding -50 pA in amplitude, even with 10 mM Ba^{2+} in the bath. Ca^{2+} currents were seldom observed in normal Ringer solution, which contained 1 mM Ca^{2+} . The Ca^{2+} current displayed a long time constant of decay (>2 s in 10 mM Ba^{2+}), activated at potentials more positive than -30 mV, reached a maximum at potentials positive to 0 mV, and was blocked by 5 μM nimodipine, suggesting that it was mediated by L-type Ca^{2+} channels. Similar nimodipine-sensitive Ca^{2+} channels were observed in bilayers into which catfish olfactory cilia membranes had been incorporated (Restrepo and Teeter, 1990). The Ca^{2+} currents showed characteristic run-down within a few minutes of obtaining the whole-cell configuration. In catfish olfactory neurons, even Na^+ currents washed out within 30 min. Although the Ca^{2+} currents were generally too small to evoke full amplitude action potentials, they may play an important role in the regulation of the Ca^{2+} -dependent K^+ currents observed in these cells. Indeed, the Ca^{2+} -activated K^+ currents, together with the delayed rectifier K^+ current, presumably play an important role in the repolarization phase of action potentials and odorant-induced depolarizations. Slowly inactivating inward currents with similar characteristics have been reported in olfactory receptor neurons isolated from amphibians (Trotier, 1986; Firestein and Werblin, 1987; Schild, 1989), lobsters (McClintock and Ache, 1989), and rats (Trombley and Westbrook, 1991).

The three types of voltage-dependent outward K^+ currents isolated in catfish olfactory neurons: a delayed rectifier current, a transient current, and a Ca^{2+} -dependent current were similar to those reported in olfactory receptor neurons of salamanders (Trotier, 1986; Firestein and Werblin, 1987) and *Xenopus* (Schild, 1989). Although delayed rectifier and transient K^+ currents were observed in isolated rat olfactory neurons, Ca^{2+} -activated K^+ currents were not present in these cells (Trombley and Westbrook, 1991; Lynch and Barry, 1991*b, c*). Single-channel currents attributable to delayed rectifier, transient, and two types of Ca^{2+} -activated K^+ channels have been reported in patches of membrane excised from mouse olfactory receptor neurons (Maue and Dionne, 1987).

In addition to the three types of outward K^+ currents, we observed an inwardly rectifying K^+ current in some cells. Inward rectifier K^+ currents have also been observed with single-channel recordings in mouse olfactory cells (Maue and Dionne, 1987) and with whole-cell recordings in rat olfactory receptor neurons (Lynch and Barry, 1991*a*), but not in isolated amphibian olfactory neurons.

Catfish olfactory receptor neurons are small and electrotonically compact, with membrane capacitances and input resistances averaging 2.1 pF and 6.6 G Ω ,

respectively. The value for the capacitance is somewhat lower than the value for the larger amphibian olfactory neurons (Trotier, 1986; Firestein and Werblin, 1987; Schild, 1989). In salamander olfactory receptor cells, 3 pA of injected current was sufficient to evoke action potentials (Firestein and Werblin, 1987). Also, it has been reported that the current through a single open channel initiates an action potential in rat olfactory receptor neurons, which, like amphibian cells, are also larger than catfish olfactory cells (Lynch and Barry, 1989). This suggests that small changes in membrane conductance in olfactory receptor cells are capable of producing large changes in membrane potential. Current-clamped catfish olfactory cells displayed marked spontaneous fluctuations in membrane potential. This phenomenon appeared to result from spontaneous channel activity. The membrane time constant of a catfish olfactory neuron with a membrane capacitance of 2 pF and input resistance of 6.6 G Ω would be less than 20 ms. Opening of a single channel which passes 1 pA of current at a resting potential of -45 mV would produce a 6.5-mV change in membrane potential with a time constant less than 20 ms. This could, in turn, activate other voltage-dependent channels and additional membrane potential deflections. The resulting cascade of spontaneous membrane fluctuations could provide the basis for both excitatory and inhibitory responses in olfactory receptor neurons, which have been reported for *Necturus* olfactory receptor cells (Dionne, 1991).

The presence of Ba²⁺, TEA⁺, or 4AP in the bath resulted in receptor cell depolarization, but neither apamin nor CTX had a direct effect on membrane potential, although all of these agents suppressed voltage-gated outward currents. Apamin and CTX are relatively specific blockers of Ca²⁺-activated K⁺ channels, while Ba²⁺, TEA⁺, and 4AP are relatively nonspecific blockers of several types of K⁺ channels (Castle et al., 1989). This suggests that Ca²⁺-dependent K⁺ channels are not activated at the resting potential. The regulation of membrane potential by Ca²⁺-activated K⁺ channels may have a special significance in olfactory receptor neurons because Ca²⁺ influx via second messenger-gated channels permeable to Ca²⁺ has been implicated in olfactory responses in fish (Bruch and Teeter, 1990; Restrepo et al., 1990), amphibians (Kurahashi, 1989; Suzuki, 1989; Sato, Hirono, Tonoike, and Takebayashi, 1991), and mammals (Breer et al., 1990; Restrepo and Teeter, 1991).

Odorant Amino Acid-activated Currents

Stimulation of catfish olfactory receptor neurons with mixtures of amino acids elicited current responses in 20% of the cells tested. Because amino acids are transported across the cell membrane by electrogenic transport systems, it is important to rule out the possibility that these responses are due to amino acid transport. Two observations make this unlikely. First, the reversal potentials of the amino acid-elicited currents were in the range from -15 to $+25$ mV, contrary to what would be expected for Na⁺-dependent amino acid transport systems, which are highly selective for Na⁺ and consequently would be expected to reverse at very positive potentials in the absence of intracellular Na⁺ (Jauch, Petersen, and Lauger, 1986; Hoyer and Gögelein, 1991). Second, olfactory neurons responded to low concentrations of amino acids (100 μ M) that would be unlikely to elicit a significant current through Na⁺-dependent amino acid transport systems, which display low affinity for amino acids (K_m is 5–20 mM) (Jauch et al., 1986; Hoyer and Gögelein, 1991).

Receptor currents elicited by a mixture of three or four amino acids which act at independent classes of receptor sites (Caprio and Byrd, 1984; Bruch and Rulli, 1988) were heterogeneous in terms of their voltage and ion dependence, time course, reversal potential, and sensitivity to drugs. Although a definitive classification of the odorant-induced responses is not possible because of the limited number of cells studied, the differences observed in these properties were substantial and consistent enough to make it unlikely that the different responses are due to a single mechanism (see below). This is consistent with recent evidence that stimulus-regulated elevations in intracellular levels of two second messengers (cAMP and IP_3), each activating different classes of ligand-gated channels are involved in olfactory transduction in catfish (cAMP: Bruch and Teeter, 1990; Goulding, Ngai, Chess, Kramer, Colicos, Axel, and Siegelbaum, 1991; IP_3 : Huque and Bruch, 1986; Restrepo et al., 1990).

Five cells responded with currents whose I - V relationship displayed a region of negative slope below -60 mV (Fig. 11). These responses were enhanced by addition of 1 mM extracellular Ca^{2+} . It is unlikely that they were mediated by the cAMP-gated conductance because these channels, although permeable to Ca^{2+} , are blocked by extracellular divalent cations (Nakamura and Gold, 1987; Bruch and Teeter, 1990; Dhallan, Yau, Schrader, and Reed, 1990; Suzuki, 1991) and removal of external Ca^{2+} would result in an increase in response amplitude rather than the decrease observed (Fig. 11). The Ca^{2+} dependence of this response would be consistent with the somewhat higher permeability for divalent than monovalent cations of the IP_3 -gated channel in catfish (Restrepo et al., 1990).

On the other hand, the outwardly rectifying responses displayed by four cells (Fig. 12) are consistent with mediation by the cAMP-gated channel. The outward rectification in the presence of extracellular divalent cations is characteristic of olfactory cyclic nucleotide-dependent currents in catfish (Bruch and Teeter, 1990; Goulding et al., 1991) and other species (Nakamura and Gold, 1987; Dhallan et al., 1990; Kurahashi, 1990) and is believed to be the result of blockage of the current in the negative potential range by external divalent cations (Suzuki, 1991). In retinal rods, extracellular Ba^{2+} is known to block the inward Na^+ current carried by the cGMP-gated channel (Colamartino, Menini, Spadavecchia, and Torre, 1990). This is consistent with our observation of a strong blocking effect of 10 mM Ba^{2+} on the outwardly rectifying responses (Fig. 12 D). In addition, Fig. 12 D shows that the outwardly rectifying responses were blocked by adaptation of the cells to forskolin, which, because of its stimulation of adenylate cyclase, should attenuate the odor responses that are mediated by cAMP (Lowe, Nakamura, and Gold, 1989). These characteristics of the outwardly rectifying responses produced by stimulation with amino acids (Fig. 12) are consistent with their mediation by odorant-induced formation of cAMP and subsequent modulation of cAMP-gated cation channels in the ciliary membranes as reported in amphibian olfactory neurons (Nakamura and Gold, 1987; Firestein and Werblin, 1989; Kurahashi, 1989; Lowe et al., 1989).

A third group of cells displayed odorant responses with a linear I - V relationship and were inhibited by amiloride (Fig. 13). Since amiloride and 3',4'-dichlorobenzamil (DCB) have been shown to inhibit the olfactory cAMP-gated channel in frog when

added to the cytoplasmic side of the membrane (Kolesnikov, Zhainazarov, and Kosolapov, 1990), inhibition by amiloride could suggest that these responses are mediated by a cyclic nucleotide-gated channel. However, responses of catfish olfactory neurons to intracellular cAMP are not blocked by extracellular amiloride or DCB (Miyamoto, Restrepo, Cragoe, and Teeter, 1992), suggesting either that the cAMP-gated conductance in catfish olfactory cells is not sensitive to amiloride or that amiloride, when added externally, does not diffuse into the cell in large enough amounts to cause inhibition of the cAMP response. This appears to be the case in the frog in which Kolesnikov et al. (1990) have shown that *internal* DCB inhibits cAMP-gated channels, while Frings, Benz, and Lindemann (1991) found that addition of *external* amiloride does not block the response of olfactory cells to forskolin (which is presumably cAMP mediated).

The distinction between the Ca^{2+} -sensitive current responses in Fig. 11 and the outwardly rectifying responses in Fig. 12 is clear. In the negative holding potential range, the responses in Fig. 11 were augmented by extracellular divalent cations, whereas the outwardly rectifying responses in Fig. 12 were blocked. Also, the *I-V* relationships and reversal potentials are different, and at negative holding potentials the responses in Fig. 11 display a much faster decay than the outwardly rectifying responses in Fig. 12. On the other hand, the distinction between the responses in Figs. 12 and 13 are less certain, although it is hard to explain the differences in *I-V* relationships, reversal potentials, and amiloride sensitivity between these two groups of responses (Figs. 12 and 13) on the basis of the same mechanism.

The magnitude of the odorant-activated current responses was quite small compared with the voltage-dependent currents. However, the responses in Figs. 11 and 13 were large enough to produce depolarizations larger than 10 mV, as assessed by comparing the voltage dependence of whole-cell current before application of stimulus with the magnitude of the odorant amino acid-induced currents at resting potential. In contrast, some of the outwardly rectifying responses (Fig. 12) are quite small at negative holding potentials. This could mean that these responses are not physiologically relevant. However, if the outwardly rectifying responses are cAMP mediated, an alternate explanation is that the concentration of amino acids used in this study is significantly lower than the K_m for amino acid activation of cAMP formation in catfish cilia (Bruch and Teeter, 1990). Further studies at different amino acid concentrations are necessary to answer this question.

In conclusion, these results, along with the report of activation of conductances with different ionic selectivities when lobster olfactory cells are stimulated with a complex mixture of odorants (Schmiedel-Jakob, Michel, Anderson, and Ache, 1990), support the hypothesis that various conductances, perhaps reflecting multiple transduction pathways, are involved in olfactory signal transduction.

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