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A β-adrenergic receptor kinase-like enzyme is involved in olfactory signal termination

(olfaction/signaling/kinase/subtypes/antibodies)

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ABSTRACT **We have previously shown that second-messenger-dependent kinases (cAMP-dependent kinase, protein kinase C) in the olfactory system are essential in terminating second-messenger signaling in response to odorants. We now document that subtype 2 of the β-adrenergic receptor kinase (βARK) is also involved in this process. By using subtype-specific antibodies to βARK-1 and βARK-2, we show that βARK-2 is preferentially expressed in the olfactory epithelium in contrast to findings in most other tissues. Heparin, an inhibitor of βARK, as well as anti-βARK-2 antibodies, (*i*) completely prevents the rapid decline of second-messenger signals (desensitization) that follows odorant stimulation and (*ii*) strongly inhibits odorant-induced phosphorylation of olfactory ciliary proteins. In contrast, βARK-1 antibodies are without effect. Inhibitors of protein kinase A and protein kinase C also block odorant-induced desensitization and phosphorylation. These data suggest that a sequential interplay of second-messenger-dependent and receptor-specific kinases is functionally involved in olfactory desensitization.**

The olfactory system responds precisely to iterative stimulation (1) and thus can continuously monitor changes in the odorous environment occurring between consecutive sniffs. This sensory behavior is due to characteristic phasic responses of receptor cells to short odor pulses (2), thereby preventing a brief stimulus from being perceived as continuous smell. The basis for this characteristic feature of olfactory neurons is a rapid termination of the odor-induced primary reaction—i.e., the second-messenger cascade initiated by an odorant-occupied receptor is rapidly turned off (3, 4). Recent studies have indicated that olfactory signaling is terminated by uncoupling the transduction cascade; this is accomplished via a negative-feedback reaction controlled by the second messenger elicited upon odor stimulation. Second-messenger-activated protein kinases [protein kinase A (PKA) or protein kinase C (PKC)] presumably phosphorylate elements of the transduction apparatus and thereby uncouple the signaling cascade (5). Subsequent experiments have shown that stimulation with odorants indeed caused significant phosphorylation of olfactory ciliary proteins, and there is some circumstantial evidence indicating that receptor proteins for odorants may be modified via second-messenger-controlled protein kinases (6). The emerging picture for signal termination in olfaction is, in many respects, reminiscent of desensitization phenomena in other systems—notably the visual and hormonal signaling pathways, where phosphorylation of receptors in an active state uncouples the reaction cascade by preventing further stimulation of G proteins (7, 8).

For the most widely studied model, the β-adrenergic system, two distinct pathways for phosphorylation and desensitization have recently been discovered. Receptor phosphorylation and cascade uncoupling may be mediated either by a cAMP-dependent protein kinase (PKA) or alternatively by a cAMP-independent kinase (β-adrenergic receptor kinase, βARK) (8–10).

In the present investigation we set out to explore whether a dual kinase pathway might also be active in olfactory desensitization. The results indicate that besides second-messenger-controlled enzymes [PKA or PKC], a βARK-like kinase, more specifically a βARK-2 subtype, is involved in rapidly terminating olfactory signaling.

MATERIALS AND METHODS

Materials. Sprague–Dawley rats were obtained from the Zentralinstitut für Versuchstierzucht, Hannover, F.R.G. Citralva (3,7-dimethyl-2,6-octadienenitrile) was obtained from International Flavors and Fragrances (Union Beach, NJ). Lilial [4-(1,1-dimethylethyl)-α-methylbenzenepropanol], ethylvanillin (3-ethoxy-4-hydroxybenzaldehyde), lyral [4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxyaldehyde], hedione (3-oxo-2-pentylcyclopentaneacetic acid methyl ester) and eugenol [2-methoxy-4-(2-propenyl)phenol] were provided by W. Steiner, Baierbrunn, F.R.G. PKA inhibitor (Walsh inhibitor) and heparin were obtained from Sigma. Calphostin C, a specific inhibitor of PKC, was purchased from Calbiochem. [γ-^{32}P]ATP was supplied by DuPont, and radioligand assay kits for cAMP and inositol 1,4,5-triphosphate ($InsP_3$) were purchased from Amersham. The purity grade of all chemicals used was >99%.

Antibodies. βARK-1 or βARK-2-specific polyclonal antibodies were prepared as described in ref. 11 by injecting rabbits with gluthathione-*S*-transferase fusion proteins containing COOH-terminal sequences of either βARK isoform. Sera were immunoaffinity-purified on antigen columns of βARK-1 or βARK-2 fusion proteins immobilized on Affi-Gel 15. Antibodies that bound to one isotype column but passed through the other were "subtype specific". Antibodies that sequentially bound to and were eluted from both types of column were subtype-nonspecific and recognized both enzyme forms. Specificity of antibodies was verified by immunoblot analysis of COS cell extracts overexpressing βARK-1 or βARK-2 (11).

Methods. *Isolation of olfactory cilia.* Partially purified preparations of chemosensory cilia from rat olfactory epithelia were produced according to the procedure described by Anholt *et al.* (12) and Chen *et al.* (13). Briefly, rat olfactory epithelia were dissected and collected in Ringer's solution (120 mM NaCl/5 mM KCl/1.6 mM K_2HPO_4/1.2 mM $MgSO_4$/25 mM $NaHCO_3$/7.5 mM glucose, pH 7.4). This

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Abbreviations: βARK, β-adrenergic receptor kinase; PKA, protein kinase A; PKC, protein kinase C; $InsP_3$, inositol 1,4,5-trisphosphate.

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procedure and all subsequent operations were done at 4°C. Cilia were detached from the epithelia by the calcium-shock procedure, replacing the buffer by Ringer's solution/10 mM $CaCl_2$. After being agitated in an end-over-end shaker for 20 min, the deciliated epithelia were removed by centrifugation at 7700 × *g* for 10 min. The supernatant was collected, and the pellet was incubated again with Ringer's solution/10 mM $CaCl_2$ and then centrifuged. The combined supernatants containing the detached cilia were centrifuged at 27,000 × *g* for 15 min. The pelleted cilia were resuspended in 10 mM Tris·HCl/3 mM $MgCl_2$/2 mM EGTA, pH 8.0 and stored at −70°C.

Phosphorylation procedure. The *in vitro* phosphorylation experiments were done by the procedure described by Wilden and Kühn (7). Odorants were dissolved in dimethyl sulfoxide, giving a stock solution of 100 mM. To obtain the appropriate final odorant concentration different volumes of the stock solution were added to the "stimulation buffer" (200 mM NaCl/10 mM EGTA/50 mM Mops/2.5 mM $MgCl_2$/1 mM dithiothreitol/0.05% sodium cholate/1 mM ATP/1 μM GTP/$CaCl_2$ to give a free Ca^{2+} concentration of 0.02 μM, pH 7.4). The odorant solutions were thoroughly mixed in an ultrasonic water bath and used immediately. Controls were always performed by using the highest dimethyl sulfoxide concentration, which never exceeded 0.05%.

The phosphorylation reaction was started by adding 20 μl of the cilia preparation (20 μg of protein) to 80 μl of prewarmed (25°C) stimulation buffer containing odorants at the appropriate concentration and 1 μCi [γ-^{32}P]ATP. After incubation for the appropriate time intervals, usually 1 sec, the reaction was stopped by adding 300 μl of an ice-cold solution of 25% trichloroacetic acid/5 mM phosphoric acid. The samples were placed on ice for 30 min followed by a thorough mixing of the precipitated proteins. Aliquots (100 μl) of each sample were spotted onto Whatman 3 MM filter paper (4 × 4 cm) and immersed in a solution of 10% trichloroacetic acid/5 mM phosphoric acid. After being thoroughly washed for 30 min, the filters were dried at room temperature for 2 hr. The radioactivity adsorbed onto the filters was quantitated by scintillation counting.

Measurement of rapid changes in second-messenger concentrations. A rapid-quench device with three syringes was used to determine the subsecond kinetics of the odorant-induced changes in second-messenger concentrations. Rapid kinetic experiments were done as described (3). Briefly, syringe I contained the olfactory cilia (0.5 μg/μl) in 70 μl of buffer (10 mM Tris·HCl/3 mM $MgCl_2$/2 mM EGTA); syringe II was filled with 360 μl of stimulation buffer containing the odorants dissolved as described above. All solutions were adapted to 37°C. Syringe III contained perchloric acid (7%) cooled to 0°C. The reaction was started by mixing the contents of syringe I and II and quenched with 300 μl of perchloric acid ejected from syringe III. Activation of the three syringes was controlled by an IBM AT computer. The quenched samples were collected and cooled on ice before analysis for second-messenger concentrations.

RESULTS

Receptor-specific kinases, like rhodopsin kinase and βARK, are efficiently blocked by polyanions, such as heparin (14). In a first approach to evaluate whether such second-messenger-independent kinases are involved in the rapid termination of olfactory signaling, olfactory cilia pretreated with heparin were assayed for kinetics of the cAMP response elicited by the fruity odorant citralva and the InsP_3 response induced by lyral. As depicted in Fig. 1*A*, upon stimulation with citralva, control samples show a very rapid and transient increase in cAMP level, thus confirming previous observations. In samples pretreated with either Walsh inhibitor or heparin, the "onset"-kinetic of the odorant-induced cAMP-signal was virtually unaffected; however the "offset"-kinetic was significantly reduced. The rapid termination of the signal is prevented by the PKA inhibitor as well as by heparin. A similar effect was seen for the InsP_3 response elicited by lyral (Fig. 1*B*); with either calphostin C, a specific blocker of PKC, or heparin signal termination was prevented. These observations indicate that desensitization of the odorant-induced second-messenger cascade is blocked by inhibitors of second-messenger-dependent kinases but is also blocked by heparin.

On the basis of the results of a previous study (6) it has been hypothesized that one mechanism of olfactory desensitization is odorant-induced phosphorylation of receptor proteins mediated via second-messenger-activated kinases. To explore the mode of heparin action, its effect on odorant-induced incorporation of [^{32}P]phosphate was analyzed. Fig. 1 *C* and *D* shows that stimulation of olfactory cilia with either citralva or lyral in the presence of [γ-^{32}P]ATP significantly increased the incorporation of $^{32}P_i$. Labeling in the presence of odorants was about three times over basal level; ≈300 pmol of P_i per mg of protein was incorporated after 1 sec. The odorant-induced incorporation of $^{32}P_i$ was almost completely blocked by the Walsh inhibitor or calphostin C, respectively; in both cases heparin also reduced the $^{32}P_i$ incorporation level but only reduced it by ≈60%. Both inhibitors together attenuated phosphorylation to the basal level (Fig. 1 *C* and *D*). Analysis of ciliary proteins incubated with [γ-^{32}P]ATP and 1 μM lyral for 1 sec indicated that a single polypeptide band (M_r 50,000) is labeled under these conditions (Fig. 1*D*, *Inset*), thus confirming recent observations (6); labeling of this protein was prevented by calphostin C. These observations suggest that, in addition to a second-messenger-controlled kinase, a heparin-sensitive kinase is involved in olfactory signal termination and odorant-induced phosphorylation of olfactory proteins.

To further characterize the involvement of a heparin-sensitive kinase in olfactory signaling, similarity of the kinase to the heparin-sensitive βARK was analyzed. Monospecific antiserum raised against βARK from rat (11) was used in the assay systems. The data in Fig. 2*A* show that the kinetics of citralva-induced cAMP signals are unchanged by a control serum (1:1000); however, the anti-βARK antiserum (1:1000) completely prevents rapid desensitization of the cAMP response. The inhibitory effect of the βARK antibodies is also observed in phosphorylation experiments (Fig. 2*B*); odorant-induced incorporation of [^{32}P]phosphate is significantly reduced by the kinase antibodies. These results suggest that a βARK-like kinase is involved in olfactory signaling.

Two closely related, yet distinct, βARK subtypes have been identified (16, 17). Although some differences concerning activity and regional distribution in the brain have been seen, the distinct physiological roles of the respective subtypes are still elusive. As a first step to evaluate whether a distinct kinase subtype was active in the olfactory system, different tissues were probed for immunoreactivity by using subtype-specific antibodies (11). The results of ELISA assays (Fig. 3*A*) show that βARK-1 predominates in erythrocytes as well as in lung and brain tissue; the samples are only weakly reactive with anti-βARK-2. In contrast, the olfactory cilia show high levels of βARK-2 reactivity but show only basal activity for βARK-1. To scrutinize the specificity of βARK subtypes in olfactory cilia, both subtype-specific antibodies were assayed over a broad range of dilutions. As depicted in Fig. 3*B*, at low antibody concentrations (high dilution) only βARK-2 reactivity is detected; only at low dilutions is some βARK-1 reactivity detectable. The specific and selective reaction of olfactory cilia with βARK-2 antibodies was confirmed by immunocytochemical approaches (unpublished observation).

The proposed specific role of a βARK-2-like kinase in olfactory cilia was confirmed in phosphorylation experiments. Fig. 4 shows that the odorant-induced incorporation

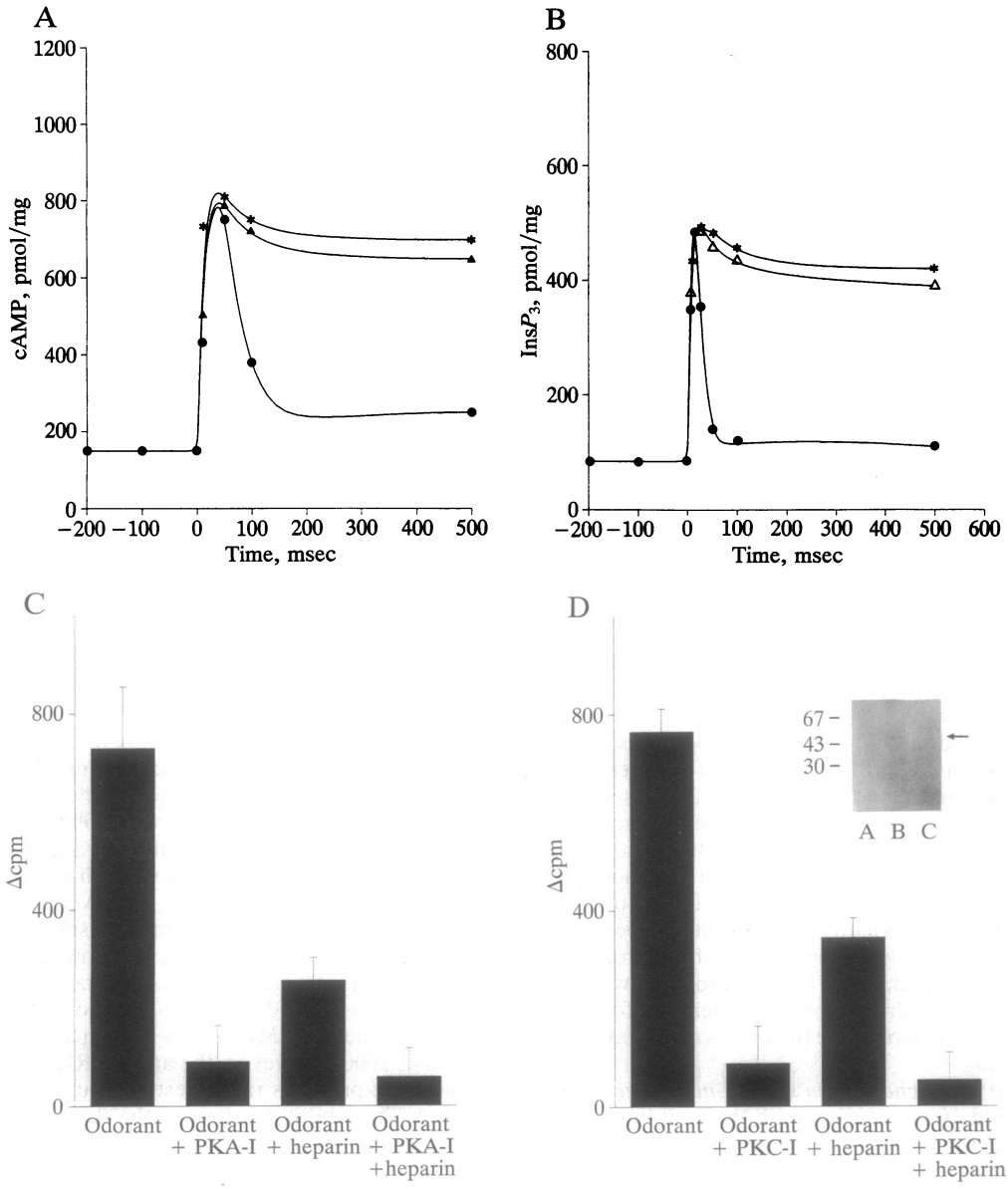

FIG. 1. Protein kinase inhibitors prevent desensitization of olfactory second-messenger signaling as well as odorant-induced protein phosphorylation. (*A*) Cilia preparations isolated from the olfactory epithelium of rat were stimulated with citralva (1 μM). In control samples a rapid and transient increase of cAMP concentration is seen, confirming reported results (3). In samples pretreated with either Walsh inhibitor (3.8 μM) or heparin (1 μM) for 15 min at 4°C, the "onset"-kinetic of the odorant-induced signal was unaffected; however, the "offset"-kinetic was significantly changed. Both inhibitors completely blocked desensitization. Data represent the means of three experiments; deviations are <10%. ●, Odorant; ▲, Walsh inhibitor; *, heparin. (*B*) Stimulation with lyral (1 μM) induced a rapid and transient response of InsP_3. Calphostin C (1 μM), the specific inhibitor of PKC or heparin, completely prevented rapid termination of the lyral-induced InsP_3 cascade; the Walsh inhibitor had no effect. Data are the means of three experiments; deviation is <10%. ●, Odorant; △, calphostin C; *, heparin. (*C*) Odorant-induced [^{32}P]phosphate incorporation into olfactory cilia was determined by incubating isolated cilia with [γ-^{32}P]ATP for 1 sec. The basal extent of ^{32}P incorporation (without odorants) during 1 sec was 250 cpm, which was subtracted from "total labeling". In the presence of the odor mix citralva/eugenol/hedione (1 μM each), which generates a cAMP signal (15), a significant increase of P_i incorporation was seen. The Walsh inhibitor (3.8 μM) reduced the degree of labeling to nearly control level; with heparin (1 μM) the $^{32}P_i$ incorporation was reduced to almost one-third. With both inhibitors the degree of inhibition was the same as that achieved by PKA inhibitor alone. Data represent the odorant-induced incorporation of $^{32}P_i$ and represent the means of three to five experiments ± SD. (*D*) Incorporation of $^{32}P_i$ induced by lyral, lilial, and ethylvanilin, odorants that induce an InsP_3 signal (15), was reduced nearly to control level by blocking PKC with calphostin C (1 μM); the Walsh inhibitor showed no effect. With heparin (1 μM) the rate of incorporation was reduced to one-third. Both inhibitors together almost completely blocked the odorant-induced phosphorylation. Data represent the means of three to five experiments ± SD. (*Inset*) Identification of ciliary proteins phosphorylated upon odorant stimulation. The phosphorylation reaction was stopped by adding SDS/sample buffer. Proteins were then separated on SDS/12.5% polyacrylamide gel, and the radiolabeled polypeptides were visualized by autoradiography. Note that only a single polypeptide band (M_r 50,000) was labeled upon odorant stimulation. Lanes: A, without odorant; B, with 1 μM lyral; C, odorant plus 1 μM calphostin C.

of [^{32}P]phosphate into ciliary proteins was hardly affected by βARK-1 antibodies, even at high concentrations, but was significantly reduced by βARK-2 antibodies in a concentration-dependent manner.

To evaluate whether a βARK-2-like kinase is, in fact, involved in desensitization of olfactory signaling, the effect of subtype-specific antibodies on the odor-induced cAMP response was monitored. The results (Fig. 5) show that control serum (1:1000), as well as the βARK-1 antibodies (1:5000), did not affect the kinetics of the second-messenger signal. However, in the presence of βARK-2-specific antibodies (1:5000) rapid termination of the cAMP signal was prevented.

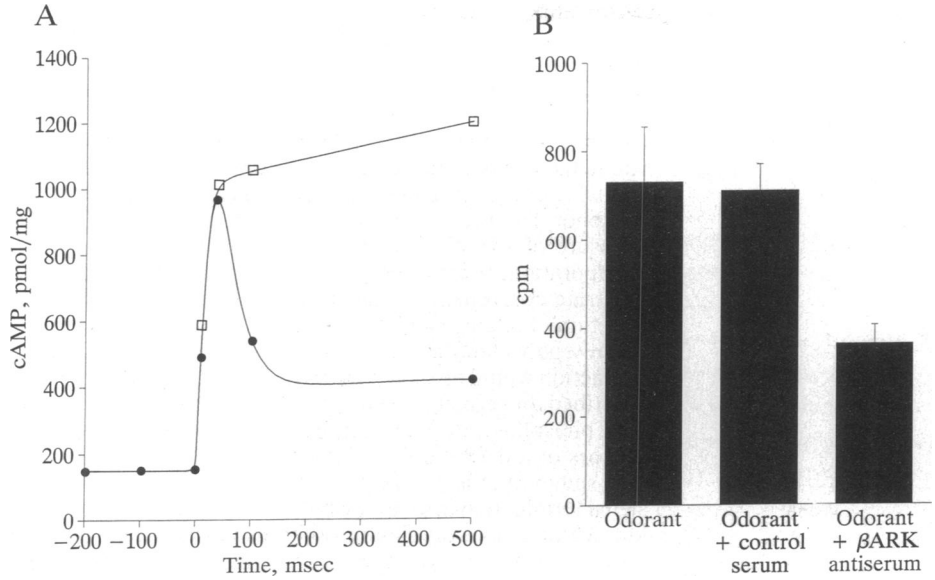

FIG. 2. Effect of anti-βARK antiserum on desensitization and phosphorylation of olfactory cilia. (*A*) Isolated olfactory cilia were preincubated either with control rabbit serum (dilution 1:1000) or with anti-βARK antiserum raised in rabbit (dilution 1:1000). Pretreated cilia were stimulated with citralva (1 μM), and the cAMP response was determined. Desensitization was completely prevented by anti-βARK antibodies. Data are the means of three experiments. ●, Odorant; □, odorant plus anti-βARK. (*B*) Attenuation of odorant-induced phosphorylation. Pretreatment of olfactory cilia with βARK-specific antibodies (1:1000) significantly reduced incorporation of $^{32}P_i$ induced by odorant stimulation; control serum had no effect. Data are the means of four experiments ± SD.

DISCUSSION

The data presented here suggest that two types of kinases are involved in turning off the olfactory transduction cascade. Inhibition of either a second-messenger-controlled kinase (PKA or PKC) or a heparin-sensitive kinase prevents olfactory desensitization as well as odorant-induced phosphorylation of olfactory proteins (putative odorant receptors; Fig. 1; ref. 6). Thus, the rapid termination of odorant-activated transduction processes in olfactory cilia appears similar to the mechanisms mediating desensitization of the β-adrenergic system, which also includes two different kinases: the cAMP-dependent protein kinase (PKA) and the heparin-sensitive β-adrenergic kinase (βARK) phosphorylate the β-adrenergic receptor at specific sites and thereby disrupt further β-adrenergic receptor/G protein coupling (16, 18). The two kinases represent alternative pathways of desensitization active at different agonist concentrations and involved in, respectively, heterologous or homologous desensitization (8). However, although agonist-occupied receptors have been conclusively shown to be phosphorylated by these kinases in the β-adrenergic receptor system (19), such proof has not been established in the olfactory system. Recent findings (Fig. 1 *Inset* and ref. 6) are, however, consistent with this hypothesis.

Some major differences also exist between the olfactory and β-adrenergic systems. The odorant-induced second-messenger response in olfactory cilia is turned off very rapidly—the cAMP or InsP_3 signal decays after only 50 msec—whereas waning of the hormone response occurs within minutes (20). The physiologically important rapidity of desensitization in olfaction probably requires specialized enzymes and a specific mechanism. In line with this notion is our discovery that in the olfactory neuroepithelium a characteristic subtype of the heparin-sensitive kinase, a βARK-2-like kinase, is preferentially expressed, in contrast to brain and peripheral tissues where the βARK-1 isoform predominates (Fig. 4; ref. 11). Furthermore, it is a βARK-2-like kinase that is specifically involved in the rapid desensitization of olfactory signaling (Fig. 5). Thus, the βARK-2-type kinase may represent another example of particular isoforms of molecular entities that build the signal-transduction apparatus in olfactory neurons. Characteristic isoforms of a G protein (G_{olf}, ref. 21) and adenylate cyclase (type III, ref. 22) have recently been described.

Not only are different kinase subtypes active in desensitizing odorant and hormone receptors but there also may be significant differences in the mechanisms. In the adrenergic system, blockade of PKA or βARK-1 partially inhibited receptor phosphorylation as well as desensitization (14). In

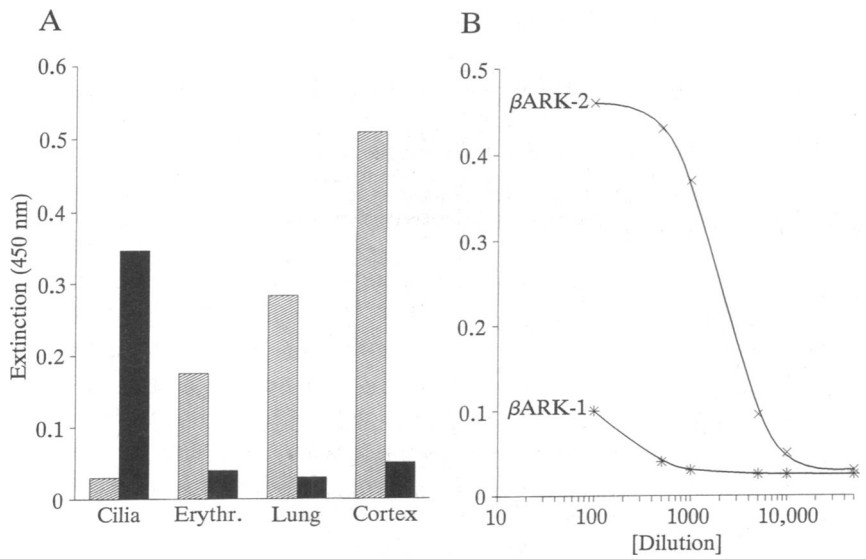

FIG. 3. Tissue-specific reactivity of antibodies discriminating between different βARK subtypes (▨, βARK-1; ■, βARK-2). (*A*) Samples from different tissues of rat were probed for immunoreactivity by using the ELISA technique and subtype-specific antibodies (dilution 1:1000). The βARK-1 subtype predominates in brain and lung tissue, as well as in erythrocytes (Erythr.); these samples display low reactivity with anti-βARK-2. In contrast, olfactory cilia show rather high levels of βARK-2 and only low activity for βARK-1. (*B*) Dose–response curve of the two subtype-specific antibodies on olfactory cilia preparation in ELISA assays. Data are the means of three separate experiments; deviation is <10%.

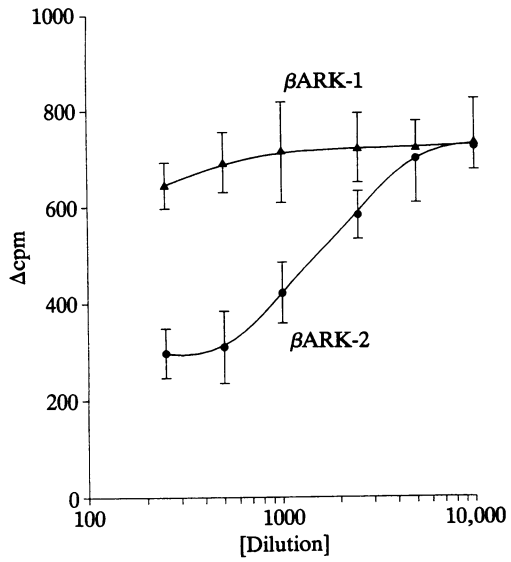

FIG. 4. Dose–response curve of the different subtype-specific anti-βARK antibodies on odorant-induced phosphorylation of olfactory cilia. Isolated olfactory cilia were pretreated with different dilutions of subtype-specific antibodies and incubated with [γ-^{32}P]ATP plus odorants for 1 sec. Note that the odorant-induced incorporation of [^{32}P]phosphate was not affected by βARK-1 antibodies; however, labeling was significantly reduced with βARK-2 antibodies. Data are the means of three experiments ± SD.

contrast, in the olfactory system inhibitors of second-messenger-dependent kinases completely blocked both phosphorylation and desensitization, whereas inhibition of βARK-2 by either heparin or anti-βARK antibodies only partially blocked phosphorylation but completely prevented desensitization (Fig. 1). Because PKA-mediated phosphorylation continues in the presence of anti-βARK antibodies or heparin, this phosphorylation is clearly insufficient for desensitization. These observations raise the possibility that the two kinases act sequentially in a reaction cascade that is initiated by activating the second-messenger-dependent kinase. Such an interrelationship between two different kinases would represent a distinctive mechanism for receptor desensitization and might be necessary for rapid signal termination. The details of how these kinases interact, however, remain elusive. Several schemes may be envisaged: (*i*) PKA could phosphorylate the odorant receptors and thereby trigger the exposure of specific sites for βARK-2-mediated phosphate incorporation. In this model, the action of βARK-2 would be substrate-controlled, a mechanism characteristic for the structurally related rhodopsin kinase (23, 24). (*ii*) PKA might directly phosphorylate and thus activate βARK-2; this type of reaction would resemble a kinase cascade, as, for example, described for regulation of metabolic pathways (25). (*iii*) PKA may phosphorylate and thereby inactivate endogenous inhibitors of a βARK-2-like kinase.

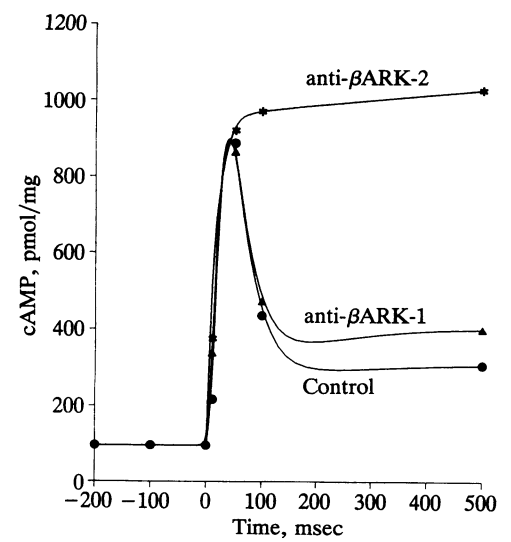

FIG. 5. Desensitization of odorant-induced second-messenger signals is specifically prevented by βARK-2 antibodies. Isolated olfactory cilia pretreated with either control serum or subtype-specific antibodies were stimulated with citralva (1 μM), and the induced second-messenger responses were determined. Note that control serum (control) and βARK-1 antibodies (1:5000) had no effect on the offset-kinetic of the odorant-induced second-messenger signal; however, with βARK-2 antibodies (dilution, 1:5000) rapid desensitization was completely prevented. Data are the means of three experiments; deviation was <10%.

In summary, these data suggest a hypothesis different from signal termination in vision, where a single kinase (rhodopsin kinase) uncouples the rhodopsin/transducin cascade (26) and different from the β-adrenergic system, where PKA and βARK-1 work in parallel to phosphorylate and desensitize the β-adrenergic receptor under certain conditions (8). In olfaction, the rapid termination of the odorant-induced signal transduction cascade may be accomplished by a coordinated, sequential action of a second-messenger-controlled kinase and a βARK-2-like kinase. Although not yet proven, the olfactory receptor proteins themselves are the most likely point of action of these kinases.

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1. Ottoson, D. (1956) *Acta Physiol. Scand.* **35**, Suppl. 122, 1–83.
2. Getchell, T. V. & Shepherd, G. M. (1978) *J. Physiol. (London)* **282**, 541–560.
3. Breer, H., Boekhoff, I. & Tareilus, E. (1990) *Nature (London)* **344**, 65–68.
4. Boekhoff, I., Tareilus, E., Strotmann, J. & Breer, H. (1990) *EMBO J.* **9**, 2453–2458.
5. Boekhoff, I. & Breer, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 471–474.
6. Boekhoff, I., Schleicher, S., Strotmann, J. & Breer, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 11983–11987.
7. Wilden, U. & Kühn, H. (1982) *Biochemistry* **21**, 3014–3022.
8. Lefkowitz, R. J., Hausdorff, W. P. & Caron, M. G. (1990) *Trends Pharmacol. Sci.* **11**, 190–194.
9. Hausdorff, W. P., Caron, M. G. & Lefkowitz, R. J. (1990) *FASEB J.* **4**, 2881–2889.
10. Dohlmann, H. G., Thorner, J., Caron, M. G. & Lefkowitz, R. J. (1991) *Annu. Rev. Biochem.* **60**, 653–688.
11. Arriza, T. M., Dawson, J. L., Simerly, R. B., Martin, L. J., Caron, M. G., Snyder, S. H. & Lefkowitz, R. J. (1992) *J. Neurosci.*, in press.
12. Anholt, R. R. H., Aebi, U. & Snyder, S. H. (1986) *J. Neurosci.* **6**, 1962–1969.
13. Chen, Z., Pace, U., Heldman, J., Shapira, A. & Lancet, D. (1986) *J. Neurosci.* **6**, 2146–2154.
14. Lohse, M. J., Benovic, J. L., Caron, M. G. & Lefkowitz, R. J. (1990) *J. Biol. Chem.* **265**, 3202–3209.
15. Breer, H. & Boekhoff, I. (1991) *Chem. Senses* **16**, 19–29.
16. Benovic, J. L., DeBlasi, A., Stone, W. C., Caron, M. G. & Lefkowitz, R. J. (1989) *Science* **246**, 235–240.
17. Benovic, J. L., Onorato, J. J., Arriza, J. L., Stone, W. C., Lohse, M., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., Caron, M. G. & Lefkowitz, R. J. (1991) *J. Biol. Chem.* **266**, 14939–14946.
18. Hausdorff, W. P., Bouvier, M., O'Dowd, B. F., Irons, G. P., Caron, M. G. & Lefkowitz, R. J. (1989) *J. Biol. Chem.* **264**, 12657–12665.
19. Sibley, D. R., Benovic, J. L., Caron, M. G. & Lefkowitz, J. (1987) *Cell* **48**, 913–922.
20. Shear, M., Insel, P. A., Melmon, K. L. & Coffino, P. (1976) *J. Biol. Chem.* **251**, 7572–7576.
21. Jones, D. T. & Reed, R. R. (1989) *J. Biol. Chem.* **262**, 14241–14249.
22. Bakalyar, H. A. & Reed, R. R. (1990) *Nature (London)* **250**, 1403–1406.
23. Kühn, H. & Dreyer, W. J. (1972) *FEBS Lett.* **20**, 1–6.
24. Bownds, M. D., Dawes, J., Miller, J. & Stahlman, M. (1972) *Nature (London)* **237**, 125–127.
25. Cohen, P. (1985) *Eur. J. Biochem.* **151**, 439–448.
26. Stryer, L. (1986) *Annu. Rev. Neurosci.* **9**, 87–119.