J. Steroid Biochem. Molec. Biol. Vol. 39, No. 4B, pp. 621–625, 1991
Printed in Great Britain.

0960-0760/91 $3.00 + 0.00

MOLECULAR REACTION CASCADES IN OLFACTORY SIGNAL TRANSDUCTION

HEINZ BREER
University Stuttgart-Hohenheim, Institute of Zoophysiology, Garbenstr. 30, 7000 Stuttgart 70, Fed. Rep. Germany

Summary—Odorant induced second messenger signals in ciliary preparations from rat olfactory epithelia were monitored in the subsecond time range using a rapid kinetic methodology. Application of micromolar concentrations of odorants induced a rapid and transient elevation of second messenger concentrations. The odorous compounds analyzed induced in a mutually exclusive way the formation of either cyclic adenosine monophosphate or inositol-triphosphate. The activating effects of odorants on intracellular signalling cascades appear to be mediated via different G-proteins. Thus, at least two different second messenger pathways appear to be involved in olfactory signal transduction. Selective inhibition of odor-induced second messenger responses by certain lectins indicate that glycoproteins appear to be involved in the perception or transduction of olfactory signals. In the presence of protein kinase inhibitors the odorant-induced second messenger response is no longer transient but persistent over a longer time period, suggesting that termination of the signal is realized via feedback phosphorylation of functional elements in the reaction cascade.

INTRODUCTION

The sense of smell is a key element for survival and adaptation in the animal world. It is well known that many organisms use chemical signals to identify their territory and their food sources; furthermore, mates and predators are recognized and discriminated via chemical signals. In vertebrates, the sense of smell is carried by olfactory receptor cells forming patches of a specialized epithelium; this olfactory epithelium covers about 5–10 cm^2 of the dorsal nasal cavity. The olfactory receptor cells are small bipolar neurons that send axons towards the olfactory bulb and dendrites towards the nasal lumen. The dendrite forms, at its apical end, a dilatation, the olfactory knob, from which a group of 5–20 cilia project into the mucus that coats the epithelium [1, 2]. A major problem of olfactory signalling concerns the question of how volatile molecules are perceived by olfactory receptor cells, and how the chemical stimulus is converted into an electrical response? The perception and sensory transduction of olfactory stimulants begin when odorants are inhaled and dissolved in the mucus. There is substantial evidence to support the notion, that the primary events of olfactory signal transduction occur in the cilia after the odorant has diffused through the mucus layer and interact with the chemosensory membrane of the cilia. Thus, the cilia may be considered as scaffolding for the chemosensory membranes providing a large expansion of the surface area with which the odorous molecules can interact. The transduction process is thought to be mediated by membrane-bound receptor proteins, which upon activation initiate a multistep reaction cascade, ultimately leading to an increase in membrane conductance and to the generation of action potentials in the olfactory nerve [3–5]. As an enormous variety of odorous compounds can be sensed and distinguished by the olfactory system, multiple mechanisms are thought to accomplish this task.

Due to great efforts over the last decade using various experimental approaches, a picture is beginning to emerge of the molecular mechanisms of the chemoelectrical signal transduction process in olfactory receptor cells. Although there is some evidence for direct channel gating by odorants [6, 7] this configuration would involve little amplification of the odor signal and may thus not be optimal for a sensory mechanism that requires high sensitivity. Transduction mechanisms which include the activation of enzymatic cascades leading to the formation of second messengers involve high amplification mechanisms.

Proceedings of the International Symposium on Recent Advances in Mammalian Pheromone Research, Paris, France, 6–9 October 1991. Sponsored by the EROX Corporation.

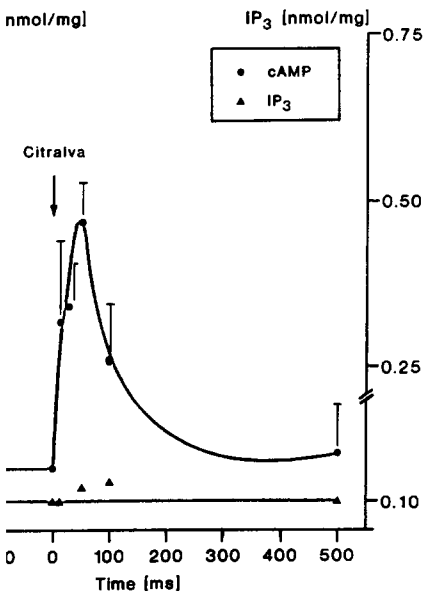
rable evidence has been accumulated that the olfactory transduction ay involve adenylyl cyclase activity. s of adenylyl cyclase activity occurs in ıry epithelium [8, 9] which was found ılated by certain odorants in a tissue- ıanner [10, 11]. The stimulation of ıe is dependent on the presence of is therefore likely to be mediated by ɔupled GTP-binding proteins (G- 12]. The resulting elevation in cyclic MP) levels is thought to elicit depolar- olfactory neurons by direct activation nucleotide-gated cation channels ıus, alterations in intracellular levels ıre transduced into alterations of the potentials of the receptor cells.

h most of the molecular elements or this concept have been identified, 'ays been a matter of debate if an reaction and changes in the concen- an intracellular messenger would be h to be causually involved in a rapid response of olfactory receptor cells ırs in the millisecond time range [16]. ensing is generally a rapid but transi- s, requiring instant response and also termination of the signal. Further- ımber of odorants obviously do not ıylyl cyclase activity suggesting that ıe formation of cAMP, additional transduction mechanisms may be involved in olfaction [11].

These fundamental questions for understanding the process of olfactory signal transduction have been approached using a fast kinetic methodology to study the odor-induced formation of second messengers in cilia preparations from rat olfactory cilia in a subsecond time course [17]. As shown in Fig. 1, application of micromolar concentrations of odorants, like citralva, induces a very rapid increase of cAMP reaching a peak after about 50 ms; at this point the cAMP concentration is several fold higher than the basal level. Thereafter, the concentration very rapidly returns to the basal level. The concentration of inositol-trisphosphate (IP_3) is not changed at all. This result indicates that odorant-induced changes in the cAMP concentration represents in fact a rapid and transient molecular signal which clearly precedes the electrical response of receptor cells [16] and thus could mediate the chemo–electrical transduction process.

In experiments using various concentrations of citralva it was shown that the peak response was clearly dose-dependent. The dose–response curve showed a very steep stimulus/response correlation for odor concentrations below 1 μM, but at higher concentrations the response further increased at a slower rate; there was no apparent saturation. Hofstee plot analysis has indicated that this observation is apparently due to two systems, a high and a low affinity system, which may be due to the activation of specific receptors at low concentrations and, in addition, activation of related systems at higher concentrations [18]. A similar cAMP response was obtained for a number of odorants, suggesting that in fact an activation of adenylyl cyclase and a rapid rise in cAMP-levels may be the molecular reaction cascade mediating the olfactory signal transduction in vertebrates. This would be in contrast to the molecular signalling in insect antennae where pheromones as well as general odorants were found to induce IP_3-responses not affecting the cAMP pathway [17].

However, after application of putrid odorants, like pyrazine, rapid and transient changes in the IP_3 level were observed in rat cilia; as in insect antennae, the concentration of cAMP was not changed in this case [18]. This result raised the question; can different odorants activate different second messenger pathways? Furthermore, there is the possibility that odorants of a



kinetics of odorant-induced changes in second ɔncentrations of isolated rat olfactory cilia. ɔtory cilia were challenged with 1 μM citralva period of time and the concentration of cAMP ɛ determined using a specific binding assay.

certain odor class may activate the same second messenger system.

In order to explore these aspects, a mixture of odorants with similar odor quality was applied. It was found that stimulation of rat olfactory cilia with a mixture of fruity odorants induced a rapid and transient concentration change for both cAMP and IP_3 [19, 20]. In order to elucidate if activation of both pathways was a new quality of the odorant mixture or if certain odorants of the same odor quality induced different second messengers, the applied odorants were assayed individually. The results of these experiments indicated that most of the applied fruity odorants (citralva, citral dimethylacetal, citronellal and citronallylacetate) induced cAMP and had no effect on IP_3; only lyral activated the IP_3 pathway with no effect on cAMP.

It is striking that apparently each individual odorant very selectively activates either cAMP or IP_3. This principal was confirmed for a number of compounds with different odor quality, including floral, herbaceous and putrid odorants. Each compound activates either cAMP or IP_3 formation. It is interesting to note that the second messenger pathways activated by certain odorants are not correlated with odor quality (Table 1).

Table 1. Odorant-induced increase in second messenger concentration and adenylate cyclase activity

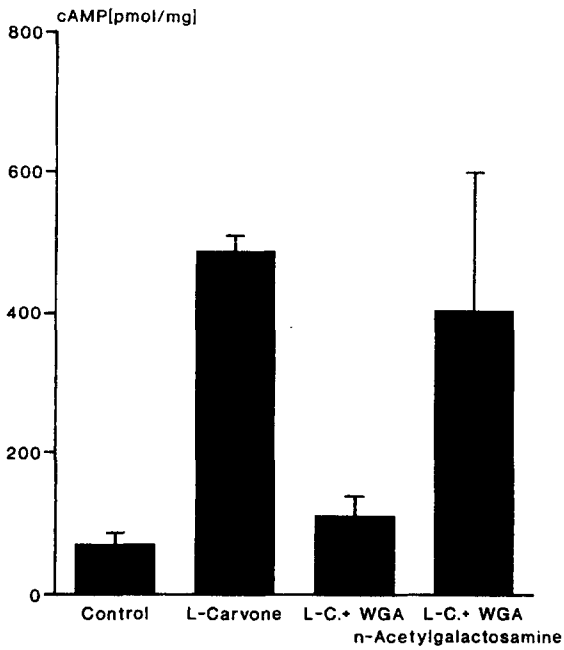
Odorant	cAMP[a]	Adenylate cyclase[b]	IP_3[a]
Fruity			
Citralva	100	100	4 ± 7
Citraldemethylacetal	63 ± 10	69 ± 10	4 ± 5
Citronellal	61 ± 7	56 ± 5	3 ± 5
Citronellylacetate	62 ± 10	50 ± 9	1 ± 1
Lyral	0	−4 ± 6	100
Floral			
Hedione	108 ± 21	60 ± 20	6 ± 7
Geraniol	62 ± 25	58 ± 3	6 ± 7
Acetophenone	36 ± 8	30 ± 4	4 ± 6
Phenylethylalcohol	16 ± 9	19 ± 4	8 ± 7
Lilial	1 ± 2	−1 ± 4	106 ± 22
Herbaceous			
Eugenol	70 ± 18	47 ± 7	4 ± 4
Isoeugenol	49 ± 13	31 ± 5	2 ± 3
Ethylvanillin	0	−3 ± 6	63 ± 15
Putrid			
Furfurylmercaptan	37 ± 9	29 ± 9	8 ± 7
Triethylamine	4 ± 2	4 ± 7	141 ± 60
Phenylethylamine	6 ± 2	0 ± 7	137 ± 78
Pyrrolidine	0	−4 ± 6	70 ± 25
Isovaleric acid	0	−6 ± 8	68 ± 24

[a]Data for cAMP and IP_3 represent values obtained after application of 1 μm odorant.
[b]Data are taken from Ref. [11].
Data for cAMP and adenylate cyclase activity are expressed as percentage of the effect induced by citralva. Data for IP_3 are expressed as percentage of concentration induced by lyral.

The presently favoured concept of olfactory signal transduction is based on the assumption that odor molecules interact with specific receptors and activate reaction cascades via G-proteins. Therefore the GTP-dependence of the rapid and transient odorant-induced second messenger signal was investigated. It was found that the odor and GTP alone had only a small effect on cAMP. Together, however, they showed a very pronounced synergistic effect. GTP could not be substituted by GDP. These observations are regarded as evidence, that the odor-induced cAMP signal is mediated via G-proteins.

Bacterial toxins, like cholera- and pertussis-toxin, catalyze the ADP-ribosylation of specific G-proteins and thus modify their function. Cholera-toxin, which activates G_s-proteins, permanently, caused an accumulation of cAMP in olfactory cilia, which was not further amplified by odorants. In contrast, pertussis-toxin showed no significant effect. When considering the activation of the IP_3 pathway, e.g. by pyrazine, it became evident that cholera-toxin neither induces the formation of IP_3 nor affects the activator effect of pyrazine. However, pertussis-toxin, which inactivates G_o- and G_i-proteins, prevented the formation of IP_3 in response to pyrazine stimulation. These results support the view, that odorant-induced activation of the cAMP systems is mediated by G_s-proteins and activation of the IP_3 systems is mediated via G_o-proteins. The critical role of G-proteins in mediating an odorant stimulus into a second messenger response suggests, of course, the existence of specific G-protein-coupled receptors for odorants. As putative odorant receptors are probably glycoproteins, attempts have been made to analyze if the specific probes for glycoconjugates, if lectins may affect the odorant-induced second messenger response [21, 22]. The results demonstrate that treatment of olfactory cilia with certain lectins, noteably Concanavalin A (ConA) and wheat germ agglutinin (WGA), significantly reduce the cAMP or IP_3 signal induced by certain odorants. The effects were specific as the appropriate hapten sugars prevented the actions of lectins. Another example for the specificity of the lectin effect was found in experiments using the two isomers of carvone, which have a different odor quality: L-carvone smells like caraway, whereas D-carvone smells like spearmint; but both induce cAMP. Analyzing the effect of different lectins it was found that cAMP formation induced by

D-carvone was markedly reduced by ConA, whereas WGA effectively interfered with L-carvone (Fig. 2). These observations may be considered as an indication that glycoproteins are involved in the recognition and/or the transduction of odorous molecules [23].



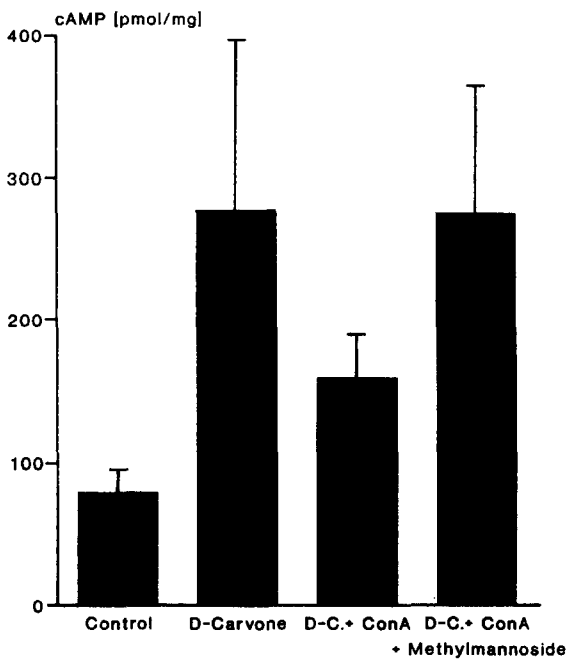


Fig. 2. Inhibitory effects of lectins on the odorant-induced second messenger signal in rat olfactory cilia. Olfactory cilia were preincubated with 5 μM lectins with and without the hapten sugars. The odor-induced cAMP formation was determined 50 ms after stimulation with the stereoisomers of carvone.

An important aspect of the second messenger response to odorant stimulation is the transient nature of the molecular signal which is particularly important for receptor cells which can be repeatedly stimulated, such as the olfactory receptor cells. This problem includes the question of how the odorant-induced second messenger cascade is turned off. Experiments using blockers of the phosphodiesterase, like IBMX, have demonstrated that the elevated cAMP concentration persists if the enzyme is inhibited; i.e. phosphodiesterase is responsible for the decay of the elevated cAMP concentration. So, a delayed stimulation of catabolic enzyme could be responsible for the decay of the cAMP signal (Fig. 3). However, no evidence was found that the phosphodiesterase was increased upon odorant stimulation suggesting that the transient nature of the cAMP signal is probably caused by a blockade of cAMP synthesis.

From the work on visual and hormonal signal transduction it is well known, that phosphorylation of receptor proteins is an important step in signal termination. Therefore the effect of kinase inhibitors on the rapid and transient odor-induced second messenger signal was analyzed. The results demonstrated that stimulation of the cAMP pathway, e.g. with citralva, in the presence of a kinase inhibitor, the Walsh

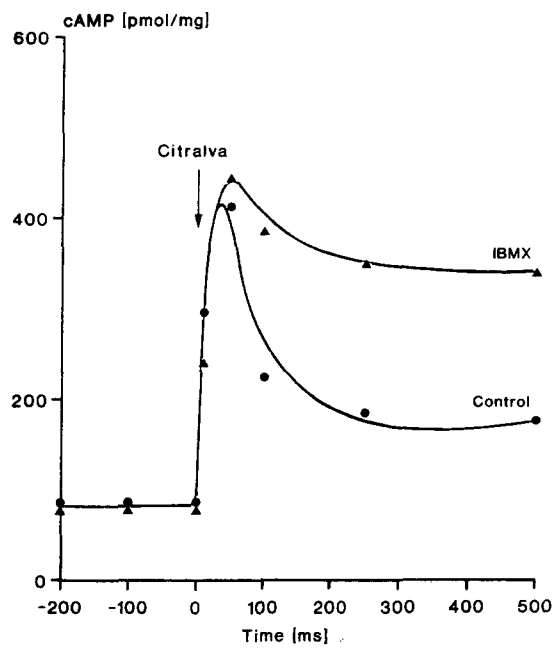


Fig. 3. The elevated cAMP level in olfactory cilia induced by odorant-stimulation persists in the presence of phosphodiesterase inhibitors, e.g. IBMX.

inhibitor, an elevated cAMP concentration further increased and persisted. Apparently, the 'turn off' reaction was blocked by the kinase inhibitor; cAMP is further synthesized at an elevated rate. A similar phenomenon was observed for the IP_3 signal in the presence of inhibitors for protein kinase C, like H7 or sphingosine. These observations support the view that inactivation of odorant-sensitive second messenger pathways is mediated via phosphorylation of one or more molecular elements of the reaction cascade. Whether these are membrane-bound receptor proteins or G-proteins or the key enzymes is presently unknown.

Acknowledgements—This work was supported by the Deutsche Forschungsgemeinschaft Br 712/7-1, 712/10-1.

REFERENCES

1. Getchell T. V.: Functional properties of vertebrate olfactory receptor neurons. *Physiol. Rev.* **66** (1986) 772–817.
2. Moulton D. and Beidler L. M.: Structure and function in the peripheral olfactory system. *Physiol. Rev.* **47** (1976) 1–52.
3. Lancet D.: Vertebrate olfactory reception. *A. Rev. Neurosci.* **9** (1986) 329–355.
4. Snyder S. H., Sklar P. B., Hwang P. M. and Pevsner J.: Molecular mechanisms of olfaction. *TINS* **12** (1989) 35–38.
5. Reed R. R.: How does the nose know? *Cell* **60,** (1990) 1–2.
6. Labarca P., Simon S. A. and Anholt R. H. H.: Activation by odorants of a multistate channel from olfactory cilia. *Proc. Natn. Acad. Sci. U.S.A.* **85** (1987) 944–948.
7. Nomura T. and Kurihara K.: Effect of changed lipid composition on responses of various odorants. *Biochemistry* **26** (1987) 6141–6148.
8. Kurihara K. and Koyama N.: High activity of adenylate cyclase in olfactory and gustatory organs. *Biochem. Biophys. Res. Commun.* **48** (1972) 30–33.
9. Menevse A., Dodd G. and Poynder T. M.: Evidence for the specific involvement of cyclic AMP in the olfactory transduction mechanism. *Biochem. Biophys. Res. Commun.* **77** (1977) 671–677.
10. Pace U., Hanski E., Salomon Y. and Lancet D.: Odorant-sensitive adenylate cyclase may mediate olfactory reception. *Nature* **316** (1985) 255–258.
11. Sklar P. B., Anholt R. H. and Snyder S. H.: The odorant sensitive adenylate cyclase of olfactory receptor cells: differential stimulation by distinct classes of odorants. *J. Biol. Chem.* **261** (1986) 15538–15543.
12. Jones D. T. and Reed R. R.: G_{olf} an olfactory neuron-specific G-protein involved in odorant signal transduction. *Science* **244** (1989) 790–795.
13. Nakamura T. and Gold G. H.: A cyclic nucleotide-gated conductance in olfactory receptor cilia. *Nature* **325** (1987) 442–444.
14. Dhallan R. S., Yau K.-W., Schrader K. A. and Reed R. R.: Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature* **347** (1990) 184–187.
15. Ludwig J., Margalit T., Eisman E., Lancet D. and Kaupp U. B.: Primary structure of cAMP-gated channel from bovine olfactory epithelium. *FEBS Lett.* **270** (1990) 24–29.
16. Firestein S. and Werblin F.: Odor-induced membrane currents in vertebrate-olfactory receptor neurons. *Science* **244** (1989) 79–89.
17. Breer H., Boekhoff I. and Tareilus E.: Rapid kinetic of second messenger formation in olfactory transduction. *Nature* **345** (1990) 65–68.
18. Boekhoff I., Tareilus E., Strotmann J. and Breer H.: Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. *EMBO Jl* **9** (1990) 2453–2458.
19. Breer H. and Boekhoff I.: Odorants of the same odor class activate different second messenger pathways. *Chem. Senses* **16** (1991) 19–29.
20. Boekhoff I. and Breer H.: Differential stimulation of second messenger pathways by distinct classes of odorants. *Neurochem. Int.* **17** (1990) 553–557.
21. Kalinoski D. L., Bruch R. C. and Brand J. G.: Differential interaction of lectins with chemosensory receptors. *Brain Res.* **418** (1987) 34–40.
22. Shirley S. G., Polak, E. H., Mather R. A. and Dodd G. H.: The effect of concanvalin A on the rat electro-olfactogram. *J. Biochem* **245** (1987) 175–184.
23. Boekhoff I.: Molekulare Reaktionskaskaden der olfaktorischen Signaltransduktion. PhD-thesis, University Stuttgart-Hohenheim (1991).
24. Hausdorff W. P., Caron M. G. and Lefkowitz R. J.: Turning off the signal: desensitivation of β-adrenergic receptor function. *FASEB J.* **4** (1990) 2881–2889.