Sodium-Gated Cation Channel Implicated in the Activation of Lobster Olfactory Receptor Neurons

ASLBEK B. ZHAINAZAROV,1 RICHARD E. DOOLIN,1,3 AND BARRY W. ACHE1,2,3
1Whitney Laboratory, 2Department of Zoology, and 3Department of Neuroscience, University of Florida, St. Augustine, Florida 32086-8623

INTRODUCTION

Na+-activated K+ channels form a family of large-conductance ion channels (for review see Dryer 1994). The biophysical properties of K+ channels activated by intracellular Na+ have been described in detail, although the physiological role of these channels is still unknown. Nonselective cation channels activated by Na+ form another group of channels that are either directly activated by Na+ or are sensitive to Na+. They include those in peptidergic nerve terminals of crab (Stuenkel et al. 1990), frog tectal neurons (Zaykin and Nistri 1995), guinea pig intestinal myocytes (Nouailhatas et al. 1994), and cultured lobster olfactory receptor neurons (Zhainazarov and Ache 1995, 1997). Whole cell recordings from crab nerve terminals (Stuenkel et al. 1990) and frog tectal neurons (Zaykin and Nistri 1995) suggest that these channels might be activated by Na+ influx through voltage-activated sodium channels and that they regulate cell excitability, but like their K+-selective counterparts, the physiological role of nonselective cation channels activated by Na+ is also not yet known.

Na+-activated channels usually require Na+ concentrations for activation that are much higher (30–80 mM) than those believed to occur intracellularly. It is not known whether or not a sufficient number of Na+ ions can enter cells through voltage-activated Na+ channels or neurotransmitter-activated nonselective cation channels to change the activity of Na+-activated channels (Dryer 1994). Little is also known about the spatial distribution of intracellular Na+ during Na+ influx through Na+-permeant ion channels, although it has been proposed that local accumulation of Na+ under the plasma membrane could be sufficient to influence the activity of Na+-dependent enzymes, transporters, and ion channels (Attwell et al. 1993; Duman et al. 1989; Kawa-hara et al. 1990; Strubing and Hescheler 1996). Olfactory transduction in lobster olfactory receptor neurons occurs in the outer dendrites, restricted cellular compartments, which are 0.1 μm in diameter (Grüner and Ache 1988). It is conceivable that local accumulation of Na+ in such a restricted space may transiently reach levels sufficiently high to activate a Na+-activated channel (see Zhainazarov and Ache 1995 1997). We therefore attempted to identify a functional role for the Na+-activated cation channel in these cells by localizing the Na+-activated channel to the outer dendrites of the cells and, secondly, by using pharmacological blockers of the channel together with ion substitution to implicate the channel in the control of odor excitability. We hypothesize that the channel amplifies the receptor current as a result of being secondarily activated by the primary odor transduction pathway.

METHODS

Animals and preparations

Adult specimens of the Caribbean spiny lobster, Panulirus argus, were obtained from the Florida Keys and maintained in the laboratory in running seawater on a mixed diet. Three different preparations were obtained from the olfactory organ (lateral antennules) of these animals (Fig. 1A).

CULTURED CELLS. Olfactory receptor cells were removed from the organ and placed in primary culture as described by Zhainazarov and Ache (1995). Inside-out and outside-out patches were excised from the soma of these cells for analysis.

ISOLATED OUTER DENDRITES. Outer dendrites of olfactory receptor cells were acutely isolated as described by Ache et al. (1995). Briefly, the tips were cut from ~100 of the olfactory sensilla. The tips, which contain only the outer dendrites of the olfactory receptor cells (Fig. 1B), were collected into poly-D-lysine-coated 30-mm plastic culture dishes containing 5 ml of Ca2+-free Panulirus saline diluted with distilled water (3/4 dilution). The outer dendrites immediately extruded from the cut end of the tips and began to form membrane vesicles, which increased in number and size with time. After 2–3 h, vesicles up to 3 μm in diameter were observed. The culture dishes containing the vesicles were mounted on the stage of an inverted microscope (Axiovert 100, Carl Zeiss), continuously perfused with Ca2+-free Panulirus saline, and viewed with phase-contrast optics at ×320 total magnification. Cell-free patches were excised from the vesicles for analysis in the inside-out configuration.

SEMINTACT ANTENNULAR PREPARATION. The olfactory organ (antennule) was excised into 1-mm long segments (Fig. 1A), which were split longitudinally to expose the somata of the olfactory receptor cells within (Fig. 1B). The portion containing the
FIG. 1.  A: drawing of a spiny lobster showing (expanded view) olfactory organ on lateral antennular filament. Organ consists of a highly ordered tuft of olfactory sensilla, also called aesthetascs, enclosed by 2 rows of guard hairs. B: drawing of a cross section of one olfactory sensillum. Inner dendrites from some 350 primary bipolar sensory receptor neurons associated with each sensillum project into a cuticular hair, where each cell gives rise to some 25 outer dendritic segments. Only outer dendrites occupy distal 80% of length of hair. Excising tip of hair (scissors) yields a pure outer dendritic membrane preparation, vesicles of which can be patch-clamped (inset, top right). Odors applied to hairs evoke receptor potentials and altered rates of discharge, both of which can be recorded by patch-clamping soma (inset, bottom right). C: diagram of a flow-through olfactometer in a layer of silicone resin in bottom of a 35-mm culture dish. A piece of olfactory organ, opened so as to expose somata of primary olfactory receptor neurons for patch-clamping, is sealed into slot so to isolate recording bath from olfactometer stream superfusing hairs. Stimulus onset time is Ïœ1.5 s (Ache et al. 1995). A variation of this design (a H-style olfactometer) used in some experiments had a stimulus onset time of Ïœ100 ms (Michel et al. 1991).

Hairlike olfactory sensilla was treated with a solution of papain (0.25 mg/ml Sigma type IV activated with 1.25 mg/ml L-cysteine) for 25 min and then with a solution of trypsin (1 mg/ml Sigma type IX in Ca²⁺-free solution) for 25 min. After rinsing, the preparation was sealed into an olfactometer so that only the olfactory sensilla were exposed to odors and/or to changes in the ionic composition of the saline that bathed them (Fig. 1C). The olfactometer was mounted on the stage of an upright microscope (Axioskop, Carl Zeiss, Germany), allowing the somata to be viewed with a ×40 water immersion objective (Zeiss 440091).

Recording and data analysis

Patch-clamp recordings were performed as described by Hamill et al. (1981). Pipettes for single-channel recording were made from borosilicate glass (Sutter Instrument BF150-86-10), coated with silicone elastomer (Sylgard, Dow-Corning) and fire-polished to a final tip diameter of <1 μm. The pipettes had resistances of 10–20 MΩ when filled with patch pipette solution and formed seals with resistances of 10–25 GΩ. Patches were perfused with various stimulus solutions using a nine-channel rotary parallel flow perfusion system (RSC-100, Biologic, France) as described previously (Zhainazarov and Ache 1995). Single-channel currents were recorded with an amplifier (Axopatch 200A, Axon Instruments), low-pass filtered at 10 kHz (–3 dB, 4-pole Bessel filter), and stored on videotape (Toshiba DX-900) for analysis. On playback, the single-channel currents were filtered at 2–4 kHz (–3 dB; 8-pole Bessel filter) and digitized at 20–40 kHz by a computer with an A/D, D/A interface (TL-1, Axon Instruments) using pClamp 6.0 software (Axon Instruments).

In some experiments the current-voltage relation of single-channel currents was determined by the application of voltage ramps (–100 to +100 mV; 350 ms) and the resulting currents recorded directly into computer memory and analyzed later by using the segmented averaging procedure (Heinemann 1995). The reversal potentials under different ionic conditions for monovalent cations were measured by determining the potential at which the open channel current was zero. The permeability of the test monovalent cations
ion X' relative to that of Na' ($P_x/P_{Na}$) was calculated from the shift of the reversal potential ($E_r$) on exchanging the intracellular Na' solution (210 mM, which contained no other monovalent, permeant cations) with a solution in which part of the Na' (120 mM) was replaced by an equivalent concentration of the test cation X', determined by using the Goldman-Hodgkin-Katz “voltage” equation (Hille 1992)

$$\Delta E_r = E_{rX} - E_{rNa} = \left(\frac{RT}{F}\right) \ln \left[\frac{P_{Na}}{P_Na}[Na^+]/(P_x[X^+]+P_{Na}[Na^+])\right]$$

where $R$ is the gas constant, $F$ the Faraday constant, $T$ the absolute temperature, $[Na^+]$, the extracellular concentration of Na', $[X^+]$, and $[Na^+]$, the intracellular concentration of the cations X' and Na', respectively.

Whole cell, current-clamp recordings were obtained by forming

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**FIG. 2.** Na' activated channel activity in inside-out patches excised from outer dendrites of lobster olfactory receptor neurons. A: trace of channel activity in a patch containing 2 channels evoked by 210 mM Na' applied to intracellular face of patch. Membrane potential, -60 mV. Upward deflection of line above trace indicates onset of stimulus. B: segments of trace in A taken where noted (a and b) and shown on an expanded time scale. C: single-channel current traces from a different patch containing 2 channels at membrane potentials indicated near to each trace. [Na'], 210 mM. D: plot of single-channel current-voltage relationship (○) taken from traces in C. Open squares are current-voltage relationship of Na' activated channels from somata. Pipette, 210 mM NaCl. Baselines are depicted by dashed lines. Traces pass filtered at 2 kHz (-3 dB).

**FIG. 3.** Dose dependence of channel activity evoked by intracellular Na' in an inside-out patches excised from outer dendrite. A: traces of channel activity induced by concentrations of intracellular Na' noted. Patch contained three Na' activated channels. Baselines; membrane potential, -60 mV; pipette, 210 mM NaCl. Traces were low-pass filtered at 1 kHz. B: plot of relationship between [Na'], and channel open probability. Solid line through data points is fit by Hill equation (see text for fit parameters). Points shown are given as mean ± SE ($n = 3$).
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TABLE 1. Comparison of the dwell-time parameters and the single-channel conductance of Na\textsuperscript{+}-activated channels from the outer dendritic membrane of lobster olfactory receptor neurons in situ and the soma membrane of these cells maintained in culture

<table>
<thead>
<tr>
<th>Source</th>
<th>$T_o$, ms</th>
<th>$T_{cl1}$, ms</th>
<th>$T_c$, ms</th>
<th>$T_b$, ms</th>
<th>$\gamma$, pS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outer dendrites</td>
<td>7.35 ± 0.71</td>
<td>0.17 ± 0.06</td>
<td>11.4 ± 2.7</td>
<td>7.87 ± 0.84</td>
<td>98.3 ± 5.6</td>
</tr>
<tr>
<td>Soma</td>
<td>7.19 ± 0.96</td>
<td>0.18 ± 0.08</td>
<td>9.1 ± 1.5</td>
<td>7.96 ± 0.97</td>
<td>103.9 ± 5.2</td>
</tr>
</tbody>
</table>

Data are given as means ± SE ($n = 3$–$5$). $[Na\textsuperscript{+}]_i$, 210 mM. Membrane potential, $-60$ mV. Number of experiments, 3–5. Low-pass filter, 2 kHz ($-3$ dB). Dwell-time parameters: $T_o$, mean open time; $T_{cl1}$, mean closed time within bursts; $T_c$, all closed times distribution mean; $T_b$, mean burst duration. $\gamma$, single-channel conductance.

tight seals (4–8 G\textOmega) on the somata of the olfactory receptor cells with pipettes pulled from borosilicate glass (Drummond Scientific, 2-00-100). The pipettes were fire polished to a tip diameter of $\sim 1$ $\mu$m (bubble number 5.0) (Mittman et al. 1987). The pipette resistance was 2–4 M\textOmega when filled with patch pipette solution. For extracellular recordings, which were actually cell-attached patch recordings under current-clamp conditions, the pipettes were filled with PS. Signals were recorded with a patch amplifier (Dagan 3900A), digitized directly into the computer and analyzed later with pClamp 6.0 software. Evoked action potentials were counted during the first second after the onset of the response.

All potentials were corrected for the liquid junction potential between the pipette and bath solution as described by Neher (1992). The recordings were referenced to a Ag-AgCl wire electrode connected to the bath solution through a 3 M KCl/agar bridge. All membrane potentials given depict the potential of the cytoplasmic face relative to the extracellular face, regardless of patch orientation. All experiments were carried out at room temperature (20–22°C).

Solutions

Unless stated otherwise, in excised patch experiments, the standard pipette solution consisted of (in mM) 210 NaCl, 11 ethylene

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Fig. 4. Na\textsuperscript{+}-activated channel activity in inside-out patches excised from soma (A) and outer dendrite (B). Channel density on outer dendrites was typically 2 orders of magnitude greater than that on soma. Time course of solution changes is shown by solid lines above current traces. Membrane potential, $-60$ mV. Pipette, 210 mM NaCl. Insets: same current traces on an expanded time scale. A: 1 active channel. B: multiple channels, (⋅⋅⋅), baselines.
glycol-bis(β-aminoethy) ether)-N,N',N'-tetraacetic acid (EGTA), 1 CaCl_2, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and 696 glucose, pH 7.4 adjusted with tris(hydroxymethyl)aminomethane (Tris base). The calculated free calcium concentration in the pipette solution was 10 nM (Chelator) (see Schoenmakers et al. 1992). Sodium-free solution consisted of (in mM) 210 LiCl, 11 EGTA, 1 CaCl_2, 696 glucose, and 10 HEPES, pH 7.4 adjusted with Tris base. In some experiments, part of the LiCl in the sodium-free solution was substituted by an equivalent concentration of NaCl as described in the text and figure legends. For whole cell recordings, patch pipettes were filled with K-acetate solution (mM): 180 K acetate, 30 NaCl, 11 EGTA, 1 CaCl_2, 10 HEPES, and 696 glucose, pH 7.2 adjusted with Tris base. For cell-attached patch-clamp recordings, patch pipettes were filled with Panulirus saline (PS), which contained (in mM) 458 NaCl, 13.4 KCl, 13.4 Na_2SO_4, 13.6 CaCl_2, 9.8 MgCl_2, 2 glucose, and 10 HEPES, pH 7.4 adjusted with Tris base. Nominally calcium-free PS was equivalent to Panulirus saline in ionic composition except it contained no added Ca^{2+}. Sodium-free PS (Li-PS or NH_4-PS) was made by substituting all of the NaCl in PS with an equivalent concentration of LiCl or NH_4Cl.

The odor was an aqueous extract of a commercial fish food TetraMarin (TET; TetraWerke, Melle, Germany), made by mixing 2 g of dry flakes in 60 ml of PS. The resulting suspension was centrifuged at 1,400 g, the supernatant filtered through Whatman paper (No. 3) and adjusted to pH 7.4. This stock solution was stored frozen in 1-ml aliquots. Working odor solutions were prepared by diluting the stock solution to the reported concentration with an appropriate saline.

All inorganic salts were obtained from Fisher Scientific. All organic chemicals were obtained from Sigma Chemical, except for HEPES, which was obtained from Research Organics.

**RESULTS**

A Na^+-activated channel occurs in the outer dendrite

Na^+ (210 mM) reversibly activated channels in inside-out patches excised from vesicles formed from isolated outer dendrites (Fig. 2, A and B). The current-voltage relation of the channels, like that of channels from the somata of the cultured cells characterized in detail (Zhainazarov and Achefree 1995, 1997), was also linear at negative potentials and showed a slight inward rectification at positive potentials (Fig. 2, C and D). The estimated slope-conductance between −90 and +10 mV was 98.3 ± 5.6 pS (SE, n = 3), which was close to the slope conductance of the soma channel. The effect of Na^+ on the outer dendritic channel was dose-dependent (Fig. 3A), similar to the soma channel (Zhainazarov and Achefree 1995, 1997).
zarov and Ache 1995, 1997). The open probability ($P_o$) of the channel increased toward a plateau level of 0.6 in a graded manner as the intracellular Na$^+$ concentration was increased from 10 to 210 mM (Fig. 3B). The data could be fit by the Hill equation (solid line, Fig. 3B)

$$P_o = \frac{P_{o,max}[Na^+]^n}{(K_{1/2} + [Na^+]^m)}$$

(2)

where $P_{o,max}$ (0.60) is an asymptotic value of $P_o$, $n$ is the Hill coefficient (2.6 ± 0.4), and $K_{1/2}$ is a half-effect concentration (49.2 ± 3.0 mM), with the membrane potential clamped at −60 mV. The dwell-time characteristics of the Na$^+$-activated channel from the outer dendrites were also similar to those of the soma channel (Table 1).

The average number of channels in membrane patches excised with patch pipettes with a tip diameter of ~1 µm (average resistance, 10 MΩ) from the cell soma and the outer dendrite was 0.05 (range, 0–1; $n = 20$; Fig. 4A) and 13.8 (range, 6–26; $n = 20$; Fig. 4B), respectively. This corresponds to a channel density on the outer dendrites (17.3 µm$^{-2}$) that is two orders of magnitude greater than that on the soma of the cells (0.06 µm$^{-2}$). The channel density was estimated by assuming that the membrane patch is a circular disk of 1.0 µm diam. The occurrence of the channel in high-density in a cellular compartment specialized for transduction suggests that the channel is involved in odor activation.

Removing extracellular Na$^+$ suppresses the response to odors

If the channel functions in odor activation, the response of the cell to odors should be dependent on extracellular Na$^+$. We therefore examined the effect of substituting extracellular Na$^+$ with Li$^+$ or NH$_4^+$ on the odor response of the cells in the semiintact antennular preparation. The 1,4,5-triphosphate (IP$_3$)-gated cation channel that serves as a primary transduction channel in lobster ORNs (Fadool and Ache 1992; Hatt and Ache 1994) is relatively nonselective for monovalent cations [<$P_{K}/P_{Na}$: Li$^+$ (1.01) > Na$^+$ (1.00) > K$^+$ (0.98) > Cs$^+$ (0.83)] (D. A. Fadool and B. W. Ache, unpublished results) so that substitution of extracellular Na$^+$ with either Li$^+$ or NH$_4^+$ should not affect the primary transduction current. Earlier, we showed that Li$^+$ and NH$_4^+$ do not activate the Na$^+$-activated channel (Zhairnazarov and Ache 1995), although they permeate through it similarly to Na$^+$ (Fig. 5). Bathing the cytoplasmic side of inside-out patches from cultured cells with 210 mM Na$^+$ or 90 mM Na$^+$ + 120 mM Li$^+$ and NH$_4^+$, gave reversal potentials (mean ± SE; $n = 3$) of 0.2 ± 0.2 mV for Na$^+$, −2.5 ± 0.2 mV for Li$^+$, and −3.4 ± 0.3 mV for NH$_4^+$, to yield relative permeability ratios $(P_{Li}/P_{Na})$ of NH$_4^+$ (1.25) > Li$^+$ (1.18) > Na$^+$ (1.00). Therefore any effect of substituting extracellular Na$^+$ with Li$^+$ or NH$_4^+$ is not the result of significant differences in the permeability of the ions.

Whole cell, current-clamp recording showed that cells in the semiintact antennular preparation are depolarized by odor (TET, 1:1.000) stimulation from 9 to 35 mV when the membrane potential was set to −70 mV. The odor-evoked receptor potential peaked within 0.5–0.8 s of its onset and began to repolarize before the stimulus terminated. Its magnitude was linear between −80 and −45 mV, and reversed polarity at an extrapolated potential of −16.5 ± 5.1 mV ($n = 3$). Substituting all of the Na$^+$ with Li$^+$ reversibly reduced the receptor potential by 46.2 ± 12.6% (SE) ($n = 6$; Fig. 4A). In the absence of odor stimulation, substituting Na$^+$ with Li$^+$ had no measurable effect on the membrane potential over the one minute interval the cells were exposed to the treatment. The same effect was observed if NH$_4^+$ was substituted for Na$^+$, indicating that the apparent dependency of the odor response on extracellular Na$^+$ was not an artifact of Li$^+$ substitution per se (see DISCUSSION). NH$_4^+$ substitution, however, itself slowly depolarized the membrane by 30 mV over minutes and eventually led to an irreversible loss of odor responsiveness, presumably because of intracellular alkalization (Ross and Boron 1981).

Ouabain (100 µm), a blocker of Na$^+/K^+$-ATPase, depolarized the membrane potential 10.4 ± 0.6 mV (SE, $n = 3$) over minutes, but did not affect the ability of the cell to respond to odors over the period of measurement. In the presence of ouabain, the peak magnitude of the receptor potential was 95 ± 19% ($n = 5$) of its pretreatment value, suggesting that it is unlikely that the immediate effect of Na$^+$ substitution on odor responsiveness was the result of perturbing the function of the Na$^+$ pump.

Ion substitution also decreased the odor-evoked discharge in cell-attached patch recordings from the cells in the semiintact antennular preparation, suggesting that the effect of ion substitution on the receptor potential was not...
imposed by the whole cell recording procedure. Cells that increased their discharge to odors (TET, 1:1,000) failed to do so when extracellular Na\(^+\) was substituted with Li\(^+\) (Fig. 7). Lithium substitution reduced the odor-evoked increase in discharge during the first second of the response from 6.4 ± 0.5 to 1.4 ± 0.4 action potentials per second \((n = 10)\). Ammonium substitution also reduced the odor-evoked increase in discharge during the first second of the response from 5.0 ± 0.3 to 0.6 ± 0.2 action potentials per second \((n = 7, \text{data not shown})\). The effects of Li\(^+\) and ammonium substitution were reversible. These results collectively indicate that Na\(^+\) is involved in the generation of odor-evoked depolarizations.

**Calmodulin inhibitors W7 and trifluoperazine block the Na\(^+\)-activated channel**

If the channel functions in odor activation, drugs that block the channel should also block odor-activation of the cells. To identify substances that reversibly block the channel, we screened substances known to be effective blockers of Ca\(^2+\) and/or nonselective cation channels, because we showed earlier that the Na\(^+\)-activated channel has a relatively high permeability for Ca\(^2+\) \((P_{Ca}/P_{Na} = 39)\) (Zhainazarov and Ache 1997). We tested amiloride, which blocks some types of sodium-selective, monovalent-cation-selective and also low-threshold calcium channels in a variety of cell types (Manev et al. 1990; Palmer 1992; Sanchez-Armass et al. 1991; Tang et al. 1988); blockers of L-type voltage-activated Ca\(^2+\) channels (diltiazem, verapamil, nifedipine, and methoxyverapamil \((D-600)\) (Hille 1992); ouabain, a blocker of the Na\(^+\),K\(^+\)-the odor-evoked increase in discharge during the first second of the response from 5.0 ± 0.3 to 0.6 ± 0.2 action ATPase (Rose and Ransom 1996), which blocks a Na\(^+\)-activated cation current in frog tectal neurons (Zaykin and Nistri 1995); and two calmodulin inhibitors, W7 and trifluoperazine, which block various Ca\(^2+\) and Na\(^+\) channels (Ehrlich et al. 1988; Greenberg et al. 1987; Ichikawa et al. 1991; Nakazawa et al. 1993).

W7 and trifluoperazine blocked the channel’s activity from both extracellular and intracellular sides of patches excised from the soma of cultured cells. Applied to the intracellular face (inside-out patches), W7 reversibly reduced channel activity at 1 \(\mu\)M and completely blocked it at 200 \(\mu\)M \((n = 5, \text{Fig. 8A})\). At 10 \(\mu\)M, W7 reduced the open probability from 0.28 ± 0.01 to 0.18 ± 0.01, but not the amplitude of the single-channel current at −60 mV \((-5.89 ± 0.01 \text{ pA vs. } -5.91 ± 0.02 \text{ pA at } 10 \mu\text{M})\). The normalized open probability \((P_o/P_{o,max})\) varied as a
function of W7 concentration (Fig. 8C). The relationship could be fit by a curve using the following equation

\[
P_o/P_{o,\text{max}} = 1 - \left[ \frac{[C]}{IC_{50}^n + [C]} \right]^{n-1}
\]

where \( P_o/P_{o,\text{max}} \) is the open probability of the channel in the absence of the blocker, \([C]\) is the blocker concentration, \(IC_{50}\) is the half-effect concentration, and \(n\) is the slope factor, yielding an \(IC_{50}\) of 15.1 ± 1.5 \(\mu\text{M}\) and an \(n\) of 1.4 ± 0.2. Applied to the extracellular face (outside-out patches), W7 also blocked the channel activity reversibly (Fig. 8B) with a similar effectiveness, yielding an \(IC_{50}\) of 16.3 ± 1.5 \(\mu\text{M}\) and an \(n\) of 1.4 ± 0.1. Trifluoperazine had a similar blocking effect on the channel activity from both extracellular and intracellular sides (Fig. 8B). As with W7, the effect of trifluoperazine was also concentration-dependent (Fig. 8C), with \(IC_{50}\) of 6.5 ± 0.5 \(\mu\text{M}\) applied intracellularly and 14.4 ± 0.9 \(\mu\text{M}\) applied extracellularly and \(n\) of 1.6 ± 0.2 intracellularly and 1.9 ± 0.2 extracellularly. Each drug had a similar effect on the channel at +40 mV (data not shown), indicating that their action is not voltage dependent. The similar effectiveness of the drugs on each side of the membrane might be due to the fact that W7 (Ichikawa et al. 1991) and, to a lesser extent, trifluoperazine (Ikegami et al. 1992) are membrane permeant.

Another calmodulin inhibitor, calmidazolium (100 \(\mu\text{M}\)), as well as calmodulin (CaM) itself (250 nM CaM in the presence of 50 \(\mu\text{M}\) free Ca\(^2+\)), had no effect on the channel. Furthermore, calmodulin was unable to reverse the blocking effects of both W7 and trifluoperazine (data not shown), so inhibition of the channel is unlikely to be mediated by calmodulin. The other potential blockers tested (see above) had no measurable effect on channel activity.

Effects of dendritic application of W7 and trifluoperazine on the response to odors

W7 and trifluoperazine reversibly decreased the odor (TET, 1:1,000)-evoked depolarization recorded in the whole cell configuration from the cells in the semi-intact antennular preparation (Fig. 9). W7 (100 \(\mu\text{M}\)) reduced the peak magnitude of the receptor potential to 30.1 ± 6.8\% (\(n = 9\)) of its pretreatment value (Fig. 9, A and C). Trifluoperazine (100 \(\mu\text{M}\)) reduced the peak magnitude of the receptor potential to 54.3 ± 9.7\% (\(n = 11\)) of its pretreatment value (Fig. 9, B and C). Both drugs maximally blocked the channel at 100 \(\mu\text{M}\) (Fig. 8). Increasing the concentration of the drugs to 300 \(\mu\text{M}\) did not further reduce the peak magnitude of the receptor potential: W7 reduced to 33.4 ± 13.4\% (\(n = 6\)) and trifluoperazine reduced to 65.7 ± 2.5\% (\(n = 3\)) of its pretreatment value. Neither drug had any significant effect on the odor-evoked receptor potential when extracellular Na\(^+\) was substituted with Li\(^+\). With 100 \(\mu\text{M}\) W7, the receptor potential was 93.4 ± 13.1\% (\(n = 3\)) of its pretreatment value, whereas with 100 \(\mu\text{M}\) trifluoperazine it was 101.2 ± 14.2\% (\(n = 3\)) of its pretreatment value. Neither drug altered the membrane potential measured in the soma in the absence of odor stimulation.

Both drugs (100 \(\mu\text{M}\)) also decreased the odor (TET, 1:1,000)-evoked discharge in cell-attached patch recordings from the cells in the semiintact antennular preparation. Bathing the sensilla with W7 reduced the odor-induced increase in the rate of discharge during the first second of the response from 10.6 ± 0.6 to 4.7 ± 0.7 action potentials per second (\(n = 6\); Fig. 10A). Bathing the sensilla with trifluoperazine reduced the odor-induced increase in the rate of discharge during the first second of the response from 10.6 ± 0.6 to 6.0 ± 0.5 action potentials per second (\(n = 6\); Fig. 10B).

None of the other drugs tested (see previous section) had...
any measurable effect on the odor-evoked discharge of the cells (data not shown).

**DISCUSSION**

Finding that a channel in the outer dendrite is specifically activated by intracellular Na⁺ in a concentration-dependent manner and that Na⁺ activates the channel in cell-free patches in the absence of both divalent cations and nucleoside triphosphates strongly implies that a Na⁺-activated channel occurs in the transduction zone of these cells. The properties of the channel recorded in the outer dendrite are identical to those described earlier in detail for a Na⁺-activated channel on the soma of cultured lobster olfactory receptor cells (Zhainazarov and Ache 1995, 1997). The presence of a Na⁺-activated channel in a cellular compartment specialized for transduction of the odor signal suggests that the channel may function in odor activation. Both lines of experimental evidence support this view: 1) pharmacological probes (W7 and trifluoperazine) that block the Na⁺-gated channel from the outside in excised patches significantly reduce the odor-evoked responses when applied focally to the outer dendrites of the intact cells in the semintact antennular preparation and 2) the response of the cells in the semintact antennular preparation to odors is specific for Na⁺. Substituting Na⁺ with either Li⁺ or NH₄⁺, both of which permeate the cell but do not activate the Na⁺-gated channel, also significantly reduces the odor-evoked responses of the cells.

We cannot exclude the possibility that W7 and trifluoperazine act upstream of the Na⁺-gated channel. If the drugs were targeting an ion channel upstream of the Na⁺-gated channel, however, such a channel would have to be both Na⁺-selective as well as antagonized by W7 and trifluoperazine. The excitatory transduction channel in the cells is thought to be an IP₃-activated cation channel (Fadool and Ache 1992; Hatt and Ache 1994) that is nonselective for monovalent cations (D. A. Fadool and B. W. Ache, unpublished data). A second, cyclic nucleotide activated transduction channel, which is thought to suppress excitation in these cells, appears to be K⁺ selective (Hatt and Ache 1994; Michel and Ache 1992). Na⁺ substitution should not significantly reduce the current carried through either of these two conductances. If the drugs were acting on some other (non-channel) element of the transduction cascade upstream of the Na⁺-gated channel, the Na⁺-selectivity of the odor-evoked response would still need to be explained. The failure of the drugs to have any significant effect on the odor-evoked receptor potential when extracellular Na⁺ was substituted with Li⁺ suggests that it is unlikely that the drugs act upstream of the Na⁺-gated channel. Li⁺ can disrupt inositol phosphate metabolism by reducing the supply of the inositol lipids used to generate the second messengers IP₃ and diacylglycerol (Berridge 1989). Therefore Li⁺ substitution could potentially reduce the ability of the cell to respond to odors by depleting the excitatory second messenger (IP₃). However, finding that substituting NH₄⁺ for Na⁺ has the same effect as Li⁺ substitution minimizes this possibility, because ammonium is not known to influence phosphoinositide metabolism. We conclude, therefore, that a substantial part of the odor-evoked depolarization is due to activation of the Na⁺-gated channel during odor stimulation.

How the channel is initially triggered is uncertain. One possibility is that Na⁺ influx from activation of the primary transduction channel triggers the channel because, as mentioned above, the second messenger–gated channel conducts Na⁺. The lack of Na⁺ influx through the IP₃-gated channel in Na⁺-free extracellular saline would prevent the Na⁺-gated channel from being activated, which in turn would generate a substantially smaller receptor potential. Intradendritic Na⁺ would have to reach tens of millimolar to trigger...
the channel, but this may not be unreasonable. The outer dendritic branches are <0.1 μm diam (Grünert and Ache 1988). Relatively small fluxes of Na⁺ presumably could produce locally large increases in Na⁺ concentration because of the high surface-to-volume ratio of such fine processes. Intracellular Na⁺ has been proposed to be elevated in a finite layer close to the plasma membrane by Na⁺ influx through nonselective cation channels to levels sufficient to block voltage-gated K⁺ channels in rat pheochromocytoma cells (Strübing and Hescheler 1996). Glutamate-induced Na⁺ influx through nonNMDA channels in rat brain astrocytes also leads to a localized rise in [Na⁺], (Jensen and Chiu 1991). Although we cannot exclude that odors directly activate the Na⁺-gated channel, we were unable to activate the channel by odors in outside-out patches (unpublished data). Thus Na⁺ influx into the outer dendrite through the primary transduction channel could reasonably be expected to trigger the channel. Alternatively, however, the Na⁺-gated channel could be triggered intracellularly by another, yet unknown mechanism in addition to its Na⁺ gating, as has been proposed for the cardiac muscarinic K⁺ channel (Sui et al. 1996). Further study is required to address how the channel is initially triggered.

Once triggered, the channel presumably would provide a positive feedback loop to amplify the triggering signal because the channel is nonselective to monovalent cations and opening of the channel would increase the intracellular Na⁺ concentration that in turn would lead to a further rise in channel activity. The activity of the channel is down-regulated by intracellular Ca‌²⁺ (Zhainazarov and Ache 1995) and the channel is highly Ca‌²⁺ permeable (P_Ca/P_Na = 39) (Zhainazarov and Ache 1997). Ca‌²⁺ influx through the channel and the subsequent rise in intracellular Ca‌²⁺ could provide important negative feedback to prevent runaway self-reactivation of the channel by Na⁺. The site of transduction in the lobster cells is relatively far (~1 mm) from the presumed site of action-potential generation in the soma (Grünert and Ache 1988). A mechanism whereby the receptor potential was secondarily amplified through activation of the Na⁺-activated channel could facilitate electrotonic spread of the signal in these cells, which lack voltage-gated channels in the outer dendrite (Hatt and Ache 1994). Persistence of a residual odor-evoked response in Na⁺-free saline and in the presence of the drugs (W7 and trifluoperazine) supports the notion of a two-stage amplification cascade. In a functionally similar manner odors have been proposed to secondarily activate a Ca‌²⁺-activated chloride conductance in vertebrate olfactory receptor cells (Kurahashi and Ache 1993; Lowe and Gold 1993), suggesting that two stage amplification may be fundamental to odor signal detection.

Involvement of the channel in odor activation would imply that Na⁺ acts as an intracellular second messenger in these cells. In contrast to the well-established role of Ca‌²⁺ as a major intracellular signal (Berridge 1993; Kennedy 1989; Miller 1998), the idea of Na⁺ serving as an intracellular signal is relatively novel, although one that is consistent with other known or suspected intracellular actions of this ion. For example, a local increase in intracellular Na⁺ and subsequent reverse operation of a neurotransmitter uptake carrier has been proposed to mediate nonvesicular release of neurotransmitters in neuronal and glial cell processes (Attwell et al. 1993). As Na⁺ also regulates adenylate cyclase activity in neurons isolated from different regions of the rat brain (Duman et al. 1989), Na⁺ could potentially function widely as an intracellular signal in other systems.

We thank E. Wiese for the preparation of the cultured cells, L. Milstead for assistance with the illustrations, and G. Cottrell for critically reading an earlier draft of the manuscript.

This work was supported by the National Institute of Deafness and Other Communications Disorders Grant DC-01655.

Address for reprint requests: A. B. Zhainazarov, Whitney Laboratory, University of Florida, 9505 Ocean Shore Blvd., St. Augustine, FL 32086-8623.

Received 18 September 1997; accepted in final form 4 November 1997.

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