A ciliary K^+ conductance sensitive to charibdotoxin underlies inhibitory responses in toad olfactory receptor neurons

Bernardo Morales[a], Pedro Labarca[a,b], Juan Bacigalupo[a,*]

[a]*Departamento de Biologia, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile*
[b]*Centro de Estudios Científicos de Santiago, Casilla 16443, Santiago, Chile*

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Abstract In olfactory neurons from *Caudiverbera caudiverbera*, a mixture of putrid odorants trigger an inhibitory, K^+-selective current and a hyperpolarizing receptor potential. The current–voltage relation resembles that of a Ca^{2+}-activated K^+ conductance; their amplitude depends on extracellular Ca^{2+}. 10 nM charibdotoxin, a blocker of K^+-selective channels, including Ca^{2+}-activated ones, reversibly abolished inhibitory currents and receptor potentials. Focal stimulation demonstrates that the underlying transduction mechanism is confined to the cilia. This represents the first evidence for inhibitory responses in vertebrate olfactory cells mediated by a ciliary CTX-sensitive K^+ conductance, most likely a Ca^{2+}-activated one.

Key words: Inhibitory response; Ca^{2+}-activated K^+ channel; Charibdotoxin; Odorant; Olfactory neuron

1. Introduction

Toad olfactory neurons generate inhibitory responses upon stimulation with odorants that do not trigger the cyclic AMP cascade in the chemosensory cilia [1]. Such inhibitory responses, reflected as a decrease in action potential firing, was traced to the development of hyperpolarizing receptor potentials as a result of activation of a TEA-sensitive, K^+-selective conductance triggered by odorants. Further work in our laboratory has suggested that olfactory cilia possess Ca^{2+}-activated K^+ channels of the BK type. Unlike those found in the soma of olfactory neurons [2,3], the BK channels derived from olfactory cilia were blocked by charibdotoxin (CTX) at nanomolar concentrations (Jorquera et al., unpublished results), indicating a toxin affinity similar to that of other BK channels [4]. The above observations prompted us to examine whether the inhibitory responses of isolated olfactory neurons exhibited sensitivity to CTX. The results obtained show that the odorant-triggered hyperpolarizing receptor potentials and inhibitory receptor currents can be blocked by nanomolar concentrations of CTX, that they are dependent on extracellular Ca^{2+}, and that odorant sensitivity is confined to the olfactory cilia. These observations provide the first evidence that inhibitory responses in vertebrate olfactory neurons are due to the activation of CTX-sensitive K^+ channels, most likely Ca^{2+}-activated, located in the olfactory cilia.

2. Materials and methods

Isolated olfactory neurons were obtained from the olfactory epithelium of the Chilean toad *Caudiverbera caudiverbera*, following established procedures [1,3,5]. The external solution contained (in mM): 120 NaCl, 2.5 KCl, 1 $CaCl_2$, 0.4 $MgCl_2$, 5 HEPES, 5 glucose, pH 7.6. Low Ca^{2+} external solution contained 10^{-8} M free Ca^{2+}, buffered with 2 mM EGTA. The pipette-filling buffer was made of (in mM): 120 KCl, 2 EGTA, 4 HEPES, 0.1 GTP, 1 Mg-ATP, pCa 7.6. The odorant mixture, composed of equimolar (1 mM) concentrations of isovaleric acid, pyrazine and triethylamine, was focally applied using a picospritzer. A multibarreled pipette connected to the picospritzer contained the different solutions, as explained in [1]. Odorant concentrations at the cellular level were estimated according to Firestein and Werblin ([6]; also see [1]). CTX (kindly donated by Dr. R. Latorrre) was added to the odorant-containing Ringer solution to a final concentration of 100 nM. Prior to the application of an odorant stimulus, the whole chamber was perfused with the carrier solution (with or without CTX), free of odorants.

Electrical recordings were obtained with a patch clamp by Warner Instruments (model PC-501A; New Haven, CT). An AT486 computer fitted with pClamp 5.5 (Axon Instruments, Inc., Burlingate, CA) was used to generate stimulation protocols and for data acquisition and analysis.

As in previous work [1,3], seal resistances were typically 10 GΩ or higher, and capacitance and series resistance were properly canceled.

A typical experimental protocol consisted of a depolarizing step from a holding potential of −70 to 0 mV. A sustained chemical stimulus was given with the picospritzer after the voltage-dependent outward current reached a plateau [1].

3. Results

In order to examine whether or not charibdotoxin (CTX) was effective in blocking odorant-triggered inhibitory currents in toad olfactory receptor cells, we tested the effect of 10 nM CTX added to an odorant-containing Ringer solution. Fig. 1A shows that while a puff of odorant solution applied locally onto the olfactory cilia induced an outward current, as previously shown [1], an identical puff of odorant solution supplemented with CTX failed to trigger the outward current when applied from a separate barrel. A subsequent stimulation with the CTX-free odorant solution again evoked an outward current, indicating that the effect of CTX was reversible. Each odorant stimulus was given during a 70 mV depolarizing step from a holding potential of −70 mV. The effect of CTX was specific for the odorant-triggered outward current, having no effect on the outward currents activated by the depolarization (Fig. 1A, inset). This result was observed in all 5 cells examined, demonstrating that the odorant-triggered inhibitory outward current is sensitive to CTX.

The effect of CTX was also tested under current-clamp conditions, in which odorants that inhibit electrical activity induce hyperpolarizing receptor potentials [1]. As shown in Fig. 1B, exposure to 10 nM CTX of the same olfactory receptor cell as in Fig. 1A sufficed to block the inhibitory receptor potential in a reversible fashion. This observation was reproduced in 2 additional cells.

*Corresponding author. Fax: (56) (2) 271-2983.

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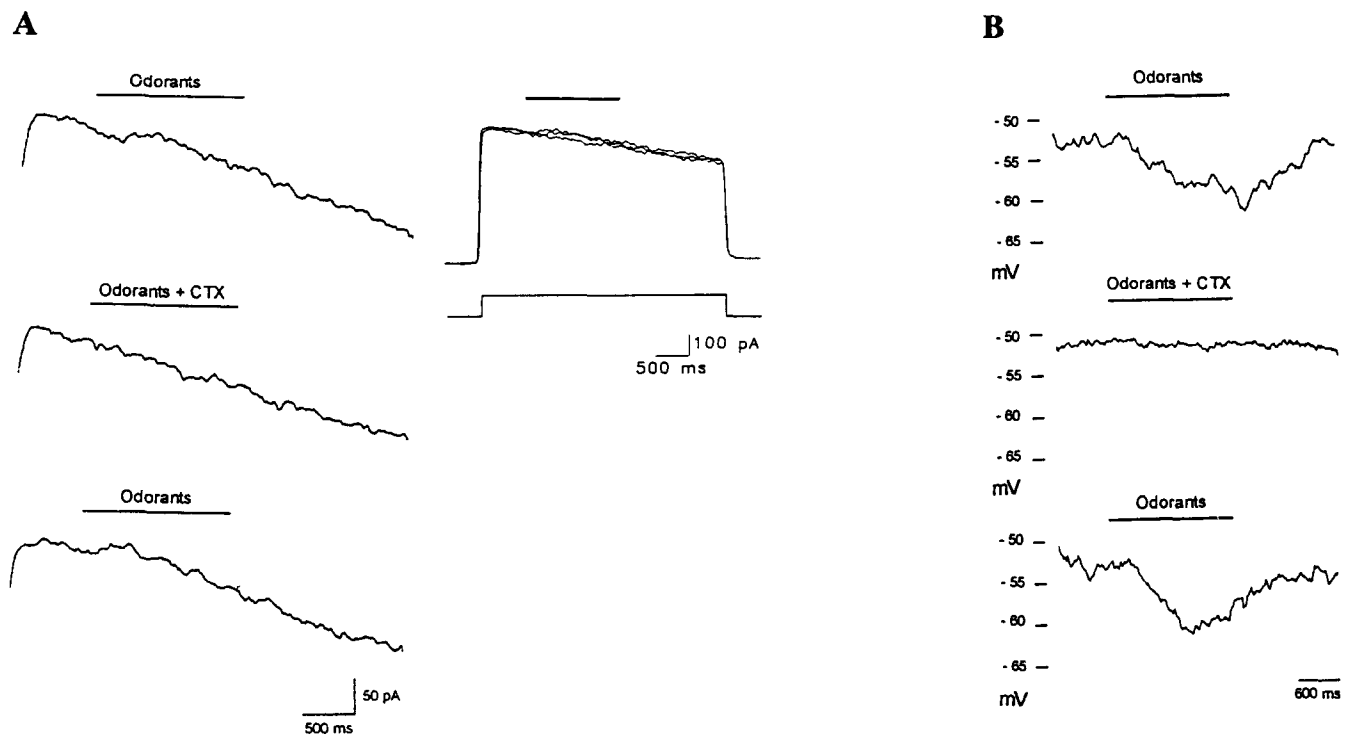

Fig. 1. Block of odorant-triggered outward currents by CTX. The cell was clamped at −70 mV and a depolarizing step to 0 mV was applied. 500 ms after the onset of the depolarization, a 60 μM odorant puff was given (bar). (A) A puff of odorant solution induced an outward current (top trace); an identical puff of odorant solution, supplemented with 10 nM CTX caused no response (middle trace); a subsequent CTX-free odorant puff evoked a new response (bottom trace). (Inset) The same 3 whole-cell currents are displayed over a longer time period and are presented superposed at a smaller vertical scale, showing no difference in the *V*-gate currents. (B) *I*-clamp recordings (I_{hold}=0 pA) show that the odorant-induced hyperpolarization (top trace) was blocked by 10 nM CTX (middle trace), recovering after CTX diffused away (bottom trace).

Recent studies in our laboratory in which chemosensory ciliary membranes were fused to planar lipid bilayers indicate that such membranes contain Ca^{2+}-activated K^+ channels blockable by nanomolar levels of CTX (Jorquera et al., unpublished results). These observations raised the possibility that the odorant-triggered, CTX-sensitive K-conductance involved in the inhibition of electrical activity is Ca^{2+}-activated. We proceeded to examine whether the *I–V* relation of the inhibitory odorant-induced current resembled the characteristic *I–V* relation of a Ca^{2+}-activated K^+-conductance [7], extending the voltage range used in our previous study to higher voltages [1]. A typical result is illustrated in Fig. 2. Fig. 2A shows odorant-triggered currents recorded at various voltages, and the *I–V* relation for this cell is presented in Fig. 2B. As shown, the odorant-triggered inhibitory current exhibited a maximum at about +20 mV, becoming smaller at more positive voltages. A similar observation was made in the 2 cells analyzed in this manner. We also examined whether the odorant-induced K^+-conductance was dependent on extracellular Ca^{2+}. For this we perfused the bath with external solution containing 10^{-8} M free Ca^{2+}. Odorant responses were abolished under these conditions in a reversible manner (Fig. 2C), indicating a requirement of extracellular Ca^{2+}. Both the peculiar shape of the *I–V* relation and the Ca^{2+} dependence support the notion that the odorant-triggered conductance could be a Ca^{2+}-activated, K^+-selective conductance.

In further studies we proceeded to determine whether the sensitivity of olfactory neurons to odorants causing inhibition of their electrical activity [1] was localized to the chemosensory membrane at the olfactory cilia, as has been shown to be the case for their sensitivity to odorants inducing excitatory responses [8,9]. We addressed this question by focally stimulating isolated olfactory neurons with the inhibitory odorant mixture onto two distant regions of the cell, the cilia and the cell body, and comparing the responses to both stimuli. Fig. 3 shows a schematic representation of an olfactory neuron, indicating the sites of stimulation. A puff of 45 μM odorants applied to the ciliary region of the cell under voltage-clamp conditions induced an outward current (top trace, labeled Odorants). This current was not induced when 10 nM CTX was added to the odorant solution and applied onto the same region of the cell (top trace, labeled Odorants+CTX). A CTX-free odorant stimulus of identical characteristics induced a much weaker response when applied onto the cell body (bottom trace) than when applied onto the cilia. Consistent results were obtained in 2 other olfactory neurons that were studied in this way.

We also followed the same experimental protocol on 2 cells under *I*-clamp conditions, finding that a much larger hyperpolarization developed when stimulation was directed onto the ciliary region than when it was directed onto the cell body (not shown). These results demonstrate a high degree of localization of the transduction machinery to the olfactory cilia.

4. Discussion

In this report we offer evidence, for the first time, that inhibitory responses in vertebrate olfactory neurons are due to the activation by odorants of a CTX-sensitive K^+-selective con-

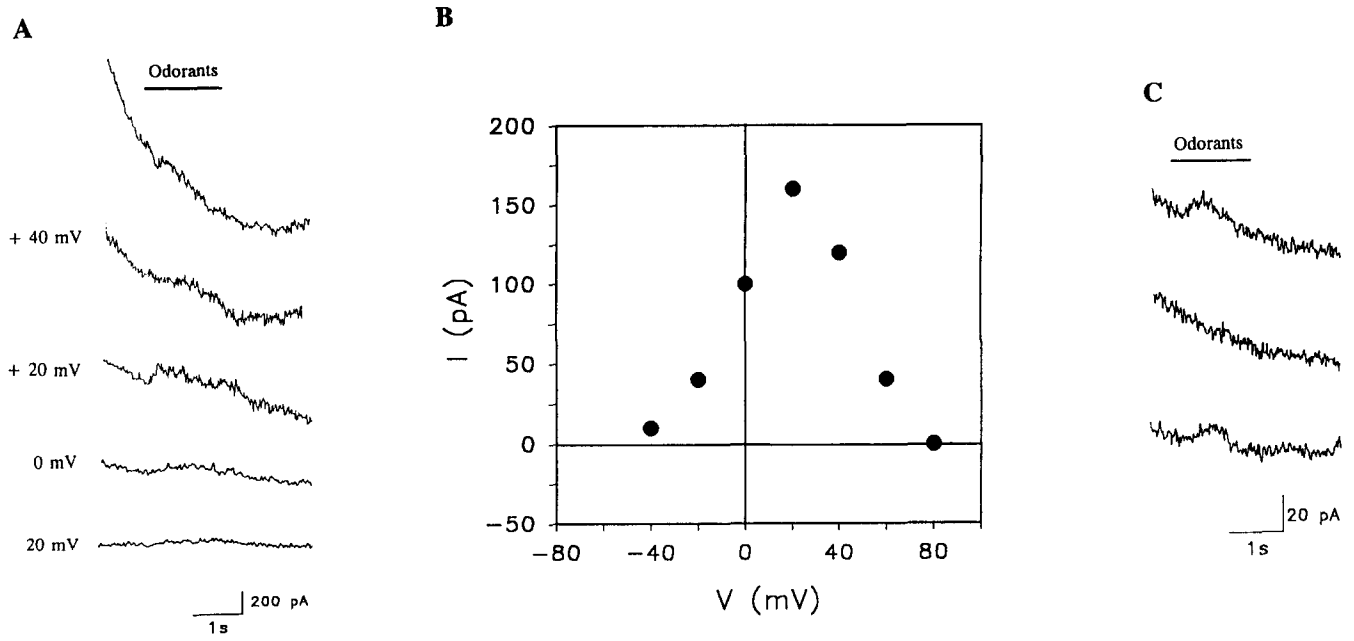

Fig. 2. *I–V* relation and Ca^{2+} sensitivity of the odorant-triggered inhibitory currents. (A) Currents, elicited by identical odorant stimuli, were recorded at the indicated holding voltages. (B) *I–V* relation of the odorant-induced currents, measured as the difference between the peak value and the extrapolated current trace. (C) Odorant-induced current (top trace), recorded in Ringer solution, was abolished under 10^{-8} M Ca^{2+} external solution; for this, the chamber was previously perfused with the low Ca^{2+} solution (middle trace). The response recovered after the Ringer solution was restored (bottom trace).

ductance, dependent on extracellular Ca^{2+}, confined to the chemosensory membrane in the olfactory cilia. This evidence, obtained in the toad *Caudiverbera caudiverbera*, may be extended to other vertebrate species. It has already been demonstrated that olfactory cells from *Xenopus*, like those of *Caudiverbera*, sustain odorant-triggered inhibitory responses mediated by a K^+-selective conductance [1].

Four lines of evidence suggest that inhibitory currents are due to the activation of Ca^{2+}-activated K^+ channels located at the olfactory cilia. First, the *I–V* relation of the odorant-triggered inhibitory current resembles that of Ca^{2+}-activated currents, including those present in olfactory cells [2,10–12], exhibiting a maximum at about +20 mV. Second, the odorant-triggered outward currents are dependent on external Ca^{2+}. Third, the presence of CTX-sensitive K^+ channels in olfactory cilia is suggested by the monitoring of CTX-sensitive, Ca^{2+}-activated K^+ channels of the BK type in planar lipid bilayers doped with purified olfactory cilia membrane fragments (Jorquera et al., unpublished). In the third place, the CTX sensitivity of inhibitory currents is consistent with the pharmacology of Ca^{2+}-activated K^+ channels, some of which are blocked by this toxin with apparent dissociation constants in the nanomolar range [4].

Inhibitory responses to odorants were reported to exhibit a characteristic delay, indicating the participation of an enzyme cascade in this transduction mechanism [1]. This would discard the idea, in principle, that odorants trigger inhibitory currents by activating directly K^+-selective channels in olfactory cilia. The notion that inhibitory currents are Ca^{2+}-activated is consistent with an enzyme cascade activated by inhibitory odorants, which would lead to the activation of Ca^{2+} channels at the ciliary membrane, giving rise to an increase in free Ca^{2+} in the lumen of olfactory cilia. This increase in Ca^{2+} would cause the activation of the Ca^{2+}-activated K^+ channels. An important open question posed by this hypothesis concerns the nature of the second messenger that mediates the inhibitory responses. Further studies directed to establish the properties of the putative ciliary Ca^{2+} channels and their pharmacology are under way. Here we offer the first evidence that a K^+-selective conductance, sensitive to nanomolar concentrations of CTX and dependent on extracellular Ca^{2+}, located differentially at the olfactory cilia, is a crucial component of inhibitory transduction in olfactory neurons.

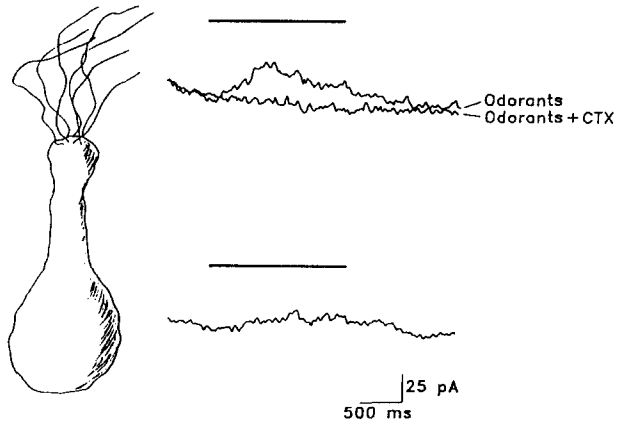

Fig. 3. Ciliary localization of inhibitory responses in toad olfactory neurons. (A) Schematic representation of the particular isolated olfactory neuron used for this experiment. The traces on the right represent whole-cell currents recorded during odorant stimulation. Each trace is located at the level of the cell at which the corresponding stimulus was applied with a multibarreled pipette. Two traces are superposed on top, one corresponding to a response to odorant stimulation and the other one to the current recorded during stimulation with odorant solution containing 10 nM CTX, which completely abolished the odorant effect.

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