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Excitation, inhibition, and suppression by odors in isolated toad and rat olfactory receptor neurons

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Sanhueza, Magdalena, Oliver Schmachtenberg, and Juan Bacigalupo. Excitation, inhibition, and suppression by odors in isolated toad and rat olfactory receptor neurons. *Am J Physiol Cell Physiol* 279: C31–C39, 2000.—Vertebrate olfactory receptor neurons (ORNs) exhibit odor-induced increases in action potential firing rate due to an excitatory cAMP-dependent current. Fish and amphibian ORNs also give inhibitory odor responses, manifested as decreases in firing rate, but the underlying mechanism is poorly understood. In the toad, an odor-induced Ca^{2+}-activated K^+ current is responsible for the hyperpolarizing receptor potential that causes inhibition. In isolated ORNs, a third manner by which odors affect firing is suppression, a direct and nonspecific reduction of voltage-gated and transduction conductances. Here we show that in whole cell voltage-clamped toad ORNs, excitatory or inhibitory currents were not strictly associated to a particular odorant mixture. Occasionally, both odor effects, in addition to suppression, were concurrently observed in a cell. We report that rat ORNs also exhibit odor-induced inhibitory currents, due to the activation of a K^+ conductance closely resembling that in the toad, suggesting that this conductance is widely distributed among vertebrates. We propose that ORNs operate as complex integrator units in the olfactory epithelium, where the first events in the process of odor discrimination take place.

olfactory transduction; excitatory current; inhibitory current; odor suppression

THOUSANDS TO MILLIONS of olfactory receptor neurons (ORNs) in the olfactory epithelium enable vertebrates to perceive a wide diversity of odor molecules (for a review, see Ref. 3). Each sensory cell is thought to express a single type or at most a few types of receptor molecule in its chemosensory cilia, where odor transduction occurs. A matter of great interest is how the olfactory system discriminates between the myriad of different odorants and how odor perception is achieved by the nervous system. Central to this problem is the question of how odors are coded and to what extent the olfactory epithelium, the olfactory bulb, and higher brain structures participate in coding. In this framework, an understanding of the physiological properties of the receptor neurons is fundamentally important.

It is well known that odors can selectively trigger a cAMP-dependent cascade in ORNs (for a review, see Ref. 30). Odor receptors activate a G protein, which in turn activates adenylyl cyclase, increasing cAMP. The cyclic nucleotide directly gates a ciliary nonselective cationic conductance. An associated Ca^{2+} influx triggers a Ca^{2+}-dependent Cl^- conductance, presumably amplifying the depolarizing effect of the cationic current (17, 20). The resulting receptor potential raises action potential firing, generating an excitatory response. In addition to cAMP, inositol trisphosphate (IP_3) has been proposed to play a role in olfactory transduction, although this remains a matter of controversy (for reviews, see Refs. 2 and 30).

In contrast, odor-induced inhibitory responses due to hyperpolarizing receptor potentials have been observed in invertebrates (21, 24) and in some vertebrate species (5, 11, 27, 31). The available information about vertebrate inhibitory chemotransduction is scarce. In the toad *Caudiverbera caudiverbera* a mixture of putrid odorants selectively triggers a hyperpolarizing Ca^{2+}-activated K^+ current that inhibits action potential firing (25, 27). However, the ionic bases for the inhibitory responses in other vertebrates remain unresolved.

In addition to odor-triggered excitation and inhibition, isolated ORNs also exhibit nonselective odor suppression, a distinct mechanism by which odors decrease ion conductances (13, 16, 19, 29). Suppression appears to be due to a direct effect of odor molecules on both transduction channels and voltage-gated channels. The effect on voltage-gated channels induces a net suppression current that contributes to the odor response, affecting spiking (29).

In the present work, we show that in a subset of isolated toad ORNs a mixture of floral and fruity odorants (*mixture I*) activated a K^+ current similar to that induced by putrid odors (*mixture II*), although the majority of the responses to *mixture I* were excitatory. We used the same odorant mixtures as in our previous work (27, 29). These mixtures were originally selected based on biochemical studies on ciliary membrane preparations, which suggested the existence of cAMP-producing and IP_3-producing odorants (1). Here we

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show, for the first time, that inhibitory currents can also be induced in rat ORNs by both odorant mixtures. Single neurons may selectively respond to a given odorant mixture with either excitation or inhibition. Previously, we reported that in the toad both types of response can occur in the same neuron on stimulation with different classes of odorants (27). In the present work we extend this result and show that, in a small fraction of ORNs, an odorant mixture concurrently elicited both excitatory and inhibitory currents, in addition to suppression currents that were observed in almost every neuron (29). Our results suggest that odor-induced inhibitory currents contribute to chemotransduction and that the underlying mechanism may be partially or entirely independent of the excitatory cascade. The activation of similar inhibitory currents in toad and rat ORNs indicates that this may be a general feature of vertebrate olfaction.

MATERIALS AND METHODS

Cell Dissociation

Toad. Adult *C. caudiverbera* were cooled in ice, killed, and pithed before dissecting out their olfactory epithelia. Epithelia were cut into small pieces (~1 mm^2) and stored up to 48 h at 4°C in a solution containing (in mM) 120 NaCl, 1 $CaCl_2$, 2 $MgCl_2$, 3 KCl, 5 glucose, 10 HEPES, and 5 sodium pyruvate, pH 7.5. This solution also contained 0.03 mg/ml leucine, 0.204 mg/ml glutamine, 0.1 IU/ml penicillin, and 1% bovine albumin. Dissociated cells were obtained by gently passing the pieces of epithelia through the tip of a fire-polished Pasteur pipette and were then transferred to the experimental chamber containing Ringer solution (see *Solutions*).

Rat. Adult Wistar rats, bred in the laboratory, were anesthetized by CO_2 inhalation and decapitated. The head capsule was opened by a sagittal section and the olfactory epithelium was removed from the dorsal posterior part of the nasal septum and from the turbinates. The epithelium was cut into pieces of 1 mm^2 thickness, stored in Leibovitz L-15 medium at 4°C, and used only on the day of the preparation. Dissociation was achieved by trituration through a fire-polished glass pipette, without the use of enzymes. Cells were let settle on coverslips coated with Pegotin (BiosChile, Santiago, Chile) for 20 min and washed with mammalian saline.

Electrical Recordings and Stimulus Application

Electrical recordings were obtained with the patch-clamp technique in its whole cell mode, using a PC-501A (Warner Instruments, Hamden, CT) and a Dagan (Minneapolis, MN) amplifier, as in our previous work (25, 27). Experimental protocols and data analysis were conducted using pCLAMP 6.0 (Axon Instruments, Foster City, CA). Recording pipettes were made from Blu Tip capillary tubes (Oxford Labware, St. Louis, MO) and drawn with a P 80/PC horizontal puller (Sutter Instruments, Novato, CA) to a tip resistance of 3–6 MΩ.

Odor stimulation was achieved with double-barreled puffer pipettes made of Θ glass (tip diameter: ~2 μm; Sutter Instruments) positioned at 20 or 40 μm from the cell. Stimuli engulfed the entire cell. Pressure pulses (range 2–15 lb/$in.^2$) were applied with a picospritzer. We estimated the delay of the puffing system to be ~20 ms (delay of the current change induced by a H_2O pulse delivered at ~5 μm distance from the recording pipette) and corrected the latencies for that value. Odor concentrations at the cell level were estimated as in Ref. 7.

Solutions

Toad. Normal Ringer contained (in mM) 115 NaCl, 1 $CaCl_2$, 1.5 $MgCl_2$, 2.5 KCl, 3 glucose, and 10 HEPES, pH 7.6. The internal solution contained (in mM) 120 KCl, 1 $CaCl_2$, 2 EGTA, 1 $MgCl_2$, 0.1 Na_2GTP, 1 MgATP, and 4 HEPES, pH 7.6, pCa 7.5.

Rat. The external solution contained (in mM) 1.3 $CaCl_2$, 1.0 $MgCl_2$, 0.7 $MgSO_4$, 5.4 KCl, 0.30 K_2HPO_4, 137 NaCl, and 1.2 Na_2HPO_4, pH 7.6. Internal solution for the rat was the same as for the toad.

Odorants

Mixture I. Mixture I contained citralva (3,7-dimethyl-2,6-octadienenitrile), citronellal (3,7-dimethyl-6-octenal), and geraniol (3,7-dimethyl-2,6-octadien-1-ol) at a concentration of 1 mM of each in the stimulus pipette.

Mixture II. Mixture II contained isovaleric acid (3-methylbutanoic acid), pyrazine (1,4-diazine), and triethylamine at concentrations of 1 or 3 mM of each within the pipette.

Odors were prepared as 100 mM stocks in distilled water and diluted in Ringer. All chemicals were obtained from Sigma (St. Louis, MO) except for citralva, which was kindly donated by Diego Restrepo (University of Colorado Health Science Center, Denver, CO). All experiments were conducted at ~22°C.

RESULTS

Activation of an Inhibitory Current by a Mixture of Floral and Fruity Odorants in Toad ORNs

Mixture I can also induce outward currents in ORNs. In whole cell voltage-clamp conditions, we examined the effect of identical puffs of *mixture I*, applied during depolarizing steps of different values, from a holding potential of −70 mV (Fig. 1). The corresponding family of odor-activated outward currents is shown in Fig. 1*A*. A 1.5-s odorant puff was applied 1 s after the onset of each voltage step; the net odor-dependent currents (I_{OD}) were obtained after subtracting the control traces. For each potential, I_{OD} has two components: a clearly distinguishable outward current (I_{out}) whose magnitude increases with voltage and a relatively small suppression current, I_s (29). I_s corresponds to a reduction in the voltage-gated outward currents and manifests itself as an inward current that precedes I_{out} and extends until the end of the step (Fig. 1*B*). To determine the exact magnitude of I_{out}, we corrected the net odor-induced current for I_s. The magnitude of I_s can be estimated by extrapolation of the first component of I_{OD} (dotted line; see legend to Fig. 1). Figure 1*B, inset,* shows the current-voltage (*I-V*) curve constructed with the peak values of I_{out} after correcting for suppression (see legend to Fig. 1). This *I-V* relation closely resembles the curve associated with the Ca^{2+}-dependent K^+ conductance activated by putrid odors in this species (25, 27). It should be noted that I_{out} cannot be a Cl^- current, because under the given ionic conditions the Cl^- reversal potential was close to 0 mV.

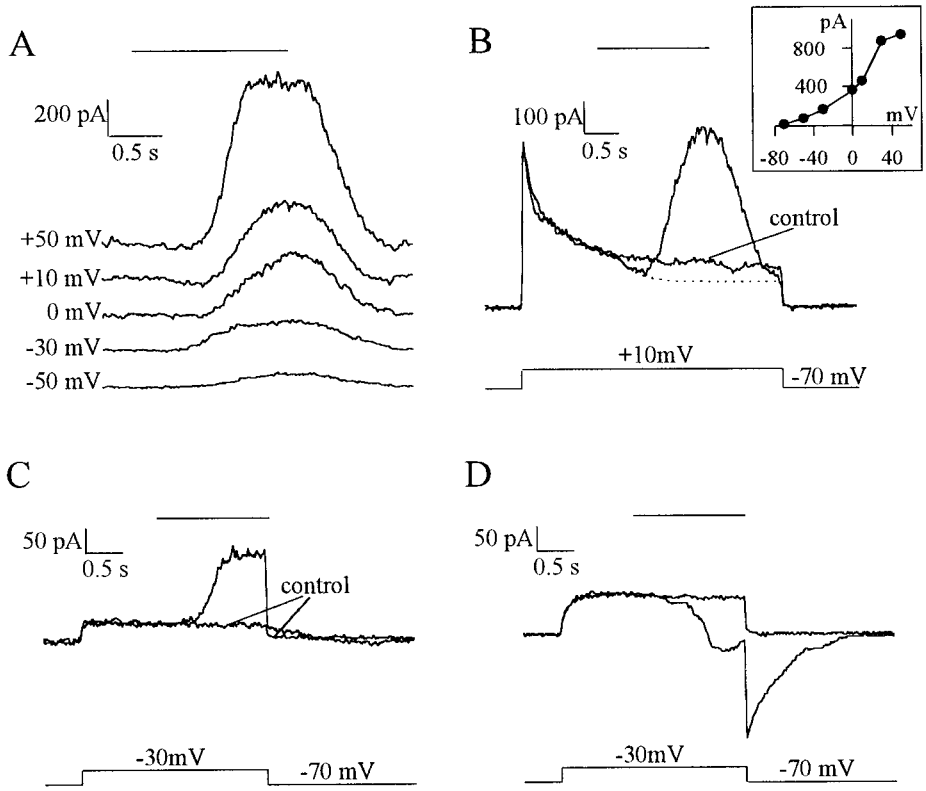

Fig. 1. Activation of inhibitory currents by floral and fruity odorants in toad olfactory receptor neurons (ORNs). *A*: family of whole cell currents induced by identical odor pulses (bar, 250 μM) applied during 4-s depolarizing steps from −70 mV to various voltages, indicated beside each trace. Control recordings with odor-free Ringer solution puffs were subtracted. Each net odor-induced current consists of a suppression current (I_s) and an outward current (I_{out}). *B*: suppression of voltage-gated currents was estimated by extrapolation (dotted line) of a single exponential fit of the early suppressive response. For more negative voltages, where I_s was not clearly visible due to the small magnitude of the voltage-gated currents and to the presence of I_{out}, we estimated I_s by its linear relationship to voltage within this range (29). *Inset*: current-voltage (*I-V*) curve of the odor-induced inhibitory current after correcting for I_s. *C*: on returning from −30 to −70 mV at the moment of maximal response, no inward tail current was observed. *D*: recording from a cell that responded only with transduction current (I_T). Note the development of a prominent tail current.

To investigate whether the cAMP-chemotransduction pathway was activated by *mixture I* in this cell, we terminated the voltage pulse when the odor response reached its peak value (Fig. 1*C*) and inspected whether or not a tail current developed. As in an earlier work (29), we considered the presence of an inward tail current on returning to −70 mV as an indication that the excitatory transduction cascade was triggered by a particular odor stimulus. This tail current develops because the transduction channels are still open and the voltage change increases the electromotive force (6). The absence of an inward tail current in Fig. 1*C* suggests that the cAMP cascade was not activated by *mixture I*. In contrast, in the example illustrated in Fig. 1*D*, corresponding to a different neuron, *mixture I* induced the excitatory transduction current (I_T) that was followed by a tail current.

Even though the majority of the responsive neurons showed the typical excitatory current associated with the activation of the cAMP pathway on stimulation with *mixture I*, the number of cells responding with an outward current was significant. From a total of 50 neurons stimulated with *mixture I*, 21 responded solely with the characteristic I_T (Fig. 1*D*), having a reversal potential near 0 mV (7, 15, 29); 5 cells responded only with I_{out} (Fig. 1, *A–C*); and 3 cells showed the activation of both I_T and I_{out}, the former indicated by the presence of the tail current (see Fig. 3). Overall, I_T was activated in 48% and I_{out} in 16% of the toad ORNs; 92% of the neurons (46 of 50 cells) displayed an observable suppression current.

The Inhibitory Current Induced by Mixture I Is Blocked by Charybdotoxin

To verify whether floral and fruity odors indeed triggered the same K^+ current activated by putrid odors in *Caudiverbera*, we applied *mixture I* in the presence of the K^+ channel blocker charybdotoxin (ChTX). ChTX blocks the odor-induced K^+ conductance (25) but not the voltage-gated K^+ conductances (4). Figure 2 shows the effect of ChTX on the outward current activated by *mixture I* in the same cell as in Fig. 1. In this example, a 1.5-s odorant puff applied during a depolarizing step to +10 mV induced an outward current of 250 pA (Fig. 2*A*). Also visible is the reduction of the voltage-gated current due to the suppression effect. From another barrel, containing the same odor stimulus supplemented with 200 nM ChTX, we applied a puff of identical intensity as in Fig. 2*A* (Fig. 2*B*). While the suppression effect remained (indicating that odors effectively reached the cell), the odor-induced outward current was completely blocked by the toxin. The ChTX effect was reversible, as illustrated by the recovery of the response recorded ~20 s later (Fig. 2*B, inset*).

Consequently, both odorant mixtures can selectively activate the K^+ current (I_{out}). In a separate study, we stimulated 50 ORNs that had lost their olfactory cilia during dissociation and observed that neither the odor-induced K^+ current nor the cAMP-dependent current were activated.

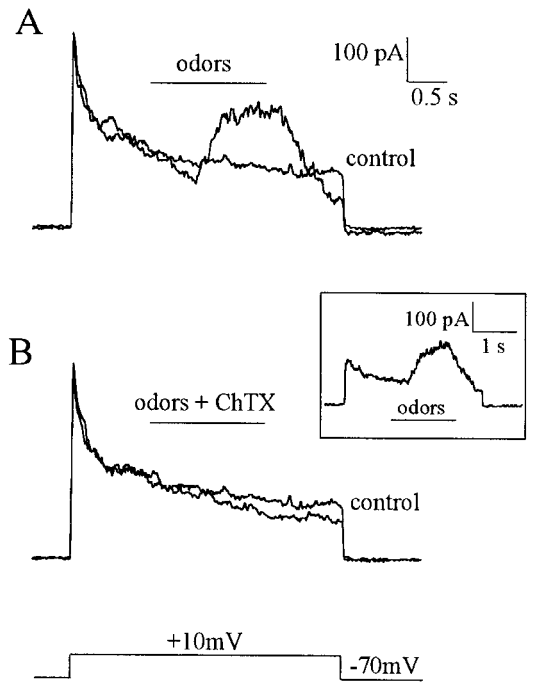

Fig. 2. Odor-induced outward current is blocked by charybdotoxin (ChTX). *A*: same cell as in Fig. 1. During a depolarizing step to +10 mV, *mixture I* triggered both I_s and I_{out}. *B*: repetition of the odor stimulus, supplemented with 200 nM ChTX in the stimulation pipette, induced suppression, but I_{out} was blocked. *Inset*: recovery of the odor response for a subsequent (20 s later) stimulation at −10 mV.

Coexistence of the Odor-Induced K^+ Current, the Excitatory Transduction Current, and the Suppression Current in the Same ORN

Figure 3*A* shows recordings from a toad cell in which stimulation with *mixture I* triggered activation of I_{out}, I_T and I_s. An odorant pulse (1.5 s) applied at 0 mV induced a biphasic current (the control trace is superimposed). At this voltage (the reversal potential of I_T), the odor-induced outward current corresponds to I_{out}. As this current diminished, I_s became visible. The presence of the characteristic tail current on returning to the holding potential indicates that the cAMP transduction cascade was also activated by the odorant puff. In the same cell, the presence of ChTX together with the odorant fully abolished the outward K^+ current, leaving the suppression effect and the tail current (Fig. 3*B*). In Fig. 3, *C* and *D*, we depict the net odor-induced responses recorded at four different membrane potentials, in the absence and in the presence of ChTX, respectively. At 0 mV and in the presence of the channel blocker, we could directly measure I_s and calculate its value for the other voltages. The *I-V* curves of the corrected odor responses (peak values) in the absence and presence of ChTX are shown in the insets of Fig. 3, *C* and *D*. In the first case, the corrected current is positive and results from the superposition of I_T and I_{out}. However, in the presence of the toxin, the *I-V* relation clearly resembles the one associated with the excitatory transduction pathway alone. To investigate whether odor-induced outward currents generated a hyperpolarizing receptor potential in this same cell, we conducted current-clamp experiments (holding current = 0 pA) and monitored the membrane potential on odor stimulation. An identical pulse of *mixture I* induced a biphasic receptor potential, consisting of a transient hyperpolarization followed by a depolarization (Fig. 3*E*); a puff of Ringer solution failed to induce any voltage change (not shown). When the odor stimulus was applied together with ChTX, the hyperpolarizing receptor potential did not develop, leaving solely the depolarizing component. The ChTX sensitivity of the odor-triggered hyperpolarization indicates that it was caused by the activation of the K^+ current. In contrast, the depolarizing receptor potential is ChTX independent, as indicated by the close superposition of the odor-induced depolarizations in the presence and absence of ChTX, and it was probably due to suppression of the voltage-gated K^+ currents (29) together with the activation of I_T.

Activation of the Odor-Induced K^+ Current in Rat ORNs

To investigate whether odor-induced inhibitory currents may also be activated in the rat, we applied pulses of odorant *mixtures I* and *II* to isolated rat ORNs. Depolarizing steps from a holding potential of −70 mV induced a pronounced early transient K^+ current (22) followed by a slowly decaying K^+ current, as illustrated in Fig. 4. This cell was stimulated by puffs of odorant *mixtures I* (Fig. 4*A*) and *II* (Fig. 4*B*). Both odor mixtures induced the characteristic cAMP-dependent I_T, as indicated by the *I-V* relations (see Fig. 4, *insets*) and the tail currents on returning to −70 mV. However, in a fraction of rat ORNs, both odor mixtures selectively activated outward currents with properties similar to those recorded in the toad. Figure 5, *A* and *B*, shows examples corresponding to two different cells, where inhibitory currents were induced by odor *mixtures I* and *II*, respectively. Even though in the first cell *mixture II* also triggered a similar but smaller outward current (not shown), *mixture I* only induced suppression in the second neuron. As in the toad, the usual I_s preceded the development of the outward currents in both cells. Figure 5, *C* and *D,* displays the net odor-induced outward currents, after subtracting the control traces (not shown) and correcting the responses in Fig. 5, *A* and *B* for I_s. The *I-V* relations of these outward currents (Fig. 5, *C* and *D*, *insets*) closely resemble those of the odor-induced I_{out} observed in the toad and cannot be explained by the activation of a Cl^- conductance (the reversal potential for Cl^- was −4 mV). In addition, the absence of a perceptible tail current on returning to the holding potential during the odor response suggests that the applied odorants did not activate the excitatory transduction pathway in either case. Moreover, the odor-induced inhibitory current present in rat cells was also blocked by ChTX (Fig. 6), further supporting the notion that the odor-induced outward current observed in the rat is a K^+ current

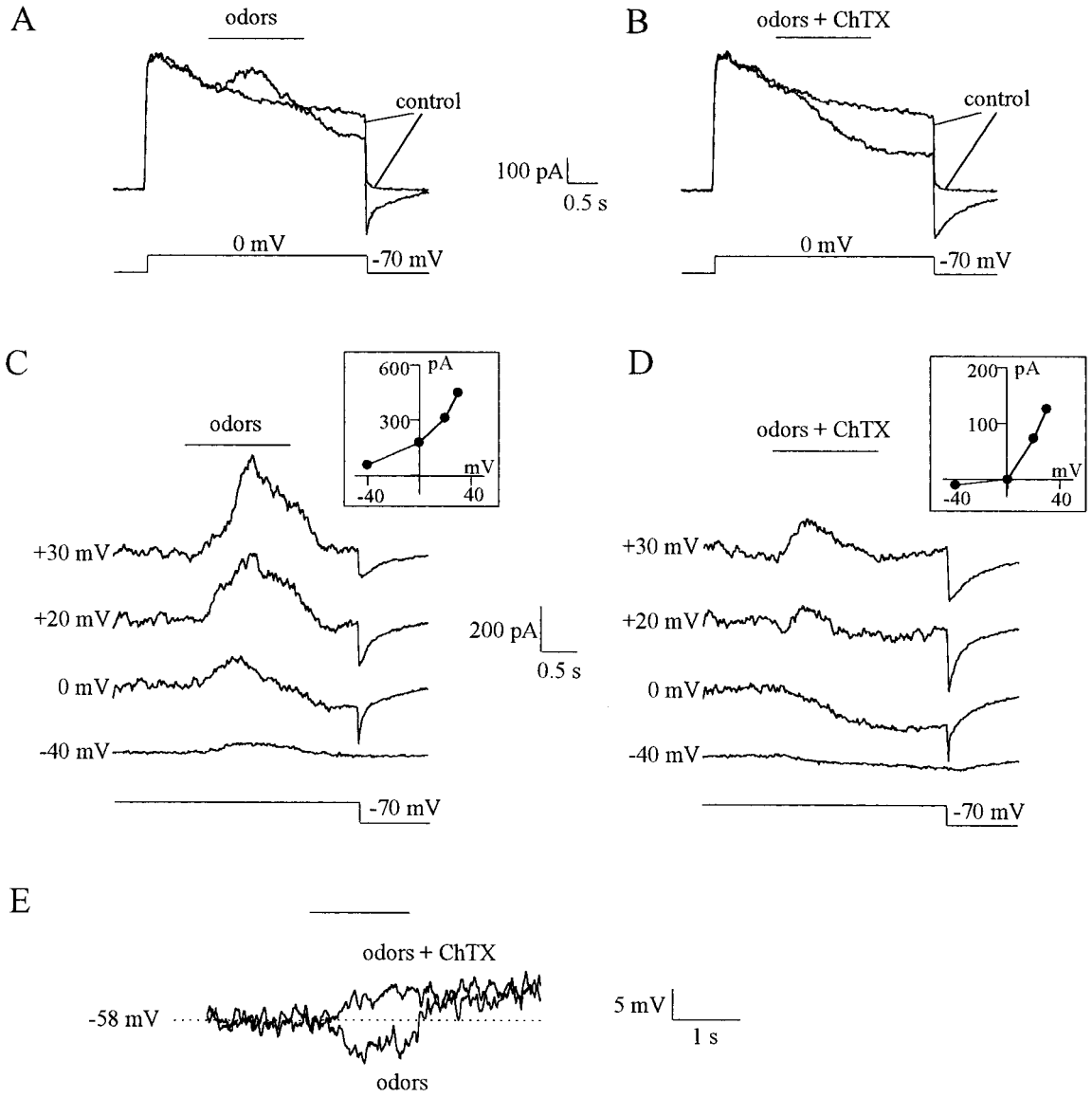

Fig. 3. Excitatory, inhibitory, and suppression currents can be concurrently activated in an ORN by an odor stimulus. *A*: at 0 mV (reversal potential of I_T), a puff of *mixture I* (bar, 200 μM) triggered a biphasic current, resulting from the superposition of I_{out} and I_s. On returning to −70 mV, a tail current developed. Control, odorant-free Ringer puff. *B*: in a subsequent stimulation in the presence of ChTX, I_{out} was abolished, leaving I_s and the tail current. *C* and *D*: net odor-induced currents at 4 different membrane potentials (indicated on *left*) with and without ChTX, obtained after subtracting the control traces. *Insets*: corresponding *I-V* curves of the odor-induced currents (peak values) after correcting for suppression. I_s was measured at 0 mV in the presence of ChTX and estimated at other voltages based on the linearity of the I_s *I-V* curve. *E*: same cell as in *A–D* is now under current clamp (holding current = 0 pA). Odor-induced membrane potential changes in the absence and presence of the toxin.

similar to that in the toad. In Fig. 6, a 3-s pulse of *mixture II* applied during a depolarizing step to −30 mV activated an outward current of ~70 pA. The end of the odor puff coincides with a transient increase of the outward current, probably corresponding to an off response (see DISCUSSION). When stimulating with odors plus ChTX (100 nM in the pipette), the odor response was pronouncedly blocked. In a subsequent ChTX-free odor stimulation, the original response recovered (not shown).

From a total of 86 rat ORNs tested, 17 cells (20%) showed the activation of I_{out}; among this latter group of neurons, 6 responded with I_{out} only to *mixture I*, 8 only to *mixture II*, and 3 to both odorant mixtures. Overall, *mixture I* triggered I_{out} in 10% (9/86) and *mixture II* in 12% (11/83) of ORNs.

As it can be observed in the different examples where I_{out} was activated in toad and rat ORNs, the latency of this inhibitory current was typically of a few hundreds of milliseconds, ranging between 60 and 600 ms. It should be noted, however, that the rapid development of I_s makes it difficult to measure the latency of I_{out} with precision, and in some cases it may have been overestimated.

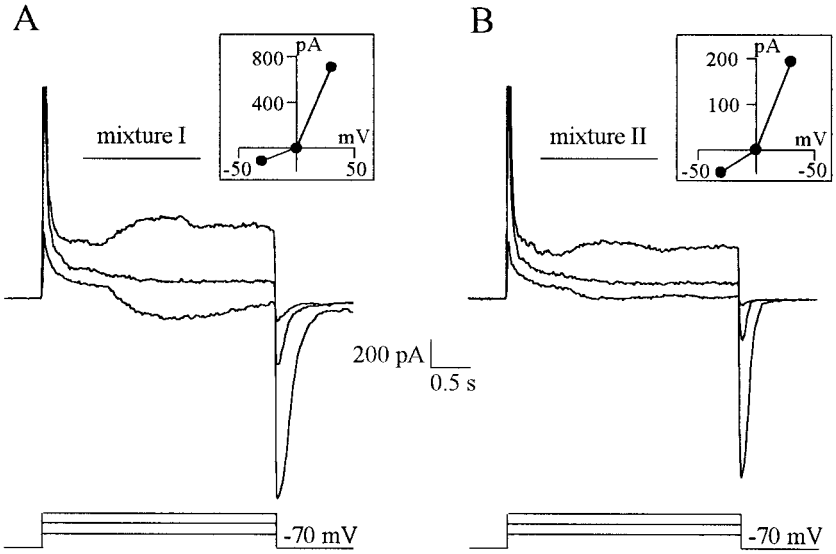

Fig. 4. Activation of the excitatory transduction current by both odorant mixtures in a rat ORN. *A*: identical odorant puffs of *mixture I* (bar) were applied during depolarizing steps from a holding potential of −70 mV. Superimposed are 3 recordings, obtained at −30, 0, and +30 mV. Note the conspicuous tail current on returning to −70 mV. The *I-V* relation of I_T, corrected for suppression, is shown in the *inset*. *B*: same as in *A*, but in this case the same neuron was stimulated by a puff of *mixture II*.

DISCUSSION

It is well established that vertebrate ORNs can selectively respond to odorants by activation of an excitatory cAMP-mediated transduction cascade. However, it has been shown that some odorants from the putrid class can activate a Ca^{2+}-dependent K^+ conductance (26), causing inhibition in *Caudiverbera* ORNs (36%; 10 of 28 neurons, in Ref. 27). The odor-induced Ca^{2+}-activated K^+ channels differ from the voltage-gated ones because they are sensitive to ChTX, whereas the latter ones are ChTX insensitive (4). In other vertebrates such as catfish (11) and *Xenopus* (31), amino acids cause inhibition as well, although nothing is known about the ionic conductance involved in these responses. In the present work we extend our previous studies and show that, in a subset of toad ORNs (16%; 8 of 50 neurons), a mixture of floral and fruity odorants (*mixture I*) can also activate the K^+ conductance, as

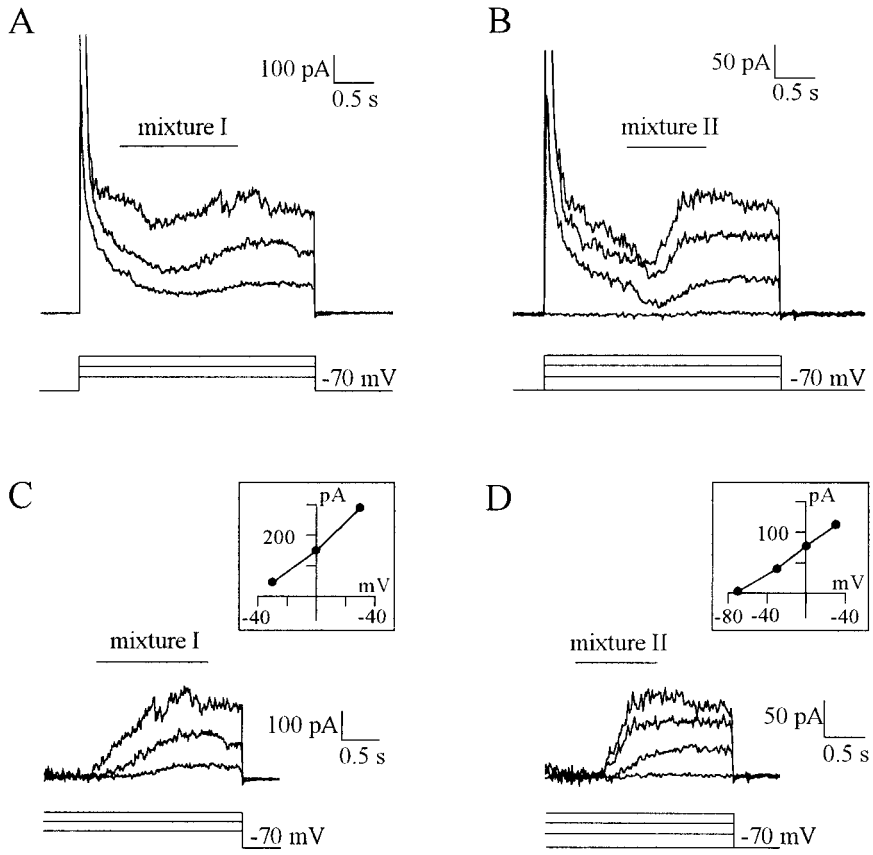

Fig. 5. Inhibitory K^+ currents can be activated by both odorant mixtures in rat ORNs. *A* and *B*: similar protocols as in Fig. 4 were applied to 2 different ORNs. Puffs of *mixture I* (*A*) and *mixture II* (*B*) induced outward currents at all tested voltages. As in the toad, odors also induced I_s, which can be distinguished in the early part of the response. No tail currents were observed on returning to −70 mV during the odor responses. *C* and *D*: odor-induced outward currents obtained after subtracting the control traces (not shown) and correcting the recordings in *A* and *B* for I_s, respectively. *Insets*: *I-V* relations of these currents.

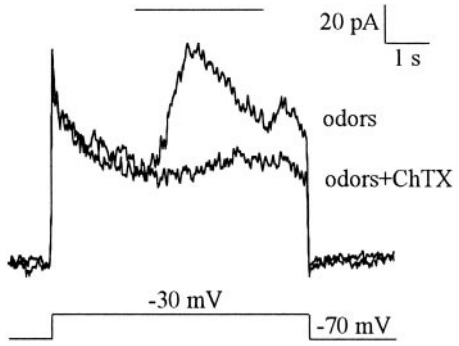

Fig. 6. Odor-induced inhibitory currents in rat ORNs are blocked by ChTX. A 3-s putrid odor stimulus activated an outward current, followed by an apparent off response. The presence of 100 nM ChTX in the stimulation pipette strongly and reversibly (not shown) reduced the odor response.

indicated by the *I-V* curve and the ChTX sensitivity of the odor-induced current. However, 24 (48%) of the 50 tested toad neurons (77% of the responsive neurons) responded to *mixture I* with the usual excitatory I_T.

Here we report that an odor-induced ChTX-sensitive K^+ current, closely resembling that in the toad, is present in rat ORNs. This inhibitory current was triggered by *mixture I*, *mixture II*, or both odorant mixtures in 20% of rat neurons. The two mixtures activated I_{out} with similar frequencies (10% and 12% of the cells) but rarely in the same ORN, suggesting that a selective mechanism is involved in the activation of this current.

In toad ORNs we usually observed inhibitory currents with stimulus concentrations in the range of 200–300 μM of *mixture I*, suggesting that the concentration threshold for triggering this type of odor response may be higher than that for inducing the excitatory transduction current. To increase the probability of observing inhibitory responses in the rat, we used odor concentrations from 0.1 to 0.5 mM of *mixture I* and from 0.3 to 1.5 mM of *mixture II*. Even though the dose dependence of the odor-induced K^+ current has been established for the toad (27), a more detailed study is required to investigate whether odor concentration constitutes a factor that determines the activation of excitatory or inhibitory currents, or of both of them simultaneously.

The *I-V* relation of the odor-activated K^+ current indicates that its amplitude is vanishingly small at membrane potentials close to the resting potential. However, the high membrane resistance of ORNs allows minute currents to cause significant changes in the membrane potential. In current-clamp experiments (holding current = 0 pA), we observed that the spontaneous action potential discharge can be inhibited by the injection of only −1 or −2 pA (not shown). These current stimuli induced hyperpolarizations of the order of 5–10 mV, comparable with the inhibitory receptor potentials caused by odorants (27). An example is shown in Fig. 3*E*, where the odor-induced K^+ current hyperpolarized the cell. Our results suggest that the activation of this hyperpolarizing current may underlie the odor-induced inhibitory responses observed in some vertebrates species (11, 31). Our observations in two phylogenetically distant animals, such as the toad and the rat, suggest that the presence of this odor-triggered conductance may be a general feature among vertebrates. Moreover, inhibitory odor responses reported in the lobster were shown to be mediated by a cAMP-dependent K^+ conductance (24), suggesting a rather general role of odor-induced K^+ currents in olfactory transduction.

It should be noted that the direction of the K^+ current and the resulting change in membrane potential in vivo depend on the K^+ concentrations in olfactory mucus and ciliary lumen. However, neither of these values has been unambiguously determined. Reuter et al. (28) recently reported that, in mammals, mucosal concentration of this ion might be higher than previously estimated (10) and, therefore, the possibility that K^+ carries a depolarizing inward current cannot be ruled out at present. Even in such a case, the odor-induced K^+ current would still have a hyperpolarizing effect if the cell had been depolarized by other components of complex odor blends, a situation that animals commonly experience in nature. In freshwater fish and some amphibians, however, external K^+ concentrations are probably lower than in mammals, thus increasing the inhibitory effect of the odor-induced K^+ currents.

Here we show that the same odorant mixture can activate depolarizing or hyperpolarizing currents in different neurons, indicating that, in absolute terms, there are no strictly excitatory or inhibitory odorants. Similarly, it has been reported that single amino acids may induce excitatory or inhibitory responses in separate ORNs of lobster (24), catfish (11), and *Xenopus* tadpoles (31).

The latency of the inhibitory current (>60 ms) suggests an indirect mechanism, as previously proposed (27). In addition, it was shown that focal application of odors to the cilia and the dendritic knob induced significantly larger currents than odor ejection onto the soma (25). Together with the fact that I_{out} has never been observed in ORNs without cilia (n = 50), these data suggest that at least some of the steps of the underlying mechanism localize to the ciliary compartment of the receptor cell. A ChTX-sensitive Ca^{2+}-dependent K^+ conductance in toad olfactory cilia was reported by Jorquera et al. (9), based on single-channel recordings from purified ciliary membranes fused to planar lipid bilayers. However, in isolated frog olfactory cilia no Ca^{2+}-dependent K^+ currents were observed (14). The issue of the exact location of odor-induced Ca^{2+}-activated K^+ channels remains therefore controversial and requires further investigation. In any case, these channels can be distinguished from the somatic voltage-gated Ca^{2+}-activated K^+ channel because of their different sensitivities to ChTX.

The off response generated in a rat ORN by a prolonged odor pulse (Fig. 6) is probably due to the removal of suppression of the odor-activated currents (16), indicating that the odor-dependent K^+ channels

remained open for some time after the odor-containing solution had been washed away.

The fact that inhibitory and excitatory responses can be elicited in the same cell by different odor stimuli has previously been noticed in lobster (23), toad (27), and fish (11). Here we show that both types of odor responses can be concurrently activated in ORNs by a pulse of odorants. The observation of odor-induced outward currents at 0 mV (the reversal potential of I_T) and also at lower voltages indicated the activation of I_{out}, and the presence of inward tail currents on returning to the holding potential evidenced the parallel activation of the cAMP-dependent I_T. As is common practice, we stimulated with odorant mixtures to increase the probability of triggering odor responses in isolated ORNs; therefore, we cannot discriminate whether a pure odorant by itself may activate both currents or whether the combined response (Fig. 3) resulted from independent effects caused by different odorants of the mixture. However, the small fraction of responsive ORNs presenting both outward currents and tail currents suggests that the mechanisms underlying I_{out} and I_T are independent. Nevertheless, the excitatory and inhibitory mechanisms may share some of the transduction stages, causing a combined response in some cases or diverging in other cases, leading either to the excitatory or the inhibitory transduction current. Local differential distribution of microdomains of chemotransduction components may exist in the cilia and somehow determine the alternative or parallel activation of the depolarizing or hyperpolarizing transduction currents. In fact, Ca^{2+} fluorescence studies in olfactory cilia during odor responses showed clear inhomogeneities in fluorescence intensity, consistent with a clustered distribution of cAMP-gated channels or other components of the olfactory cascade (18). Further studies are required to investigate this possibility and to establish the conditions under which the excitatory or the inhibitory odor-induced currents, or both of them, become activated.

Odor concentrations used by us and other workers on isolated ORNs are higher than those detected by the olfactory epithelium, where they have been estimated to be in the nanomolar or picomolar range (8). Therefore, our observations are valid for isolated ORNs, but the physiological relevance for vertebrate olfaction of the phenomena that we are describing remains to be determined.

The observations described in the present work may have implications for the differential effects of odor mixtures compared with the effects of their individual components, a phenomenon recently reported for individual fish olfactory ORNs using extracellular recording techniques (12). Such studies show that odors having comparable effects (either excitatory or inhibitory) when applied individually may occasionally generate a smaller response or even not generate a response at all when applied in blends ("mixture suppression"). Further information on the mechanism of inhibitory chemotransduction and on the way both pathways interact may shed light on this and possible other olfactory-related phenomena.

In conclusion, the present study shows that odorants of very different chemical structure are able to induce excitatory or inhibitory responses in ORNs from the toad and from the rat. Our data suggest that, comparable to the excitatory odor response, the inhibitory response is based on a selective mechanism that operates in the olfactory cilia. Although both types of responses appear to be independent, they may be simultaneously observed in an ORN, and the possibility that both partially share the same transduction cascade cannot be discarded at present.

The coexistence of excitatory and inhibitory responses in a neuron suggests that ORNs operate as primary integrative centers of chemical signals in the olfactory system. The effect of odor-induced K^+ currents on the membrane potential may depend on the particular odor blend that the animal is smelling at a given time. The consequences of such currents and their significance in olfactory processing may also vary with the animal species. We propose that chemotransducing excitation, inhibition, and probably also nonselective suppression together contribute to define the spiking behavior of the ORN in vivo.

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