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AN ELECTROPHYSIOLOGICAL CHARACTERIZATION OF CILIATED OLFACTORY RECEPTOR CELLS OF THE COHO SALMON *ONCORHYNCHUS KISUTCH*

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Summary

Electrical properties of ciliated olfactory receptor cells isolated from coho salmon *(Oncorhynchus kisutch)* were studied using the whole-cell mode of the patch-clamp recording technique.

1. Voltage-dependent currents could be separated into two inward and three outward conductances, including a Na^+ current, Ca^{2+} current and three K^+ currents.

2. The components of the outward current varied with the life stage of the salmon from which cells had been isolated. In cells isolated from juvenile fish (parr), a Ca^{2+}-dependent K^+ current dominated the outward current, whereas in cells isolated from older fish (i.e. fish that had undergone smoltification), a transient K^+ current became prominent.

3. Differences in response characteristics of outward currents to internal dialysis with cyclic GMP (but not cyclic AMP) were also correlated to the life stage of salmon. Under conditions in which the Ca^{2+}-activated current was blocked, relaxation of the outward current was slowed by dialysis with cyclic GMP only in cells isolated from smolts and sea-run fish, but not in those isolated from mature spawners.

4. From these results, we suggest that hormone modulation of olfactory receptor cell development or differentiation may play a role in establishing these differences.

Introduction

Pacific salmon are known for their remarkable ability to use olfactory cues to home to the exact spawning grounds in which they were hatched in order to complete their life cycle (Fig. 1). Extensive behavioral and neurophysiological investigations have pinpointed specific and potent olfactory stimuli for salmonids, including amino acids, bile acids, kin odorants and synthetic chemicals (reviewed by Hasler and Scholz, 1983; Hara *et al.* 1984). Because such behaviorally relevant

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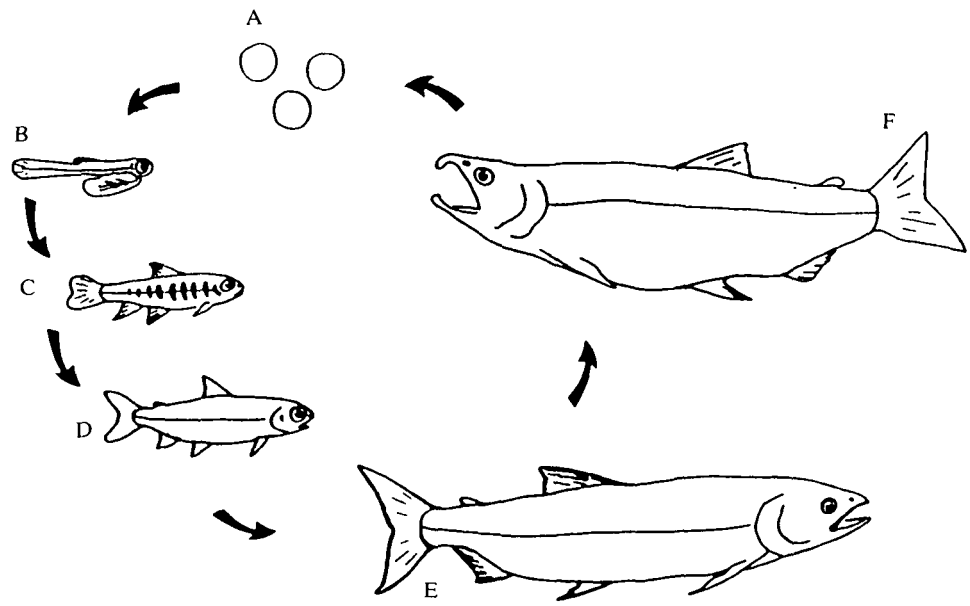

Fig. 1. The life history of the coho salmon, *Oncorhynchus kisutch*. (A) Eggs are laid each fall and hatch in freshwater streams. (B) Hatchlings (alevins) reside in the gravel, absorbing nutrients from their yolk sac. (C) Parr continue to live and grow in freshwater streams until the following spring, when they undergo a behavioral and physiological metamorphosis called smoltification that enables them to live in salt water. (D) Smolts migrate downstream to begin life in the open ocean as sea-run salmon (E). Two to three years later, mature spawning individuals (F) complete extraordinary migrations, often over thousands of miles, to return to the exact tributary where they were hatched, to spawn and so complete their life cycle.

stimuli have already been identified, salmonids are an ideal vertebrate for studying mechanisms underlying olfactory transduction.

The following study provides a characterization of the electrical properties of olfactory receptor neurons isolated from coho salmon (*Oncorhynchus kisutch* Walbaum) on which future studies using natural odorant stimulation will be based. We describe five distinct voltage-gated conductances, which include a transient inward Na^+ current, an inward Ca^{2+} current and three outward K^+ currents. Additionally, our data indicate that the relative composition of these currents varies among cells isolated from animals of different life stages.

Some of these data have appeared in preliminary form (Nevitt, 1987).

Materials and methods

Experimental animals

The results described here are based on data collected from over 150 cells isolated from 44 fish. For most experiments, coho salmon parr or smolts (31

individuals) were obtained either from the Northwest Fisheries Center, National Marine Fisheries Service, Seattle, Washington, or from the Pacific Biological Station, Nanaimo, British Columbia. Fish were kept either outdoors or under a photoperiod regime approximating seasonal changes in day length, and all animals were housed in 1–1.5 m diameter fiberglass tanks supplied with running Lake Washington water at year-round ambient temperature. In early experiments, olfactory neurons isolated from triploid coho salmon were studied because of the technical convenience offered by the larger olfactory receptor cell size (approximately 15 μm soma diameter) in these animals. However, because neither the olfactory nor the homing abilities of triploid salmon have been determined, most experiments were performed on cells isolated from standard diploid salmon stocks. Sea-run fish (7 individuals) were collected in the Straits of Juan de Fuca off Tatoosh Island from July to early October, 1987, using standard line-fishing gear. Spawning adult fish (6 individuals) were obtained from the University of Washington School of Fisheries salmon run during the fall of 1987 and 1988.

Tissue isolation

Animals were killed by a blow to the head followed by decapitation. Olfactory rosette tissue was removed from nasal sacs and placed in chilled buffered salmon Ringer's solution (see below) for up to 24 h. To dissociate cells, lamellae were dissected from each other, transferred to a divalent-ion-free Ringer's solution containing 1 mmol l^{-1} EGTA, and cut into fine pieces. Tissue fragments were triturated 3–5 times using a fire-polished Pasteur pipet. After 15–20 min, isolated receptor cells and cell clumps were washed in salmon Ringer and transferred to sterile, polystyrene dishes. Cells and cell aggregates were allowed to settle without the use of anchoring agents. Cells were generally stored for 1–8 h at 8°C for electrophysiological examination, but retained their distinct bipolar morphology for up to 24 h following dissociation.

Electrical recording

The whole-cell mode of the patch-clamp recording technique was employed as described by Hamill *et al.* (1981). Patch pipets were prepared from standard VWR micropipet glass using a two-stage pull, and were not fire-polished. Pipet resistances were 5–10 MΩ in salmon Ringer's solution, and 5–20 GΩ seals were routinely obtained by gentle application of suction. Membrane rupture was monitored electrically as a large increase in capacitance. Input resistances ranged from 5 to 12 GΩ (7.5±2.0, N=11) as calculated by the slope of the steady-state current–voltage (I–V) relationship in the potential range of −120 to −70 mV, where time-dependent currents were not activated. Series resistance (10–20 MΩ) was not compensated. Most recordings lasted 10–15 min, although recordings from some cells could last for up to 1 h. During longer recordings, both loss of current and gradual shifts in apparent surface potential occurred and receptor cells tended to lose their distinct morphology. For these reasons, care was taken to limit data collection to the first 10 min following membrane rupture. All voltage

recordings were referenced to a silver wire isolated from the bath *via* an agar bridge. Current records were filtered at 2 kHz using a filter with an eight-pole Bessel characteristic. Data were digitized, stored and processed using a standard laboratory microcomputer. All values are given as mean±S.E.M.

Solutions

Normal external Ringer's solution had the following composition (mmol l^{-1}): 130 NaCl, 5 KCl, 3 $CaCl_2$, 1 $MgCl_2$, 5.5 glucose and 10 Hepes buffered to pH 7.3–7.4. Sr^{2+} Ringer was prepared by substituting 10 mmol l^{-1} $SrCl_2$ for 3 mmol l^{-1} $CaCl_2$ and reducing NaCl to 116 mmol l^{-1}. Zero-Na^+ Ringer was prepared by substituting choline chloride for NaCl on an equimolar basis. Zero-divalent-ion solution for dissociation procedures was prepared by substituting divalent salts with NaCl and adding 1 mmol l^{-1} EGTA. Internal pipet solution consisted of (mmol l^{-1}): 140 KCl, 2 NaCl, 2 $MgCl_2$, 1 EGTA, 0.1 cyclic AMP (or 30–70 μmol l^{-1} cyclic GMP, Figs 7 and 8), 4 MgATP, 2 theophylline, and was buffered to pH 7.2 with 10 mmol l^{-1} Hepes. To eliminate outward currents, CsCl was substituted for KCl on an equimolar basis for some experiments.

Results

In salmon, as in other teleosts, odors are detected by both microvillar and ciliated olfactory receptor cells. Once isolated, ciliated olfactory receptor neurons maintained a bipolar shape consisting of a rounded cell body approximately 10 μm in diameter (9.7±0.5 μm, N=9), a long dendritic process (16.1±3.4 μm, N=9), in which several non-motile cilia were embedded, and an axon. However, microvillar receptor cells were difficult to distinguish reliably from support cells and, consequently, electrical investigation was limited to ciliated receptor neurons.

During the formation of a gigaohm seal, spontaneous electrical activity was typically observed. Such spiking activity was never observed in Na^+-free solution, suggesting that the Na^+ current is an important contributor to the upstroke of the action potential in these cells. After the whole-cell recording configuration had been established, olfactory receptor cells had an input resistance of 5–12 GΩ under standard recording conditions (7.5±2.0 GΩ, N=11). In whole-cell current-clamp mode, action potentials were brief and clearly overshot zero potential. Under voltage-clamp conditions, delivering depolarizing steps from holding potentials more negative than −60 mV resulted in the activation of a fast inward current, followed by a pronounced outward current. The major currents contributing to these voltage-clamp responses are described below.

Inward currents

Na^+ current

Our results indicate that the fast transient inward current is carried primarily by Na^+. Fig. 2 illustrates a family of Na^+ current traces (Fig. 2A) and the corresponding current–voltage relationship (Fig. 2B), recorded with Cs^+ substituted

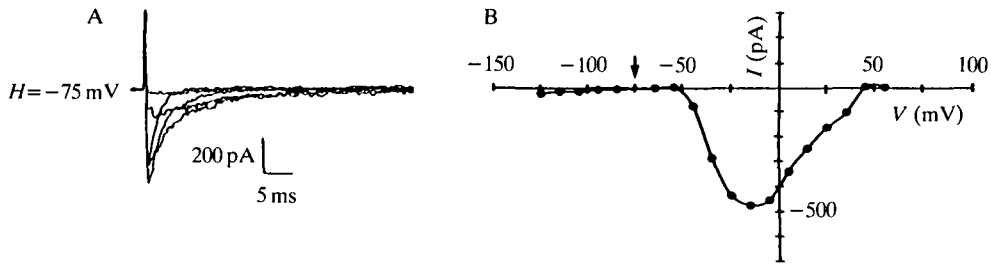

Fig. 2. The transient inward Na^+ current. (A) A family of current traces recorded by first holding the cell at −75 mV and then stepping it to the following potentials (mV): −55, −45, −25, −15, −5. (B) The corresponding current–voltage relationship for the peak Na^+ current. Currents were recorded in standard external solution; Cs^+ replaced K^+ on an equimolar basis in the internal solution to block outward currents.

for K^+ in the internal recording solution. This current was abolished by replacement of external Na^+ with choline and was blocked by 1 μmol l^{-1} tetrodotoxin (TTX). The activation threshold for detectable current was between −50 mV and −40 mV (−45.8±0.8 mV, N=24). Peak current occurred between −36 mV and −15 mV (−25.8±1.2 mV, N=26), activated within 1 ms, and 50 % inactivation times were roughly 3 ms. Peak Na^+ current typically ranged from 500 to 1000 pA [holding potential (H)≥−85 mV]; however, in one cell, no inward current was observed. Because we were rarely able to record from cells at more negative holding potentials, these values probably underestimate the actual current densities (see Fig. 3).

A steady-state inactivation curve was obtained by plotting the maximal peak currents activated by stepping the cell to −24 mV against the potential of a 100 ms variable prepulse (Fig. 3A). The current showed 50 % inactivation at −64 mV and saturated at −105 mV (Fig. 3B).

Fig. 3C,D illustrates recovery from inactivation for the Na^+ current. The cell was first stepped from a holding potential of −70 mV to a voltage that elicited maximal activation of the Na^+ current (−20 mV). This voltage step was then followed by a second, identical voltage step, applied after a variable time delay (Fig. 3C). Recovery from inactivation had a time constant of 38 ms at this voltage (arrow, Fig. 3D).

Ca^{2+} current

In addition to the Na^+ current, we recorded a second, much smaller, inward current from olfactory receptor neurons. This current could best be resolved under conditions in which outward currents were blocked by replacing internal K^+ with Cs^+, by eliminating the inward Na^+ current with 0-Na^+ external solution, and by replacing Ca^{2+} with Sr^{2+} in the external solution. Fig. 4A shows a family of current traces elicited from a cell stepped to a series of more positive voltages from a holding potential of −40 mV. The corresponding current–voltage relationship is

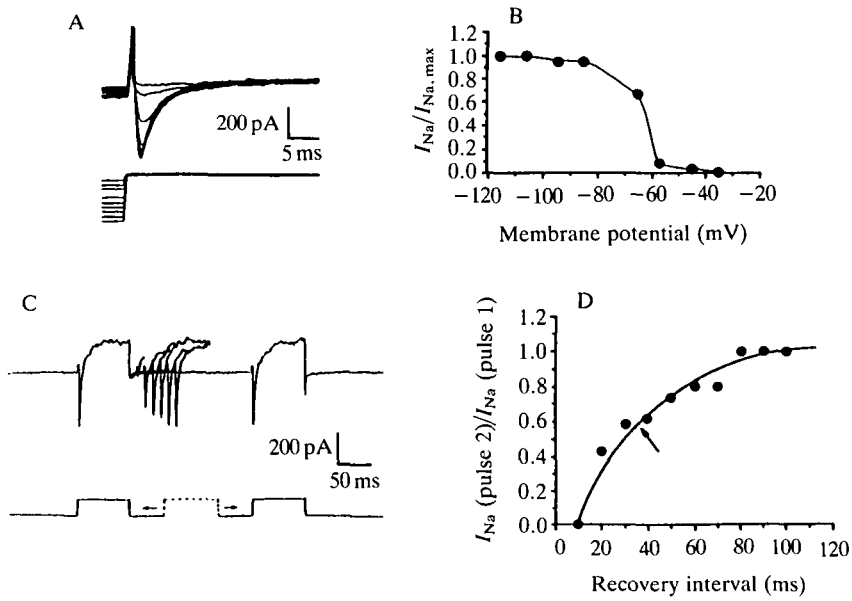

Fig. 3. Characterization of the Na^+ current. (A) Na^+ current traces recorded by stepping the cell to −24 mV against a variable prepulse (−125 to −35 mV). Solutions are as in Fig. 2B. (B) The inactivation curve plotted from the current values shown in A. Solutions as in Fig. 2. (C) Na^+ current traces showing recovery from inactivation in a different cell held at −70 mV. Here, two depolarizing steps to −20 mV were separated by varying time intervals. Standard internal pipet solution was used. (D) The recovery from inactivation is plotted from the current records shown in C. The time constant of recovery is indicated by the arrow (τ_H=38 ms).

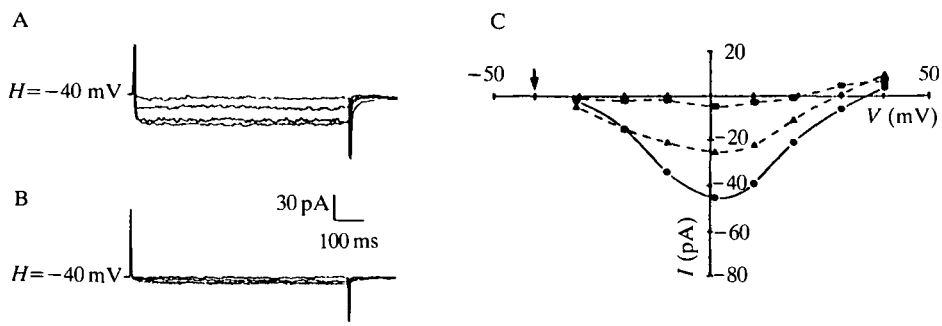

Fig. 4. Time-dependent washout of Ca^{2+} currents. (A) A family of current traces recorded immediately following the establishment of the whole-cell configuration (time=0). The cell was held at −40 mV and depolarizing steps were delivered (steps to −30, −20, 0 and +10 mV are shown). The corresponding current–voltage relationship is plotted in C (circles). Steps were repeated at time=2 min 15 s and the current–voltage relationship was again plotted (C, triangles). (B) Current traces as in A for time=5 min. The corresponding current–voltage relationship was plotted in C (squares). For all records, Sr^{2+} replaced Ca^{2+} in the external Ringer; outward currents were blocked by replacing K^+ with Cs^+ on an equimolar basis in the pipet solution.

plotted in Fig. 4C (circles), illustrating that the threshold for activation of this current was in a more positive voltage range than that of the Na^+ current (−35 mV to −30 mV, four observations). The persistence of this current in the absence of Na^+ in the external Ringer implies that it is carried through Ca^{2+} channels. Peak Ca^{2+} currents never exceeded 100 pA and were typically quite small (i.e. <30 pA).

As has been observed for many Ca^{2+} currents (Kostyuk, 1980; Hagiwara and Byerly, 1981), this current showed a time-dependent washout during whole-cell recording (Fig. 4B). Immediately following the establishment of the whole-cell configuration, a Ca^{2+} current was elicited, reaching a maximal amplitude of 45 pA at +2 mV (Fig. 4C, circles). Within 3 min, the maximal amplitude had already decreased to 56 % of its original value (Fig. 4C, triangles). By 5 min, the current was no longer detectable (Fig. 4B, squares in Fig. 4C). Attempts to slow this washout by dialysing cells with cyclic AMP, MgATP and theophylline during recordings did not alter the time course of current disappearance. In contrast, noticeable Na^+ current washout was only apparent during extremely long (1 h) recordings (data not shown).

Outward currents

In addition to inward currents, three outward currents were observed in response to depolarization. Replacement of K^+ with Cs^+ blocked all of these currents by more than 90 % (e.g. Fig. 5C, squares), suggesting that K^+ served as the current carrier. Relative contributions of K^+ currents varied in a manner that appeared to be correlated to age-specific or seasonal differences between animals from which cells were isolated. When two or more currents were present in the same cell, separate conductances were generally easily distinguishable on the basis of either voltage-dependence and kinetics of activation and inactivation or washout characteristics.

Ca^{2+}-activated K^+ current

A slowly activating K^+ current was observed in all cells isolated from fish of every life stage tested. In cells isolated from parr (see Fig. 1), this current typically made up more than 80 % of the total outward current (Fig. 5A). Threshold for activation of this current was in a slightly more positive voltage range than that of the Na^+ current (−45 to −25 mV, −34.3±1.3 mV, N=14), and peak amplitude was typically reached between 10 and 30 mV (22.1±1.7 mV, N=14). Peak currents ranged from 200 to 1000 pA, activated within 100–200 ms, and did not inactivate. Peak currents elicited declined dramatically as the voltage was stepped to more positive potentials (Fig. 5A; circles in Fig. 5C).

Several experiments indicated that this K^+ current was activated by elevated internal Ca^{2+} concentration resulting from Ca^{2+} influx through voltage-dependent Ca^{2+} channels. First, peak currents occurred in a voltage range similar to that of the peak Ca^{2+} current. Fig. 5A shows a family of current traces recorded from a cell held at −85 mV and depolarized to the voltages shown just after establishment of the whole-cell configuration. The current–voltage relationship is plotted in

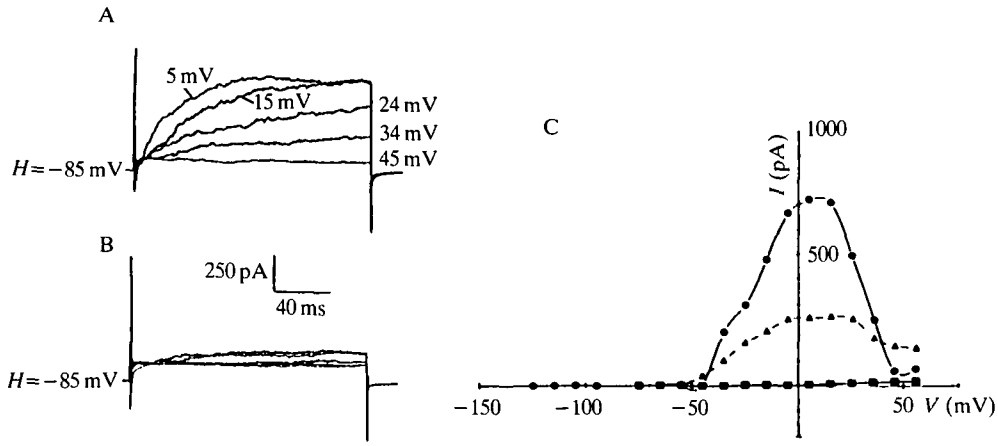

Fig. 5. Time-dependent washout of the Ca^{2+}-activated K^+ current. (A) A family of current traces recorded from a cell isolated from a parr, immediately following the establishment of the whole-cell configuration (time=0). The cell was held at −85 mV and depolarized to the voltages indicated. (B) A family of current traces recorded from the same cell at time=12 min. Voltage steps are as in A. (C) The corresponding peak current–voltage relationship plotted for A (circles) and B (triangles). Outward currents were completely blocked when Cs^+ replaced K^+ in the internal pipet solution (squares).

Fig. 5C (circles). The current reached a maximum amplitude (731 pA) when depolarized to 5 mV, coinciding with the voltage range of activation of the peak current through Ca^{2+} channels described above. However, at more positive voltages, less current was elicited, giving the current–voltage relationship a bell-shaped appearance. Second, like the Ca^{2+} current, the Ca^{2+}-activated K^+ current was subject to a time-dependent washout. Fig. 5C illustrates that within 12 min of establishing the whole-cell configuration, the Ca^{2+}-activated K^+ current was reduced to 270 pA, revealing a smaller, voltage-activated component of the outward current (Fig. 5B, triangles in Fig. 5C). Finally, the Ca^{2+}-activated K^+ current could not be activated when Sr^{2+} replaced Ca^{2+} in the external Ringer, suggesting that extracellular calcium was needed to activate this current.

Other K^+ currents

When Ca^{2+} was replaced by Sr^{2+} in the external solution to block the Ca^{2+}-activated K^+ current, additional outward currents became apparent, and the relative magnitudes of these currents were correlated to the life stage of the animal from which olfactory receptor cells had been isolated.

Fig. 6A,B each show a family of current traces elicited from two different cells isolated from fish of the same genetic stock before (Fig. 6A, parr) and after (Fig. 6B, smolt) smoltification. These recordings were made within 3 min of establishing the whole-cell configuration, and the corresponding current–voltage relationships are plotted in Fig. 6C. As Fig. 6A illustrates, a small, non-

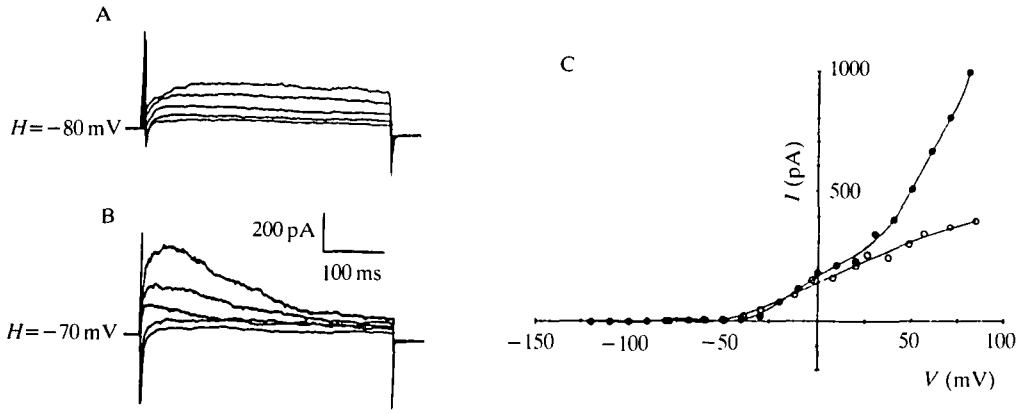

Fig. 6. Outward currents: life-stage differences. (A) A family of current traces recorded from a cell isolated from a parr. Recordings were made within 3 min of establishing the whole-cell configuration. For these records, external Sr^{2+} Ringer was used to block the Ca^{2+}-activated K^+ current. The cell was held at −80 mV and briefly stepped to different holding potentials (steps to −20, −10, +20, +40 and +60 mV are shown). (B) A family of current traces from a ciliated olfactory receptor cell isolated from a smolt, recorded under similar conditions to those in A. The cell was held at −70 mV and stepped to different holding potentials (steps to −23, −12, +8, +26 and +56 mV are shown). (C) The corresponding peak current–voltage relationship for the families of current traces in A (cell from parr, open circles) and in B (cell from smolt, filled circles).

inactivating voltage-dependent K^+ current was typically elicited in cells isolated from parr (Fig. 6C, open circles). Peak amplitudes of this current typically ranged from 50 to 190 pA (97.4±16.3 pA; $H=-75$ mV stepped to +45 mV, $N=8$; measured at $t=10$ min after the establishment of the whole-cell configuration).

However, in cells isolated from older fish, an additional outward current became prominent (Fig. 6B; filled circles in Fig. 6C). This K^+ current resembles the A current described in molluscan neurons (e.g. Connor and Stevens, 1971*a*). Threshold for activation occurred between −33 and −20 mV (−29±0.7 mV, $N=21$), and activation time was roughly 10–25 ms (12.6±1.8 ms; $N=10$). This current was completely inactivated at holding voltages equal to or more positive than −30 mV. Peak amplitudes ranged from 200 to 560 pA (449±64.8 pA; $H=-70$ to −80 mV stepped to +40 mV, $N=10$; measured at $t=10$ min) and did not significantly decrease throughout the time course of most recordings.

Effects of cyclic GMP

Because cyclic nucleotides have been implicated in the olfactory transduction pathways of other vertebrate systems (e.g. Nakamura and Gold, 1987), several experiments were conducted to test whether intracellular dialysis with either cyclic AMP or cyclic GMP would modify whole-cell currents.

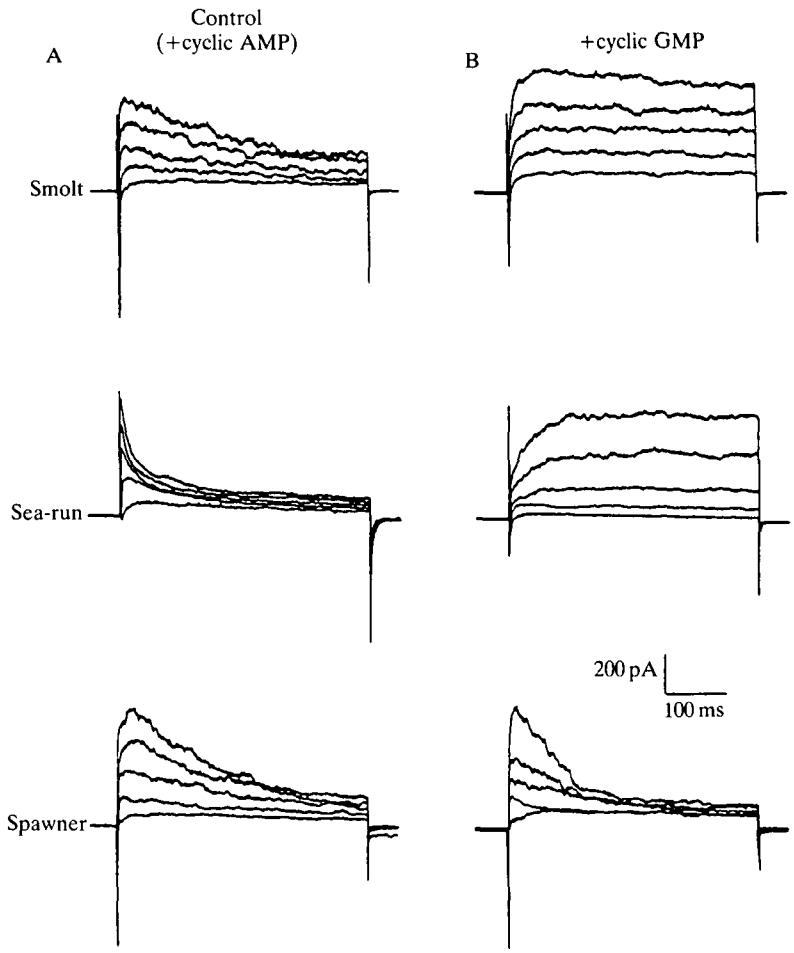

Fig. 7. Effects of cyclic nucleotide dialysis: life-stage differences. (A) Families of outward current traces recorded from cells isolated from a smolt, sea-run or mature fish (spawner) using standard pipet solution which contained 0.1 mmol l^{-1} cyclic AMP. In all cases, Sr^{2+} replaced Ca^{2+} in the external solution. Current traces shown were activated by delivering depolarizing steps to −20, 0, +20, +40 and +60 mV from a holding potential of −80 mV. (B) Families of outward current traces recorded from cells isolated from the same life stages and following identical procedures to those in A. External and internal solutions were identical to those in A, except that 40–70 μmol l^{-1} cyclic GMP replaced cyclic AMP in the pipet solution.

Fig. 7A shows three families of current traces recorded from receptor cells isolated from fish of different ages (smolt, sea-run and spawner) under conditions in which Sr^{2+} replaced Ca^{2+} on an equimolar basis in the external solution and the standard pipet solution contained 0.1 mmol l^{-1} cyclic AMP. Cells were held at −80 mV and stepped to the voltages shown. Under these control conditions, the rapidly inactivating K^+ conductance was the predominant component of the outward current. Fig. 7B shows three additional families of current traces

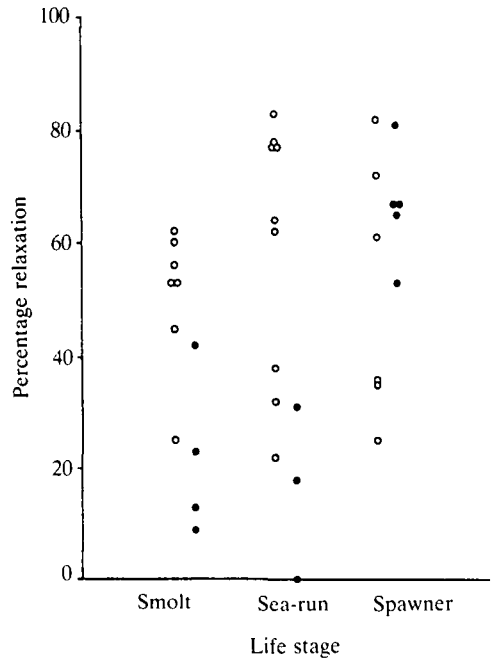

Fig. 8. Cyclic GMP significantly slows the relaxation rate of the outward current in cells isolated from immature fish (smolts, sea-runs), but not from spawners. Differences between percentage relaxation in cyclic-GMP-dialysed cells (filled circles) and cyclic-AMP-dialysed cells (open circles) were significant in the case of immature fish (smolts and sea-runs considered together) (Mann–Whitney *U*-test, $P<0.005$) but not in cells isolated from mature spawners ($P=0.360$).

recorded from cells isolated from the same animals. Recordings were made under identical conditions to those in Fig. 7A, except that cyclic GMP replaced cyclic AMP in the pipet solution. Again, as voltage steps were delivered, an outward current was activated. With cyclic GMP dialysis, however, relaxation was slowed in cells isolated from smolts and sea-run fish, but apparently not in cells isolated from mature spawners.

We compared the extent of current relaxation between treatments by measuring the magnitude of current activated just before the termination of a 400 ms voltage step as a percentage of peak current activation (holding voltage = −80 mV, stepped to +20 mV). In recordings made of cells isolated from nonspawning, immature fish (smolts and sea-run fish considered together), cyclic GMP dialysis significantly slowed relaxation of the outward current as compared to control cells (Fig. 8). In contrast, relaxation rates of recordings made of cyclic-GMP-dialysed and control cells isolated from mature spawners were not statistically different (Fig. 8). Taken together, these results suggest that cyclic GMP affects the relaxation characteristics of the outward current of olfactory receptor neurons and that this effect is life-stage specific.

Discussion

The results presented here show that olfactory receptor cells isolated from salmon have broadly similar ionic conductances to olfactory cells isolated from other organisms studied to date (Trotier, 1986; Maue and Dionne, 1987; Firestein and Werblin, 1987; Schild, 1989). Our data also suggest that much of the variability observed with respect to outward currents is associated with the life stage of the fish from which olfactory receptor cells are isolated.

Inward currents

The inward Na^+ current displayed rapid activation and inactivation, and was responsible for the upstroke of the action potential. However, this current was completely blocked by micromolar concentrations of TTX, suggesting a channel type that is pharmacologically different from the primarily TTX-insensitive Na^+ currents that have been described in amphibian olfactory receptor neurons (Trotier, 1986; Firestein and Werblin, 1987; but see also Schild, 1989). Trotier has suggested that the TTX-sensitive component of the Na^+ current observed in some cells may reflect a variability in axon fragments persisting following dissociation, but our study of TTX sensitivity was not extensive enough either to support or to refute this hypothesis.

Although Ca^{2+} channels have also been demonstrated in olfactory receptor cells, we were only able to observe a Ca^{2+} current by delivering depolarizing steps from a holding potential more positive than or equal to −40 mV. This observation is in sharp contrast to Ca^{2+} currents reported in amphibian olfactory receptor neurons, which were elicited by delivering depolarizing steps from holding potentials of −80 mV to −100 mV (Trotier, 1986; Firestein and Werblin, 1987; Schild, 1989). Interestingly, in each of these cases as well as in salmon olfactory receptor cells, peak current occurred in roughly the same voltage range (0–30 mV) but, since our observations were made using Sr^{2+} as the ionic carrier, inactivation characteristics are difficult to compare.

Further physiological characterization of the Ca^{2+} current was hampered by a time-dependent washout during whole-cell recordings. The phenomenon suggests that soluble metabolites essential to the functioning of Ca^{2+} channels rapidly diffuse out of the cytoplasm once the whole-cell recording mode has been established (see Kostyuk, 1980; Hagiwara and Byerly, 1981). Our attempts to compensate for this washout by dialysing cells with cyclic AMP, ATP or theophylline during recordings did not dramatically alter the time course of current disappearance (see Doroshenko *et al.* 1984). However, since cells were observed to generate action potentials in current–clamp mode even after the Ca^{2+} current should have been completely washed away, it is unlikely that an influx of Ca^{2+} is necessary to generate an action potential in these cells.

Ca^{2+} may serve a regulatory function, either in modulating other currents or in the odorant transduction pathway itself. For example, the data in this paper as well as that of Trotier (1986), Maue and Dionne (1987), Firestein and Werblin (1987)

and Schild (1989) suggest that Ca^{2+} modulates a K^+ channel in olfactory receptor neurons. Extracellular recordings of *in vivo* preparations of lamprey olfactory receptor neurons have also shown that the magnitude of the receptor response to L-arginine is a specific function of the Ca^{2+} concentration in the external solution (Suzuki, 1978*a*). Furthermore, an increase in L-arginine binding has been correlated with increasing extracellular Ca^{2+} concentration (Suzuki, 1978*b*). Interestingly, sockeye fry have also been shown to discriminate behaviorally between natural water sources solely on the basis of minute differences in Ca^{2+} concentration (10^{-6} mol l^{-1} *vs* 10^{-7} mol l^{-1}; Bodznick, 1978). Finally, an inositol 1,4,5-trisphosphate–Ca^{2+}-mediated cascade has been shown to be involved in catfish olfactory transduction (Huque and Bruch, 1986; Restrepo *et al.* 1990). Together these results suggest that a Ca^{2+} influx may be significant in the modulation of odorant responsiveness at the level of the receptor cell.

Outward currents: life-stage differences

Our results describe three separate K^+ conductances in ciliated olfactory receptor cells and further suggest that variation in these outward currents is correlated with life-stage differences. For example, the Ca^{2+}-activated K^+ current consistently comprised most of the outward current in cells isolated from parr, but was also present in cells isolated from every life stage. The function of this current may be to facilitate repolarization following depolarization in olfactory receptor neurons, as it is known to do in other cells (for reviews, see Meech, 1980; Marty, 1989), and it may also play a role in the modulation of bursting during periods of strong odorant stimulation (Conner and Stevens, 1971*b*; see also discussion in Trotier, 1986).

Although the transient K^+ current was typically pronounced only in cells isolated from older coho (i.e. fish that had developed past smoltification: smolts, sea-run individuals or mature spawners), other evidence suggests that this current is probably not necessarily a specific adaptation for regulating spike or burst frequency in the marine as opposed to the freshwater environment. For example, studies of olfactory receptor cells isolated from the freshwater-dwelling parr of chinook salmon (*Oncorhynchus tschawyska*, G. Nevitt, unpublished data) also indicate a substantial transient K^+ current comparable to that observed in cells from coho sea-run and mature spawning salmon. Moreover, transient K^+ currents have been described in olfactory receptor cells of freshwater salamanders and frogs (Trotier, 1986; Firestein and Werblin, 1987; Schild, 1989). Interestingly, in two cells isolated from a coho parr, we were able to record substantial transient K^+ currents (i.e. >300 pA when stepped to +45 mV), but only under conditions in which depolarizing pulses were interrupted by prolonged hyperpolarizing intervals (−75 to −85 mV; >1 min). Unfortunately, technical difficulties with cell run-down and maintaining viable cells during prolonged hyperpolarizations hindered further investigation of this phenomenon.

Based on these combined results as well as on the results of other researchers (e.g. Bayer *et al.* 1989; Siegelbaum *et al.* 1986; Cuello, 1987; Schumann and

Gardner, 1989; Soliven *et al.* 1989; reviewed by Brown, 1990), we hypothesize that hormone modulation during smoltification may play a role in establishing variation in outward currents. Extensive research has shown, for example, that seasonal surges in thyroid hormone (T_3 and T_4) activity are strongly correlated with the onset of smoltification in coho salmon, and other studies have implicated the involvement of additional hormones, including growth hormone, cortisol, prolactin and possibly atrial natriuretic hormone (Uemura *et al.* 1990), during the saltwater transformation (for reviews, see Folmar and Dickhoff, 1980; Hoar, 1988). Although effects of acute exposure to these hormones were not studied here, hormone modulation could also be involved in either the expression of the transient K^+ current (Moody and Landsman, 1983; Simoncini and Moody, 1990) or the proliferation of a new population of receptor cells that express this current. Local application of thyroid hormone has been shown, for example, to induce extensive proliferation of olfactory receptor cells in *Xenopus laevis* tadpoles (Burd, 1990), but the electrophysiological properties of these cells have not yet been examined.

The possibility that differences in dissociation could additionally account for some of the electrical variability reported here seems unlikely because the same dissociation procedure was followed throughout the course of these experiments, and variation was specific to outward currents. Interestingly, considerable variability in apparent outward current densities has also been noted in olfactory receptor cells isolated from salamander (Trotier, 1986; Firestein and Werblin, 1987), but these studies do not present detailed information about the age or reproductive state of the animals that were used in experiments, making comparisons between salamander and salmon olfactory receptor cells difficult.

Effects of cyclic GMP

Experiments with cyclic GMP additionally suggest that the excitability of ciliated olfactory receptor neurons may be modulated during other phases of the life cycle such as the reproductive period. Although preliminary, the results presented here clearly show a life-stage dependence in the sensitivity of olfactory receptor neurons to a cyclic nucleotide, which has also been implicated as a second messenger in both olfactory transduction (Nakamura and Gold, 1987; but see also Pace *et al.* 1985; Sklar *et al.* 1986) and hormone modulation in a variety of other systems (e.g. Fischmeister and Hartzell, 1987; Chen *et al.* 1988; Gotow, 1988; Morton and Truman, 1988). Interestingly, from results of studies of whole-tissue voltage-clamp of bullfrog olfactory epithelium, Persaud *et al.* (1988) have suggested that cyclic GMP may play a role during olfactory transduction by activating outward K^+ currents. However, until the effects of biologically important and behaviorally tested odorants on possible second messengers in this system have been determined, we are cautious in assigning a specific role to either cyclic AMP or cyclic GMP in the olfactory transduction process in salmon.

The home-stream migratory phase is marked by a hormonally induced general physiological deterioration in salmon, and this degeneration could be reflected in

the lack of responsiveness of olfactory receptor cells to dialysis with cyclic GMP. However, we feel that this explanation is inadequate for several reasons. First, cells isolated from mature spawners were morphologically and electrically intact compared with cells isolated from younger fish. Second, since olfactory acuity is likely to be important throughout courtship and mating (see Sorensen *et al.* 1987, 1990), it is improbable that this system would be hormonally triggered to shut down at the time when fish were collected for this study (i.e. before spawning). Alternatively, the cyclic-GMP-induced reduction in outward current relaxation could again be important in mediating interspike interval or burst frequency in salt water, since this effect was observed only in fish that were either physiologically preparing to enter or already inhabiting salt water. The mechanisms by which cyclic GMP modulates the transient K^+ current, or possibly an underlying voltage-dependent K^+ current, were not, however, addressed in this study.

The results presented here suggest that a background knowledge of the life history characteristics and olfactory sensitivities of the specific organism being studied is critical to understanding functional aspects of peripheral olfaction. The system described here is ideal in that a detailed knowledge of olfactory stimuli and endocrinology is already available. Future studies are aimed at characterizing how olfactory receptor cells isolated from salmon respond to behaviorally and electrophysiologically tested olfactory stimuli and will further examine mechanisms by which hormones may modulate these cells during smoltification and the home-stream migration. Such an integrative approach should prove fruitful in elucidating cellular mechanisms underlying olfactory function and behavior in this unique and fascinating animal.

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