Vol. 154, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1988 Pages 437-442

LARGE OLFACTORY RESPONSES OF THE CARP AFTER COMPLETE REMOVAL OF OLFACTORY CILIA

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Received May 30, 1988

SUMMARY: To study the role of olfactory cilia on olfactory reception, the carp olfactory cilia were removed by modified "ethanol-calcium shock" and the bulbar responses were recorded before and after deciliation. Large olfactory responses to various amino acids were observed after complete deciliation. The relation between magnitude of olfactory response and alanine concentration before and after deciliation was essentially unchanged. The present results suggests that the olfactory cilia may not be necessary for receptor neuron function in the carp.

Receptor sites for odorants are considered to be on olfactory cilia or olfactory knobs. Recently it was reported that various odorants activate adenylate cyclase located in frog olfactory cilia (1, 2) and application of cyclic nucleotides to a patch of ciliary membrane increases the membrane conductance (3). These results suggested that adenylate cyclase located in olfactory cilia is concerned with olfactory transduction. The present study demonstrates that in the carp, large olfactory responses to amino acids are still produced after the olfactory cilia have been removed completely from the whole area of the olfactory epithelium by modified "ethanol-calcium shock". These results suggest that the cilia may not be necessary for receptor neuron function in the carp.

METHODS

Deciliation procedure — At the beginning, a part of the olfactory epithelium at abdominal region (shaded area in schematic drawing for carp olfactory rosette) was surgically removed. The olfactory cilia were removed by the modified "calcium-ethanol shock" (4). A 5 mM phosphate buffer solution of pH 8.0 containing 5 mM EGTA, 10 % ethanol and 0.2 M sucrose was applied to the epithelium at a flow rate of 8 ml/sec for 50 sec. The jet stream of the solution was applied through 5 nozzles to reach all region of the olfactory epithelium. Then Ca-solution (100 mM $CaCl_2$) was applied to the olfactory epithelium for 20

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0006-291X/88 $1.50

min at a low flow rate (50 ml/min). Mild deciliation treatment (Fig. 1(c)) was carried out by application of treatment solution at a low flow rate (4 ml/sec).

Scanning electron microscopic (SEM) observation After the olfactory responses were recorded, the olfactory organ was fixed and dehydrated according to a method described by Lewis et al (5). The fixed olfactory epithelia were examined with SEM (S-430, Hitachi).

Recording of carp bulbar response Carps were anesthetized with ketamine HCl (10 mg/100 g body weight) as described by Kauer (6), then immobilized with d-tubocurarine (1 mg per 100 g), and locally anesthetized with lidocaine at the wound and head fixation points. The olfactory responses were recorded from olfactory bulb according to Yoshii and Kurihara (7). Briefly, stimulant-induced brain waves were recorded from twin tungsten electrodes inserted to olfactory bulb and integrated by electric integrator (time constant 0.3 sec).

RESULTS

The carp olfactory organ is composed of about 36 of the olfactory discs on which olfactory cells exist (see Fig. 1). It is difficult to apply stream of deciliating solution at a constant pressure to the whole discs. Hence at the beginning of each experiment a part of the olfactory epithelium at abdominal

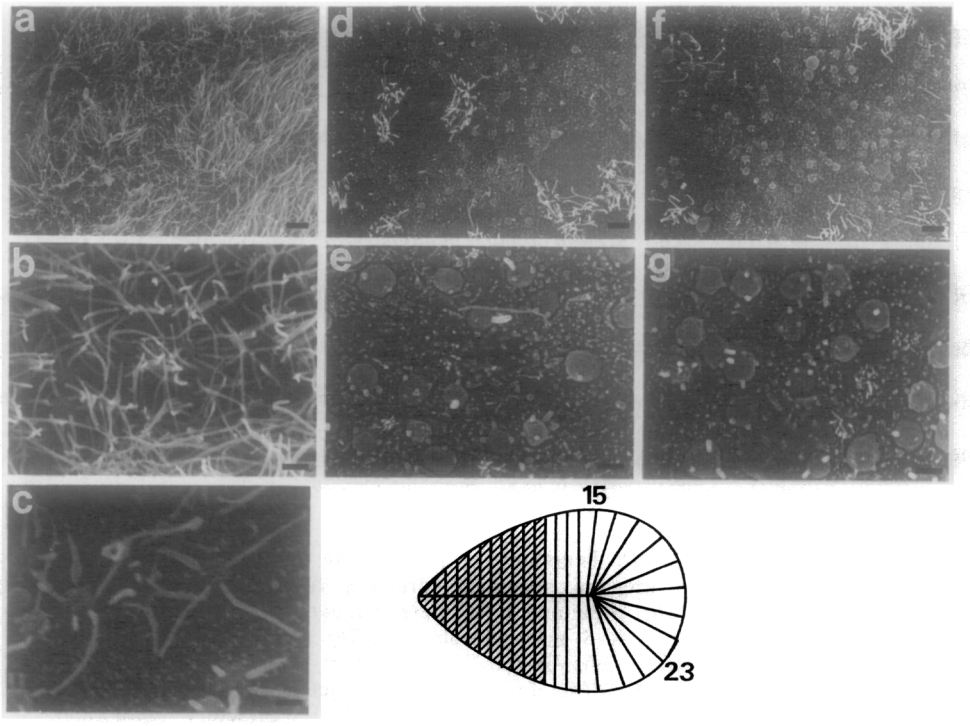


Fig. 1. Effect of the calcium-ethanol treatment on the carp olfactory epithelium. (a), (b); intact olfactory epithelium. (c); olfactory epithelium subjected to mild deciliation treatment. (d), (e) deciliated olfactory epithelium on the 15th olfactory disc indicated in the figure. (f), (g); deciliated olfactory epithelium on the 23th disc. Bar indicates 3 μm in Figs. (a), (d) and (f) and 1 μm in Figs. (b), (c), (e) and (g).

region (shaded area in Fig. 1) was surgically removed to decrease area of the epithelium to which the deciliation solution is applied. The deciliating solution was applied to the preparations having 10 to 23 olfactory cilia which remained after the section.

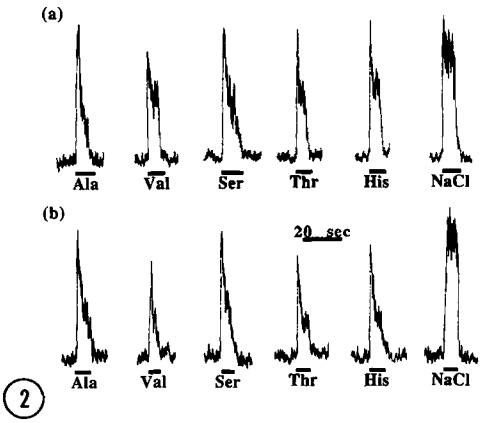
Figs. 1 a and b show SEM micrographs of intact olfactory epithelium at low and high magnification. There are many long non-sensory cilia of epithelial cells. The olfactory cilia, which are relatively short, exist between the non-sensory cilia, although the olfactory cilia are not easily seen in the intact preparations. The olfactory cilia can easily be seen in preparations subjected to mild deciliation treatment (Fig. 1c). Here the olfactory cilia are shortened but mostly remain.

In order to determine the conditions that the olfactory cilia are completely eliminated by as mild treatment as possible, various conditions such as Ca concentration, EGTA concentration, a flow rate and treatment time were changed. After deciliation treatment, the olfactory bulbar responses to amino acids were recorded and then the olfactory epithelia of the preparations were examined with SEM. Deciliation conditions to eliminate the olfactory cilia completely and to give large olfactory responses were finally determined.

SEM micrographs of one disc of the olfactory epithelium subjected to deciliation treatment are shown in Figs. d and e (low and high magnification). Those of another disc are shown in Figs. f and g. As seen from the micrographs, the olfactory cilia are completely removed, and many deciliated olfactory knobs and a few shortened non-sensory cilia are seen. All discs of the olfactory epithelium in each preparation were examined with SEM. An intact olfactory cell has 5 - 8 cilia in average whose mean length is 4.5 μm. The extent of deciliation was evaluated as follows: SEM micrographs of typical area of all discs were taken and length of cilia of typical 10 olfactory cells in each micrograph was measured. The sum of ciliary length of olfactory cells examined (10 cells in all discs) was compared with that of intact olfactory cells. In this paper, the word of "complete deciliation" is used when the sum of ciliary length after deciliation is less than 3 % of that of intact cilia. Data on the olfactory responses from preparations whose cilia were incompletely removed were excluded.

Fig. 2a shows typical bulbar responses to amino acids and NaCl before deciliation treatment. The responses to these stimuli diminished immediately after deciliation treatment, but recovered within 30-60 min. Fig. 2b shows the responses recorded 60 min after treatment. We obtained 6 preparations whose olfactory cilia were removed completely and olfactory responses recovered after deciliation treatment. The mean magnitudes of responses to 1 mM alanine, 1 mM serine, 3 mM arginine and 50 mM NaCl after treatment were 71 ± 30, 49 ± 21, 41 ± 21, and 91 ± 33 % of respective control responses. These values are surprisingly high considering the possibility that the receptor membranes might be injured by "calcium-ethanol shock". In some preparations, the responses to the stimuli tested recovered over 80 % of the control response. For example in the preparation shown in Fig. 2b, magnitudes of the response to alanine, valine, serine, threonine, histidine and NaCl after deciliation are 98, 90, 99, 80, 86 and 104 % of respective control responses.

Fig. 3 shows the relation between magnitude of the response and concentration of alanine before and after deciliation treatment. The figure indicates that the relation is essentially unchanged by treatment, although the magnitude of the response is a little lowered by treatment.



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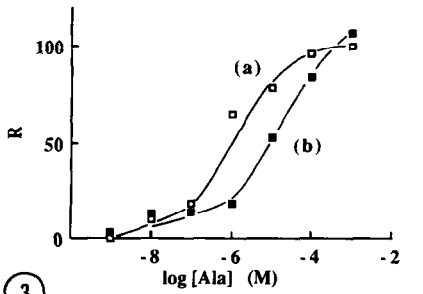

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Fig. 2. Olfactory bulbar responses before (a) and 60 min after (b) deciliation treatment. The olfactory epithelium was stimulated by 1 mM alanine, 1 mM serine, 3 mM threonine, 3 mM histidine, 3 mM valine and 50 mM NaCl.

Fig. 3. Relative magnitude of olfactory responses as a function of alanine concentrations before (a) and after (b) deciliation. Plotted response (R) was calculated relative to a response to 10^{-3} M alanine before treatment and multiplied by 100. Peak height of the summated bulbar response is taken as magnitude of the response.

DISCUSSION

Admek et al. reported that removal of the frog olfactory cilia with Triton X-100 greatly decreased electro-olfactograms (8). This results do not always imply that the cilia are necessary for the olfactory reception since the receptor membranes might be injured by treatment. In fact, we often encountered the case where no olfactory response appeared after the olfactory epithelium was subjected to hard treatment. Tucker removed the turtle olfactory cilia with Triton X-100 and found that the olfactory neural response to amyl acetate was not greatly impaired after deciliation (9). This study did not, however, examine the whole area of the olfactory epithelium to determine the extent of ciliary removal. The present study demonstrates that large olfactory responses were produced after complete removal of olfactory cilia from whole epithelium. The possibility that binding of the stimuli to the remaining root of the cilia is sufficient to produce a large olfactory response is not completely excluded, but the possibility that the cilia are not necessary for receptor neuron function is much more likely. The latter possibility is consistent with the fact that cells carrying no cilia such as the trigeminal nerve, the vomeronasal organ (10) and the neuroblastoma cell (11, 12) respond to various odorants. In addition, the following fact suggests that an increase in conductance of olfactory ciliary membrane does not contribute the receptor potential.

In a previous paper (7), we showed that removal of ions from the carp olfactory epithelium led to complete diminish of the olfactory responses and addition of ions recovered the responses. Perfusion of the epithelium with 1 mM KCl solution produced a large olfactory response. This suggested that activation of channels at the apical membranes of olfactory cells is not concerned with generation of the receptor potential since the equilibrium potential of K^+ across the membranes is calculated to be -127 mM (the intracellular K^+ concentration is assumed to be 140 mM). Thus it is unlikely that the carp olfactory response is induced by an increase of ionic permeability at the apical membranes including ciliary membranes. Hence activation of cyclic nucleotides-sensitive channels in ciliary membranes does not seem to contribute the olfactory receptor potential in the carp. In separate papers (13, 14), we showed that the lipid bilayer membranes

exhibit membrane potential changes in response to odorants similarly to olfactory cells. In the following paper (15), we show that the ion dependence of the responses of the lipid membranes to odorants is closely related to that in the carp olfactory system, suggesting that the initial process of generation of the receptor potential in olfactory cells closely resembles that in the lipid membranes.

ACKNOWLEDGMENTS

This work was supported by grants from Ministry of Education of Japan and Nissan Science Foundation.

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