Presynaptic Afferent Inhibition of Lobster Olfactory Receptor Cells: Reduced Action-Potential Propagation Into Axon Terminals

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Wachowiak, Matt and Lawrence B. Cohen. Presynaptic afferent inhibition of lobster olfactory receptor cells: reduced action-potential propagation into axon terminals. J. Neurophysiol. 80: 1011–1015, 1998. Action-potential propagation into the axon terminals of olfactory receptor cells was measured with the use of voltage-sensitive dye imaging in the isolated spiny lobster brain. Conditioning shocks to the olfactory nerve, known to cause long-lasting suppression of olfactory lobe neurons, allowed the selective imaging of activity in receptor cell axon terminals. In normal saline the optical signal from axon terminals evoked by a test stimulus was brief (40 ms) and small in amplitude. In the presence of low-Ca 2+/high-Mg 2+ saline designed to reduce synaptic transmission, the test response was unchanged in time course but increased significantly in amplitude (57 ± 16%, mean ± SE). This increase suggests that propagation into receptor cell axon terminals is normally suppressed after a conditioning shock; this suppression is presumably synaptically mediated. Thus our results show that presynaptic inhibition occurs at the first synapse in the olfactory pathway and that the inhibition is mediated, at least in part, via suppression of action-potential propagation into the presynaptic terminal.

INTRODUCTION

Presynaptic inhibition of transmitter release from primary afferent fibers is a well-established strategy for regulating sensory input to the CNS, occurring in vertebrates and invertebrates ( Rudomin 1990; Watson 1992 ). Presynaptic inhibition enables regulation of input from specific sets of afferent fibers and can sharpen receptive fields, prevent habituation, and maintain the sensitivity of inputs across a wide dynamic range ( Burrows and Matheson 1994; Watson 1992 ). One mechanism for presynaptic inhibition is a reduction in size or penetration of the action potential into the nerve terminal ( e.g., Wall 1994; Zhang and Jackson 1993 ). Another is an effect on subsequent events that control transmitter release ( e.g., calcium influx into the terminal ) ( Wu and Saggau 1997 ).

There are suggestions that presynaptic afferent inhibition may play a role in olfaction. Presumptive inhibitory interneurons contact receptor axons in the cockroach ( Distler and Boeckh 1997 ), and inhibitory D2 dopamine receptors are found in the olfactory nerve layer of the rat olfactory bulb ( Nickell et al. 1991 ). Physiological data from several studies suggest presynaptic inhibition. In the spiny lobster, Panulirus argus, olfactory receptor cells are hyperpolarized by histamine, a known inhibitory transmitter in olfactory glomeruli ( Bayer et al. 1989; McClintock and Ache 1989 ). In vertebrates, mitral cells and periglomerular interneurons show strong suppression after a conditioning nerve shock, which cannot be attributed to activation of other known interneurons ( Aroniadou-Anderjeska et al. 1997; Freeman 1974; Mori et al. 1984 ). However, no direct physiological evidence exists, largely because electrode measurements of transmembrane potential in pre- or postsynaptic sites are prohibited because of the small diameter of the neuronal processes.

We used voltage-sensitive dyes to directly measure action potentials in the axon terminals of olfactory receptor neurons in the spiny lobster and also conditioning olfactory nerve shocks and low-Ca 2+/high-Mg 2+ block to suppress activation of postsynaptic neurons. Our results suggest that olfactory lobe interneurons mediate presynaptic inhibition of olfactory receptor cells, either by reducing the height of action potentials or by blocking propagation into terminal branches.

METHODS

Recordings were obtained in an isolated brain preparation ( Wachowiak and Ache 1994 ). The brain was perfused with oxygenated Panulirus saline containing ( in mM ) 460 NaCl, 13 KCl, 13 CaCl 2, 10 MgCl 2, 14 Na 2 SO 4, 1.7 glucose, and 3 N-2-hydroxyethyl piperazine-N’-2-ethane sulfonic acid ( HEPES ), pH 7.4. Paired electrical shocks [ 250–800 ms interstimulus interval ( ISI ) ] were delivered to the olfactory ( antennular ) nerve. For the low-Ca 2+/high-Mg 2+ substitution experiments, the saline was transiently replaced with saline containing 1 mM CaCl 2 and 22 mM MgCl 2.

Brains were stained with a voltage-sensitive dye, di-2-ANEPPHQ ( JFW-2081, 0.06–0.1 mg/ml; synthesized by J. P. Wuskell and L. M. Loew, University of Connecticut Health Center, Farmington, CT ) or di-4-ANEPPS ( Molecular Probes, Eugene, OR; 0.05 mg/ml ) by introducing 1–2 ml dye into the perfusion line or by applying dye ( di-2-ANEPPHQ: 0.24 mg/ml ) into the bath for 20 min. Dye perfusion resulted in uniform staining of the brain, whereas bath application stained only the outermost 50–100 μm of tissue. One brain was stained by bath application and perfusion.

To record optical signals, light from a tungsten halogen lamp was passed through a 520 ± 45 nm interference filter, reflected from a 590-nm, long-pass dichroic mirror and focused on the object plane. The photocurrents from each diode were amplified separately, band-pass filtered ( 0.06–500 Hz ), and digitized at 1,010 Hz ( Wu and Cohen 1993 ) under the control of NeuroPlex software ( OptImaging, LLC, Fairfield, CT ) on an IBM PC computer. In the five preparations, the mean photocurrent from the diodes was about 10 nA. Afterward, acquisition signals were digi-
R E S U L T S

Figure 1 shows optical and electrical responses to paired-pulse stimulation of the antennular nerve at three levels of the olfactory pathway. In the olfactory nerve (Fig. 1A) the optical signals evoked by the first (conditioning) and second (test) stimuli consist of identical spikelike waveforms reflecting action potentials in receptor cell axons. In the olfactory lobe (Fig. 1B), the optical signal evoked by the conditioning stimulus consists of a large, fast component and a smaller, slow component. The test response consists of a single, small-amplitude fast component, similar in duration to that in the olfactory nerve. Electrical recordings from olfactory lobe interneurons show no intracellular response to the test stimulus (Fig. 1B, top trace), indicating a total suppression of postsynaptic activity after the conditioning stimulus. This suppression was observed in intracellular recordings from all known types of olfactory lobe interneurons and lasts from 1 s up to 30 s (Wachowiak et al. 1996, 1997). Complete suppression is also seen in the accessory lobe, a second-order olfactory neuropile, where the test stimulus elicits no response in either the optical or intracellular recordings (Fig. 1C).

We compared responses in the olfactory lobe to paired-pulse stimulation in normal and low-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline designed to reduce synaptic transmission. As controls, we monitored optical signals in both the antennular nerve and also in the accessory lobe, which receives polysynaptic but not direct input from olfactory afferents. Optical signals in the antennular nerve were unchanged in low-Ca\(^{2+}\)/high-

![Diagram](http://jn.physiology.org/)

FIG. 1. Schematic drawing of the lobster brain (bottom right) and electrical (intracellular) and optical signals recorded in response to paired electrical stimulation of the antennular (olfactory) nerve. (Clockwise from lower left) A: optical signal from the nerve. B: time of the antennular nerve stimulus. Olfactory receptor cell axons entering the olfactory lobe are shown in the drawing; di-2-ANEPHQ, bath-applied. B: optical signal (bottom: di-2-ANEPHQ, perfused) from the olfactory lobe and intracellular recording (top) from an olfactory lobe projection neuron. C: optical signal (bottom: di-4-ANEPPS, perfused) from the accessory lobe and intracellular recording (top) from an accessory lobe projection neuron. All optical traces were taken from single photodiodes with no additional filtering. Optical and intracellular records are from different preparations. A, B, and C are from different preparations, and different interstimulus intervals (ISIs) were used. In this and subsequent figures the vertical calibration bar represents (either or both) the intracellular potential measured with a microelectrode and/or the fractional fluorescence change, \(\Delta F/F\), in the optical measurement.
of the antennular nerve, a protocol that allows us to selectively image action-potential propagation into primary olfactory receptor axon terminals. In >200 neurons tested in previous intracellular studies, all known types of olfactory lobe interneurons are completely unresponsive to the second of a stimulus pair at the ISIs used in this study (Wachowiak et al. 1996, 1997). In addition, in the present study we observed no optical response to the second (test) pulse in the accessory lobe (see Fig. 1), which receives second-order fibers but no primary afferent input (Wachowiak et al. 1996). We thus conclude that the optical signal in the olfactory lobe evoked by a test stimulus reflects activity only in the terminals of primary olfactory receptor axons.

**FIG. 2.** A: low-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline does not affect the optical signal measured in the antennular nerve. Traces are spatial average of 5 diodes and were digitally high-pass filtered at 1.3 Hz; di-2-ANEPHQ, bath applied. B: low-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline eliminates the accessory lobe signal. Spatial average of 4 diodes; di-4-ANEPPS, perfused. C: intracellular recording from a single olfactory lobe projection neuron. Low-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline eliminates the spiking and hyperpolarizing response of projection neurons. A slow-onset, prolonged depolarization persists in low-Ca\(^{2+}\)/high-Mg\(^{2+}\). Optical signals were not recorded in this preparation.

**DISCUSSION**

We measured voltage-sensitive dye signals from the lobster olfactory lobe in response to paired-pulse stimulation to the electrical response of single olfactory lobe projection neurons in low-Ca\(^{2+}\)/high-Mg\(^{2+}\) (Fig. 2C, bottom trace).

**FIG. 3.** Effect of low-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline on the optical signal recorded from the olfactory lobe. A: test response amplitude is increased in low-Ca\(^{2+}\)/high-Mg\(^{2+}\). The conditioning response amplitude is reduced and a slow depolarizing component is seen. Spatial average of 9 diodes, high-pass filter, 2.6 Hz; di-2-ANEPHQ, perfused. B: traces from a different preparation showing the increase in the test response amplitude (top right) and decrease in the conditioning response (left). Heavy traces, normal saline; light traces, low-Ca\(^{2+}\)/high-Mg\(^{2+}\). Test responses also shown (bottom) with the vertical scales adjusted so that the peak amplitudes in normal and low-Ca\(^{2+}\)/high-Mg\(^{2+}\) salines are equal, illustrating that the kinetics of the test response are unchanged. Spatial average of 7 diodes. High-pass filter, 4.2 Hz; di-2-ANEPHQ, perfused.
We used low-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline to block postsynaptic activity initiated by the first (conditioning) pulse, indicated by the fact that low-Ca\(^{2+}\)/high-Mg\(^{2+}\) significantly reduced the amplitude of the conditioning response. The persistence of a slow, prolonged component in the conditioning response that parallels the slow olfactory lobe projection neuron response in low-Ca\(^{2+}\)/high-Mg\(^{2+}\) indicates that synaptic transmission was not blocked completely under these conditions. However, the intracellular recordings from projection neurons show that monosynaptic transmission was severely attenuated, whereas the lack of response in the optical signal from the accessory lobe shows that transmission across multiple synapses was eliminated (see Fig. 2).

Our finding that the test response is larger under conditions of reduced synaptic transmission (low-Ca\(^{2+}\)/high Mg\(^{2+}\) saline) implies that in normal saline, action-potential propagation into the receptor cell axon terminal is inhibited by interneurons activated by the conditioning pulse. An alternative explanation is that the increase in the test response in low-Ca\(^{2+}\)/high-Mg\(^{2+}\) saline is caused by a reduction of a Ca\(^{2+}\)-activated potassium current in receptor cell axon terminals, which would prolong and possibly increase the amplitude of the optical signal. However the test response increased significantly in amplitude but not in duration, and thus we believe this explanation is unlikely. We conclude that the strong paired-pulse suppression of olfactory lobe neurons is at least in part a result of presynaptic inhibition of action-potential propagation into olfactory receptor axon terminals.

Synaptically mediated propagation block occurs in several systems (Debanne et al. 1997; Wall 1993; Zhang and Johnson 1993), and a modeling study indicates that at branch points propagation can be blocked by relatively small conductance increases (Segev 1990). In the lobster, glomeruli are columnar and arranged radially around the olfactory lobe, and receptor cell axons branch and penetrate through three glomerular layers (Schmidt and Ache 1992). Thus inhibition in the outermost layer could potentially block propagation into the deeper layers, which contain the processes of olfactory lobe output neurons.

In vertebrates indirect evidence exists for presynaptic inhibition of olfactory receptor cells (see introduction), although no anatomic contacts onto receptor cell axons were found (Pinching and Powell 1971). Our results show that in the lobster, presynaptic inhibition of olfactory afferents can be substantial. The functional role of presynaptic afferent inhibition in other systems includes gain control of input across a wide dynamic range (Burrows and Matheson 1994) and refining the tuning of afferent input (Levine and Murphy 1980), functions that are also presumed critical to olfactory coding. Future work will focus on characterizing the synaptic pathways underlying presynaptic afferent inhibition in the lobster and the functional role of this phenomenon in olfactory processing, using pharmacological probes to the known inhibitory transmitters in the olfactory lobe.

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### Table 1. Effect of low-Ca\(^{2+}\)/high-Mg\(^{2+}\) on test response amplitude

<table>
<thead>
<tr>
<th>Prep</th>
<th>ISI (ms)</th>
<th>Staining method</th>
<th>Condition</th>
<th>Test/cond.</th>
<th>Test(<em>{\text{inj}})/test(</em>{\text{norm}})</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>250</td>
<td>Bath, perfusion</td>
<td>Pre</td>
<td>0.92</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Low Ca(^{2+})</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Post</td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>250</td>
<td>Bath</td>
<td>Pre</td>
<td>0.65</td>
<td>1.29</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Low Ca(^{2+})</td>
<td>1.01</td>
<td></td>
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<tr>
<td></td>
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<td></td>
<td>Post</td>
<td>0.53</td>
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</tr>
<tr>
<td>3</td>
<td>300</td>
<td>Perfusion</td>
<td>Pre</td>
<td>0.43</td>
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<tr>
<td>4</td>
<td>600</td>
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<td>Pre</td>
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<td>1.94</td>
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<td></td>
<td></td>
<td></td>
<td>Post</td>
<td>0.06</td>
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</table>

ISI is interstimulus interval; test/cond. is ratio of test to conditioning response amplitude before, during, and after low-Ca\(^{2+}\)/high-Mg\(^{2+}\) perfusion; test\(_{\text{inj}}\)/test\(_{\text{norm}}\) is ratio of test response amplitude in low-Ca\(^{2+}\)/high-Mg\(^{2+}\) to that in normal saline. The saline response was measured as the mean of the responses before and after perfusion. Although the absolute amplitude of the responses decreased over the course of the experiment, the test/cond. ratios were the same before and after perfusion.
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NOTE ADDED IN PROOF
Keller et al. 1998 recently reported evidence supporting the contribution of presynaptic inhibition to paired-pulse depression in the rat olfactory bulb. They did not address the issue of whether this inhibition results from suppression of action potential propagation or from action on subsequent events that control transmitter release.

REFERENCES


