Sensitivity and Transduction Mechanisms of Responses to General Odorants in Turtle Vomeronasal System

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ABSTRACT (a) The responses of the vomeronasal organ to general odorants in the turtle, Geoclemys reevesii, were measured by recording the accessory olfactory bulbular responses. The threshold concentrations of the vomeronasal responses to various odorants were similar to those in main olfactory bulbular responses, indicating that vomeronasal cells lacking cilia and olfactory cells having many cilia have similar sensitivities to general odorants. (b) The vomeronasal epithelium was perfused with 100 mM NaCl solution and the salt-free solution and the effects of NaCl on the vomeronasal responses to various odorants were examined. There was no essential difference between the concentration–response curves for n-amyl acetate and menthone dissolved in 100 mM NaCl solution and those dissolved in the salt-free solution in the whole concentration range examined. The ratios of the magnitudes of vomeronasal responses in the salt-free solution to those in 100 mM NaCl solution were between 1.01 and 1.10 for seven odorants tested. (c) The magnitudes of responses to the odorants were unchanged by changes in NaCl concentrations. The replacement of Na⁺ with organic cations such as choline⁺, Bis-Tris propane²⁺, and N-acetyl-d-glucosamine⁺ did not affect the magnitudes of the responses to the odorants. The Na channel blocker amiloride also did not affect the responses. (d) The vomeronasal responses were practically unchanged by changes in CaCl₂ concentration. The Ca channel blockers diltiazem and verapamil did not affect the responses. (e) The replacement of Cl⁻ with SO₄²⁻ did not affect the magnitudes of the vomeronasal responses. (f) The present results suggest that ion transport across the apical membranes of vomeronasal receptor cells does not contribute to the responses to odorants in the turtle.

INTRODUCTION
The vomeronasal organ is a chemoreceptor that is distinguished from the main olfactory organ. In many vertebrates, the vomeronasal system plays important roles in the perception of chemical stimuli related to feeding behavior, social behavior, and reproductive behavior (Halpern, 1987; Wysocki and Meredith, 1987). For instance, garter snakes use the vomeronasal system to recognize prey odors, and the chemoat-
tractant for the snakes was recently purified from earthworm (Jiang, Inouchi, Wang, and Halpern, 1990).

Both the vomeronasal and olfactory receptor cells are bipolar neurons. The dendrites of olfactory receptor cells project to the epithelial surface, where they terminate in olfactory knobs from which several cilia extend into the mucus layer. In contrast, the dendrites of the vomeronasal receptor cells lack cilia and possess microvilli (Graziadei and Tucker, 1970; Hatanaka, Matsuzaki, and Shibuya, 1982).

Tucker (1963, 1971) reported that vomeronasal nerves in the turtle respond to various odorants. Hatanaka, Shibuya, and Inouchi (1988) recorded the turtle accessory olfactory bulb wave induced by odor stimuli and showed that the turtle vomeronasal organ responds to many kinds of odorants including nonvolatile chemicals. These results demonstrated that the turtle vomeronasal organ senses general odorants as well as chemical stimuli of a social nature. However, no quantitative studies on sensitivities of the vomeronasal system to general odorants have been carried out.

In recent years, several studies have been carried out on the transduction mechanisms of the main olfactory system. Pace, Hanski, Salomon, and Lancet (1985) reported that a preparation of cilia from frog olfactory epithelium contains adenylate cyclase at nearly 15 times specific activity of the whole olfactory epithelium, and that odorants activate the enzyme in the cilia in the presence of GTP. Nakamura and Gold (1987) showed that excised patches of ciliary plasma membrane contain a conductance that is gated directly by cyclic AMP and cyclic GMP. Injection of the cyclic nucleotides into isolated olfactory cells of the frog (Suzuki, 1989a, b), the salamander (Trotier, Rosin, and MacLeod, 1989), and the newt (Kurahashi, 1990) led to depolarization of the cells. These results suggested that activation of the cyclic nucleotide–gated channels of olfactory ciliary membranes contribute to generation of the receptor potential. Since vomeronasal cells have no cilia, it is interesting to examine the odor sensitivity and the transduction mechanism in the vomeronasal system.

In this study we have observed that the turtle vomeronasal and olfactory systems have similar sensitivities to various general odorants. We have also observed that vomeronasal responses to all odorants tested are practically unchanged by perfusing the vomeronasal epithelium with salt-free solution or by replacing Na⁺ with less permeable cations. These results suggest that ion transport across the apical membranes of vomeronasal cells does not contribute to responses to odorants in the turtle.

**MATERIALS AND METHODS**

**Recordings of Main and Accessory Olfactory Bulbar Responses**

Turtles, *Geoclemys reevesii*, weighing 200–800 g were used in this study. Turtles were weakly anesthetized with an intraperitoneal injection of urethane solution (150 mg/100 g body weight), then immobilized by an intramuscular injection of d-tubocurarine (0.6 mg/100 g body weight) and locally anesthetized with lidocaine at the wound and head fixation points. A hole was made with a dental drill on the dorsal surface of the skull to expose the brain, and the dura mater of the accessory olfactory bulb was removed carefully. During recording of the brain
wave, the surface of brain was covered with liquid paraffin to prevent drying of the brain. Activities of accessory olfactory bulbar responses were recorded as described previously (Yoshii and Kurihara, 1983). To eliminate the possible effect of the main olfactory bulb activity (Hatanaka et al., 1988), the olfactory nerve was cut before entry to the main olfactory bulb. Stimulation-induced brain waves were recorded from twin tungsten electrodes inserted into the olfactory bulb and integrated by an electric integrator (time constant of 0.5 s). The recording was carried out at ±18°C.

Activity of the main olfactory bulbar responses was recorded as described previously (Shoji, Kashiwayanagi, and Kurihara, 1991).

Perfusion of Vomeronasal Epithelium

The adapting and stimulating solutions were applied to the vomeronasal epithelium through a stainless steel tube. The vomeronasal epithelium was first perfused for 1 min with a 10 mM EDTA solution of pH 8.0 containing 200 mM mannitol for 1 min to eliminate divalent cations and then with adapting solution containing a given concentration of a salt and mannitol, which was added to keep the osmolarity of 200 mosmol/liter for 3 min. 200 mosmol is equivalent to the osmolarity of Ringer solution. An odorant dissolved in the adapting solution was then applied to the epithelium. The pure water used was prepared by deionization with an ion exchange column and filtration through a Milli-Q Water System (Millipore Corp., Bedford, MA). The concentration of Ca²⁺ in the pure water used, measured with a Zeeman-effects atomic absorption spectrophotometer (Hitachi 170-70) was <4x10⁻⁸ M. The composition of the Ringer solution was 116 mM NaCl, 4 mM KCl, 2 mM CaCl₂, and 0.5 mM NaH₂PO₄-Na₂HPO₄, pH 7.2.

Chemicals

All chemicals used were of the best grade available. n-Amyl acetate, menthone, 1,8-cineole, β-ionone, citral, n-nonanol, cyclohexanone, choline chloride, amiloride, diltiazem, and verapamil were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Bis-Tris [2,2-Bis(hydroxymethyl)-2,2',2''-nitrilotrittihanol] (Bis-Tris propane) and N-acetyl-D-glucosamine were purchased from Naclai Tesque, Inc. (Kyoto, Japan).

RESULTS

Fig. 1 a shows the summated accessory olfactory bulbar responses to n-amyl acetate dissolved in Ringer solution. As seen from the figure, the responses were large at onset of stimulation and decreased to a spontaneous level during stimulation. In this article, peak height of the response to an odorant is taken as the magnitude of the response. For comparison, the main olfactory bulbar responses to odorants were recorded. Fig. 1 b shows the summated main olfactory bulbar response to n-amyl acetate. Fig. 2 shows the vomeronasal and olfactory responses to various odorants dissolved in Ringer solution as a function of odorant concentrations. As seen from the figure, the minimum concentrations (thresholds) of the vomeronasal responses are similar to those of olfactory responses in spite of the lack of cilia, suggesting that the vomeronasal and the olfactory cells have similar sensitivities to odorants.

To see the effects of salts on the odor responses in the vomeronasal system, the epithelium was perfused with an adapting solution containing a given concentration of a salt and mannitol added to keep osmolarity constant before application of odorants and then an odorant dissolved in the adapting solution was applied to the
epithelium. Adapting solution usually contains salts and induces salt response. The responses to salts themselves are, however, declined to a spontaneous level during perfusion of the epithelium with the adapting solution. Hence a response induced by an odorant solution does not contain a response to a salt itself. Fig. 3 shows relative magnitudes of the vomeronasal responses to \( n \)-amyl acetate and menthone as a function of odorant concentrations in the salt-free solution (200 mM mannitol solution) and 100 mM NaCl solution. Here magnitude of the response is calculated relative to the response to 5 mM \( n \)-amyl acetate dissolved in Ringer solution. The responses start to appear between \( 10^{-8} \) and \( 10^{-7} \) M and increase with an increase in odorant concentrations. As seen from the figure, there is no essential difference between the responses in the salt-free solution and those in 100 mM NaCl solution in the whole concentration range examined.

Table I represents ratios between magnitudes of the vomeronasal responses to various odorants in the salt-free solution and those in the 100 mM NaCl solution. As seen from the table, the responses to seven odorants tested are not greatly changed by removal of NaCl; the ratios are between 1.01 and 1.10.

Fig. 4 shows the magnitude of the responses to \( n \)-amyl acetate and menthone as a function of NaCl concentration in perfusing solution where the magnitude of the response is calculated relative to that to 5 mM \( n \)-amyl acetate dissolved in Ringer solution. As seen from the figure, the responses to the odorants are practically unchanged by a variation in NaCl concentration.

Table II represents the magnitudes of the response to 5 mM \( n \)-amyl acetate and 0.5 mM menthone dissolved in 100 mM choline chloride, 100 mM \( N \)-acetyl-D-glucosamine chloride, 100 mM Bis-Tris propane dichloride, and 50 mM \( \text{Na}_2\text{SO}_4 \), where the magnitude of the response to each odorant in 100 mM NaCl is taken as...
unity. As seen from the table, the replacement of NaCl with these organic salts or Na₂SO₄ does not affect the responses to n-amyl acetate and menthone.

Fig. 5 shows the magnitude of the responses to 5 mM n-amyl acetate and 0.5 mM menthone as a function of CaCl₂ concentration, where the magnitude of the response is calculated relative to the magnitude of the response to 5 mM n-amyl acetate dissolved in Ringer solution. As seen from the figure, the responses to n-amyl acetate vomeronasal responses and menthone were practically independent of CaCl₂ concentration. Furthermore, the responses were unchanged by the presence of 5 mM EGTA-2 Na in 200 mM mannitol solution (data not shown).

The effects of a Na channel blocker, amiloride, and Ca channel blockers, diltiazem and verapamil, on the magnitudes of vomeronasal responses were tested. Table III represents the magnitudes of the responses to 5 mM n-amyl acetate and 0.5 mM

![Diagram](attachment:image.png)

**FIGURE 2.** The relative magnitude of the responses to accessory olfactory bulbar responses (a and b), and main olfactory bulbar responses (c and d) as a function of concentrations of various odorants dissolved in Ringer solution. The magnitude of response is calculated relative to the response to 10 mM n-amyl acetate. Each point represents the mean ± SE of data obtained from at least three preparations.
menthone in the presence of these blockers. As seen from the table, the magnitudes of responses are unchanged by the presence of 0.5 mM amiloride, 0.5 mM diltiazem, and 0.5 mM verapamil.

**DISCUSSION**

The sensitivity of the vomeronasal system to various general odorants and the effects of changed ionic environment on the accessory olfactory bulb responses to odorants were examined in this study. The accessory olfactory bulb responses reflect less directly the receptor responses, but can be measured stably and reproducibly for a long time (e.g., 1 d). It was demonstrated that there is good correlation between electroolfactogram (EOG), neural responses, and bulbar responses (Byrd

**TABLE 1**

<table>
<thead>
<tr>
<th>Odorant</th>
<th>Ratio (± SE) (Range)</th>
<th>n</th>
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<tbody>
<tr>
<td>0.5 mM menthone</td>
<td>1.05 ± 0.05 (0.86–1.23)</td>
<td>7</td>
</tr>
<tr>
<td>0.1 mM citral</td>
<td>1.10 ± 0.06 (0.92–1.16)</td>
<td>4</td>
</tr>
<tr>
<td>10 mM cyclohexanone</td>
<td>1.09 ± 0.02 (1.05–1.14)</td>
<td>4</td>
</tr>
<tr>
<td>0.1 mM n-nonanol</td>
<td>1.03 ± 0.03 (0.96–1.06)</td>
<td>4</td>
</tr>
<tr>
<td>0.05 mM β-ionone</td>
<td>1.03 ± 0.03 (0.94–1.08)</td>
<td>4</td>
</tr>
<tr>
<td>5 mM n-amyl acetate</td>
<td>1.04 ± 0.04 (0.86–1.23)</td>
<td>7</td>
</tr>
<tr>
<td>1 mM cineole</td>
<td>1.01 ± 0.02 (0.96–1.06)</td>
<td>4</td>
</tr>
</tbody>
</table>

Data presented are mean ± SE of n preparations. Values in parenthesis represent ranges of values.
and Caprio, 1982; Hara, 1982). Tucker and Shibuya (1965) measured both the EOG and the neural response of the turtle, Terrapene carolina, to n-amyl acetate and showed that both responses behave similarly under various conditions as long as the solution resistivity is not changed. Shoji et al. (1991) also showed that the ion

![Figure 4](image-url)  
**Figure 4.** Dependence of vomeronasal responses to 5 mM n-amyl acetate and 0.5 mM menthone on NaCl concentration. The magnitude of the response is calculated relative to the response to 5 mM n-amyl acetate dissolved in Ringer solution. Points at the leftmost side represent data in the salt-free solution. Each point is the mean ± SE of data obtained from four preparations.

| **TABLE II** |  
| Effects of Replacement of NaCl with 100 mM Choline Chloride (Choline Cl), 100 mM Bis-Tris Propane Dichloride (Bis-Tris Propane Cl₂), 100 mM N-Acetyl-D-Glucosamine Chloride (N-Acetyl-D-Glucosamine Cl), and 50 mM Na₂SO₄ on the Vomeronasal Responses to 5 mM n-Amyl Acetate and 0.5 mM Menthone Where the Magnitude of the Response in the Presence of 100 mM NaCl Is Taken as Unity |  
| **n-Amyl acetate** | **Menthone** |  
| Choline Cl | 0.95 ± 0.04 (0.86–1.06) | 1.02 ± 0.03 (0.94–1.06) |  
| Bis-Tris propane Cl₂ | 1.08 ± 0.08 (0.88–1.12) | 1.06 ± 0.08 (0.89–1.25) |  
| N-Acetyl-D-glucosamine Cl | 1.00 ± 0.08 (0.82–1.18) | 0.93 ± 0.06 (0.83–1.06) |  
| Na₂SO₄ | 0.88 ± 0.05 (0.78–0.94) | 0.96 ± 0.06 (0.85–1.06) |  

The values in the table are mean ± SE of four preparations. The values in parenthesis represent ranges of values.
dependence of olfactory nerve response to n-amyl acetate is essentially equal to that of the olfactory bulb response.

In the vomeronasal system, no quantitative study on the sensitivity of responses to general odorants has been carried out. The present results show that the sensitivities of vomeronasal responses to various odorants are similar to those of the olfactory responses, although the vomeronasal cells have no cilia. It was shown that removal of cilia from the carp olfactory cells did not affect the magnitudes of olfactory responses

**TABLE III**

*Effects of 0.5 mM Amiloride, 0.5 mM Diltiazem, and 0.5 mM Verapamil on the Vomeronasal Responses to 5 mM n-Amyl Acetate and 0.5 mM Menthone Where the Magnitude of the Response in the Salt-free Solution Is Taken as Unity*

<table>
<thead>
<tr>
<th></th>
<th>n-Amyl Acetate</th>
<th>Menthone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiloride</td>
<td>0.99 ± 0.05 (0.81–1.12)</td>
<td>1.03 ± 0.07 (0.89–1.31)</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>1.03 ± 0.02 (0.99–1.10)</td>
<td>1.06 ± 0.07 (0.81–1.16)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>0.94 ± 0.07 (0.74–1.17)</td>
<td>1.08 ± 0.08 (0.88–1.31)</td>
</tr>
</tbody>
</table>

The values in the table are mean ± SE of five preparations. The values in parenthesis represent ranges of values.
to various amino acids (Kashiwayanagi, Shoji, and Kurihara, 1988). These results suggested that cilia are not essential for high sensitivity to odorants. It is interesting to note that the turtle trigeminal nerves respond very sensitively to various odorants in spite of lack of cilia (Tucker, 1971).

Tucker and Shibuya (1965) examined the effects of the changed ionic environment on the turtle olfactory responses and showed that the neural response to n-amyl acetate was unchanged by the removal of NaCl from the Ringer solution perfusing the olfactory epithelium. They also showed that EOG in the response to n-amyl acetate was markedly enhanced by removal of NaCl in Ringer solution. This was because the magnitude of EOG is a function of solution resistivity and an increase in solution resistivity due to removal of NaCl leads to an increase in the magnitude of EOG (Tucker and Shibuya, 1965; Leveteau, Andriason, Trotier, and MacLeod, 1989). We (Shoji, Kashiwayanagi, and Kurihara, 1991) also examined the effects of the changed ionic environment on the turtle olfactory responses to 13 odorants and showed that the responses to all odorants examined were essentially unchanged by perfusing the epithelium with the salt-free solution. Here we observed that the olfactory system responded to very low concentrations of salts (NaCl > 10^{-6} M, KCl > 10^{-5} M, CaCl_2 > 10^{-7} M) after perfusing of the epithelium with the salt-free solution. These results indicated that the perfusion with the salt-free solution sufficiently eliminated the salts from the surface of the epithelium.

The present results on the effects of changed ionic environment on the vomeronasal responses to odorants are similar to those on the olfactory responses. That is, there is no essential difference between the magnitudes of the responses to all odorants examined in the salt-free solution and those in 100 mM NaCl. The magnitudes of the responses to odorants are unchanged by changes in NaCl or CaCl_2 concentrations. The replacement of Na^+ with less permeable organic cations does not affect the responses to odorants. It is also shown that the replacement of Cl^- with SO_4^{2-} does not affect the responses. These results suggest that activation of cation or anion channels by odorants does not contribute to the vomeronasal responses to odorants.

The present results suggest that the ionic permeability of vomeronasal receptor membranes does not contribute to generation of odor responses in the turtle. The transduction mechanism of the vomeronasal responses is unknown at present, but three possible mechanisms can be considered. Activation of adenylate cyclase or phosphoinositide turnover in response to odorants occurs at the receptor membranes and the second messengers produced activate ionic channels located at cell membranes below the tight junction. A second possible mechanism is that the microvillus membrane is permeable to odorants and the odorants directly trigger release of Ca ion from its store within the cell. A third possible mechanism is a physicochemical one (Kurihara, Yoshii, and Kashiwayanagi, 1986). That is, adsorption of odorants on the receptor membranes leads to changes in the phase boundary potential at the receptor membranes, which brings about depolarization at the receptor membranes. The depolarization is electrotonically propagated to the cell body membranes, which activates ionic channels at the membranes. It is unknown at present which mechanism holds in the vomeronasal transduction. Further study will be needed to explore the transduction mechanism.
REFERENCES


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