DISCUSSION

1 Targets of odor inhibitory action

1.1 Inhibitory effects on cAMP cascade

Kurahashi and co-workers (1994) showed that the suppression of the transduction current by odorants has a short latency, and suggested that odorants directly suppress CNG-channel. The short latency of suppression was also observed in my study (Figure 16). Some of my results provided corroborating evidence that odorants inhibit CNG-channel directly. First, odor stimulation suppressed the IBMX-induced current (Figure 12). Competitive effects of stimulus-receptor binding have been proposed to be a mechanism of suppression of odor-induced current by odorants (Derby et al., 1991). Odor suppression of the IBMX-induced current in my study, however, suggested that the suppression cannot only be due to competitive effects and but the target of suppression is downstream of receptor binding in the cAMP-pathway. Second, the conductance increase induced by intracellular injection of cAMP was also suppressed by odorants (Figure 13). The injection of cAMP directly raises the concentration while application of IBMX raises the intracellular concentration of cAMP by inhibiting the breakdown of cAMP in the cell,. Thus odor suppression of the current induced by injection of cAMP demonstrated that the suppression could not be due to the inhibition of adenylate cyclase, but suggested that the target of the suppression was after the accumulation of intracellular cAMP. Finally, odor stimulation suppressed the current that was induced by application of 8-Br-cGMP (Figure 14). The 8-Br-cGMP is a membrane-permeable and unhydrolysable analogue of cyclic GMP (Zimmerman et al., 1985; Nawy, 1999) and has been reported to activate CNG-channels in olfactory receptor neurons (Firestein et al., 1991). Therefore, the suppression of the current induced by the application of 8-Br-cGMP ruled out the possibility that this

suppression was caused only by degeneration of intracellular cAMP, and meant that the suppression of transduction current was due to suppression of activation of the CNG-channel itself. Furthermore, the ratio of suppressed current when the current was activated by application of 8-Br-cGMP (0.47 ± 0.26; n=3) was very similar to that when the current was activated by application of IBMX (0.53 ± 0.08; n=6) ($P>0.75$; one-way ANOVA) (Figure 15). Thus the inhibition of CNG-channel seems to be a major cause of the suppression of cAMP-induced current.

1.2 Inhibitory effects on other conductances

Ca^{2+}-activated chloride current contributes to the transduction current, however the result that 10 mM anisole suppressed almost all the IBMX-induced current (Figure 16) suggests that anisole also decreased the chloride current as well. However, I cannot conclude that odorants directly suppressed the chloride conductance, because inhibition of CNG-channel alone would cause a decrease in intracellular calcium and inactivate chloride conductance.

While odor stimulation suppressed the transduction current, it did not suppress the basal conductance that maintains the cell in a hyperpolarized state. Odor stimulation did not suppress the TEA-inhibited basal potassium conductance, as shown in Figure 12. If odorants did suppress the basal potassium conductance, application of odorants to resting cells would cause depolarization. Such depolarization, however, has not been observed. As shown in Figure 11, odor stimulation did not cause such a depolarization even when it was able to suppress the current induced by IBMX.

2 Dose-range of odor inhibitory effect

It has been reported that olfactory neuron sensitivities for various odorants range from millimolar to picomolar concentrations (Firestein et al., 1993; Getchell, 1986; Getchell and Shepherd, 1978). However, I observed that an inhibitory effect of anisole on IBMX-induced currents was restricted in high dose-range (Figure 16). There are several possible explanations for the differences between the dose-ranges for inhibition and excitation.

One possibility is that the inhibitory effect of an odorant might work functionally at higher dose-range than the excitatory response. In the case of the lobster (Ache and Zhainazarov, 1995), the dose-ranges of excitatory and inhibitory responses are nearly identical (Michel and Ache, 1994) and both systems involve dual second messenger pathways, which work in parallel to form the cell's response potential. On the other hand, the inhibitory effect in the newt olfactory neurons appears to work only by inhibiting excitatory transduction system, as shown in this study. Thus the inhibitory effect seems simply to play a supporting in making odor representation in ORNs. The inhibitory effect in newts might not work in overall range in which odor stimulation have the excitatory effects, but play a restricted role only at higher dose-range in newt olfactory receptor neurons.

Another potential cause for the higher dose-range may be due to the nature of my experiments. I have attempted to explore the dose-dependency of odor suppression of a large steady current induced by continuous application of 0.25mM IBMX (Figure 16) in order to observe dose-dependency of odor suppressing transduction current clearly. In this case the dose range of suppressive odorant may be inappropriate for estimating physiological dose-range, because excess cAMP was continuously produced by IBMX application. For exploring physiological dose-range of the odor

inhibitory effect, it will be necessary to study further more the odor inhibitory effect in more physiological condition. In particular, I need to compare between responses to mixture odor and those to individual component odor (Figure 22), and to estimate the inhibitory effect of an odorant on responses to other odorants, as studied in some reports (Michel and Ache, 1994; Derby et al., 1991).

3 Inhibitory responses that caused by odor suppression of cAMP-induced current

Inhibitory responses have been identified as an inhibition of excitation in the cell (reviewed by Getchell, 1986; Ache and Zhainazarov, 1995). Some recent studies in amphibians have described odor-induced hyperpolarizing current, which is triggered by increase in intracellular calcium, as a cellular mechanism that causes inhibition (Morales et al., 1994, 1995, and 1997). Studies on excitatory transduction system in vertebrates, however, have shown that increases in intracellular calcium elicit chloride conductance exclusively (Kleene and Gesteland, 1991b), and cause depolarization of the cilia (Kurahashi and Yau, 1993). The cause of the discrepancy between these studies remains unclear, but one hypothesis is that the hyperpolarizing current might not coexist in the same cell with a standard cAMP-cascade (Vogler and Schild, 1999). On the other hand, results in my study suggest another possibility that the odor hyperpolarization could be due to odor suppression of cAMP-induced current. It is difficult to examine whether the entire hyperpolarization is due to this mechanism. Nevertheless, the odor suppression would elicit hyperpolarization of the olfactory receptor neurons when the cells have high level of intracellular cAMP, which would be caused by either background stimulation of odorant (Figure 22) or by high basal

activity of adenylate cyclase (Kleene et al., 1994),

4 Odor representation in ORNs

4.1 Mixture suppression

It has been generally accepted that odor sensation is represented by the pattern of activated neurons in the olfactory epithelium and that the activation of each olfactory receptor neuron is determined by the excitatory specificity of a particular odors (reviewed by Mori and Yoshihara, 1995; Hildebrand and Shephered, 1997).

On the other hand, psychological (Cain, 1975; Laing et al., 1984; Berglund and Olsson, 1993a, 1993b; Laing et al., 1989a), behavioral (Monkey; Laska and Hudson, 1993: Rat; Laing et al., 1989b: Lobster; Daniel and Derby, 1991), and physiological (Bell et al., 1987) studies have shown that odor representation cannot be formed by the excitatory pathway alone. Laing and coworkers (Jinks and Laing, 1999a, 1999b), for example, have shown that the capacity of human to identify the components of odor mixture has limitation. This phenomenon that increasing the number of odorants in a mixture leads to increased loss of odorant identity has also been known as termed "mixture suppression" or "masking" in the other studies (reviewed by Laing et al., 1989a). This limitation of capacity for analyzing mixture odor was not improved by varying test method, type of odorants, or training and experience of subjects. Even flavourists and perfumers, for example, are known to exhibit the same limitation as subjects with no special training in odor discrimination and identification. From such results, Laing and coworkers suggested that the mechanisms of cognition are not the basis of the limitation (Jinks and Laing, 1999a, 1999b). They proposed a hypothesis, as more likely mechanism, that limitations on forming representation in

ORNs restrict the ability to identify components in an odor mixture.

In lobster behavioral studies (Daniel and Derby, 1991), such mixture suppression has also been described; moreover, Ache and coworkers (reviewed by Ache and Zhainazarov, 1995) have shown the corresponding physiological mechanism for the mixture suppression. They showed that single olfactory cells, stimulated by an odorant, can have their responses to that odorant suppressed by the presence of another odorant (Michel and Ache, 1994). From their physiological studies on transduction cascade in lobster ORNs, they concluded that the reciprocal suppression between odorants is due to co-existence of inhibitory and excitatory pathwayd that have different odorant specificity (Figure 4) (Fadool and Ache, 1992; Michel and Ache, 1992). Accordingly, they suggested that odor representation in ORNs would be formed with inhibitory effect of odorant as well as excitatory effect. As compared to the studies in lobsters, however, we lack the direct evidence that odor inhibitory effect involves in forming odor representation in ORNs and reciprocal suppression of odorants leads to the mixture suppression.

4.2 Putative representation of mixture odorant in ORNs

The results in my study provided the evidence that the mutual suppression occurs in ORNs in. An odorant decreased excitatory responses to another odorant in individual neurons, as shown in Fig 22. EOG results (Figure 25) that responses to mixture odorant were smaller than that to individual odorant consist with the former results from solitary cells, which also supports mutual suppression in ORNs.

Furthermore, the results in my study also suggested how mixture odorants are encoded in ORNs. Application of an odorant that caused less

excitatory response leaded to the greater suppression of responses to another odorant (Figure 23 and 24). This nature can also be deduced from the fact that odor inhibitory effect worked in a non-cell-specific manner (Figure 20 and 21) and was antagonized by excitatory effect of odorants (Figure 17 and 18). Because odor inhibitory effect works uniformly among cells, application of an odorant would more greatly work inhibitory when the cell has less excitatory specificity to the odorant.

As a result of such suppression, a population of ORNs activated by mixture odorant cannot follow additivity of those by individual odorant. Rather than the addition, the population of the cells activated by mixture odorant would be restricted to an overlapping part of the activated ORNs (Figure 26). In EOG recordings, the results that the extent of mixture suppression for a dissimilar-odorant-pair was greater than that for a similar-odorant-pair were consistent with the putative representation of mixture odorant. Overlapping part of activated neurons for dissimilar-pair can be assumed to be smaller than that for the similar-pair, so that mixture suppression for dissimilar-pair would be greater. It is known that mixture suppression can be observed more frequently as a pair of odorants is dissimilar in psychophysical studies (Laing et al., 1984). EOG results seem to be equivalent to these psychophysical observations.