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Two Second Messengers Mediate Amino Acid Responses in Olfactory Sensory Neurons of the Salamander, *Necturus maculosus*

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Abstract

Odor transduction mediated by the adenylyl cyclase/cAMP pathway has been well studied, but it is still uncertain whether this pathway mediates the transduction of all odors in vertebrates. We isolated olfactory sensory neurons from the salamander *Necturus maculosus* and used calcium imaging with the indicator dye fura-2 to examine olfactory responses elicited by amino acids. The properties of approximately two-thirds of the odor responses suggested they were mediated by the adenylyl cyclase/cAMP pathway, but one-third of the responses were not mimicked by cAMP analogs nor blocked by inhibition of adenylyl cyclase, suggesting that these odor responses were mediated differently. Responses that were unaffected by inhibition of adenylyl cyclase were blocked by neomycin, an inhibitor of phospholipase C, implying that they were transduced by activation of phospholipase C. Some cells which responded to more than one amino acid appeared to employ both pathways, but each was used to transduce different odors. In addition, many responses that were mediated by the adenylyl cyclase/cAMP pathway were enhanced following inhibition of phospholipase C, suggesting that the phospholipase C pathway has a role not only in odor transduction, but also in the modulation of olfactory responses.

Introduction

Odor responses are transduced by G-protein-coupled olfactory receptor proteins (ORs) that regulate intracellular biochemical pathways in olfactory sensory neurons (OSNs) (Schild and Restrepo, 1998). In addition, odor responses can be modulated through the actions of G-protein-coupled receptors, for example by neuroactive compounds such as hormones (Kawai *et al.*, 1999; Eisthen *et al.*, 2000; Wirsig-Wiechmann *et al.*, 2001). In invertebrate OSNs, at least two different intracellular pathways mediate odor transduction; one uses adenylyl cyclase (AC) and its product cyclic adenosine monophosphate (cAMP) (Michel and Ache, 1992), the other phospholipase C (PLC) and its product inositol 1,4,5-trisphosphate (IP_3) (Fadool and Ache, 1992). By comparison, odor transduction in vertebrate OSNs is generally thought to be mediated by the AC/cAMP pathway alone (Gold, 1999). Although PLC and its product IP_3 have been implicated in odor transduction in vertebrates such as fish (Huque and Bruch, 1986; Restrepo *et al.*, 1990, 1993; Miyamoto *et al.*, 1992), amphibians (Kashiwayanagi, 1996) and mammals (Boekhoff *et al.*, 1990; Rawson *et al.*, 1997; Lischka *et al.*, 1999), the activation of PLC in response to odor may be indirect, with modulation of the odor response rather than transduction as its consequence. A critical test of this issue has been difficult. The goal of this study was to examine how odor responses in the salamander, *Necturus maculosus*, depend on the AC/cAMP and PLC pathways. *Necturus* is a neotenic amphibian that reaches lengths of 35–50 cm as an adult and has cells that are considerably larger than those found in mammals or other vertebrates.

In most cases, odors produce depolarizing receptor potentials that open voltage-gated Na^+ and Ca^{2+} channels to elicit action potentials. In *Necturus* voltage-gated Ca^{2+} channels of OSNs are located on the dendrite and cell body, allowing Ca^{2+} influx through these channels to elevate intracellular [Ca^{2+}] throughout the cell. In addition, cytoplasmic [Ca^{2+}] can rise in OSNs when cAMP-gated cation channels in ciliary membranes or IP_3-gated cation channels are opened. As a result, Ca^{2+} is an effective reporter of depolarizing odor responses.

Using imaging techniques with the Ca^{2+}-sensitive dye, fura-2, and OSNs isolated from *Necturus*, we find evidence that both the AC/cAMP and PLC pathways are used to transduce odor responses. In addition, our data indicate that these pathways can interact and that the PLC pathway can modulate the activity of the other.

Materials and methods

Adult *N. maculosus* were obtained from commercial vendors and housed in aerated aquarium tanks at 10°C. The nasal

tissues were removed by blunt dissection from animals that had been chilled on ice and decapitated. Olfactory sensory neurons were dissociated from the tissue without proteolytic enzymes as described previously (Dionne, 1992). Briefly, the olfactory epithelium was peeled from underlying connective tissue and cut into small pieces in amphibian physiological saline (APS). The pieces were then incubated in a low [Ca^{2+}] saline for 18 min and placed in normal extracellular saline with DNase for 10 min, then gently swirled. After swirling and allowing 10–30 s for debris to settle, isolated OSNs could be found in the supernatant. The supernatant was plated onto Cell-Tak (Collaborative Biochemical Products) or concanavalin-A-coated dishes, where the cells were allowed 20 min to settle and adhere to the substrate. The saline was then replaced with fura-2 loading solution containing 5 μM fura-2AM plus 0.02% pluronic F-127 for 10–30 min, after which the cells were washed with APS (10 min) and used for experimentation. Loaded cells were viewed with a compound microscope (either a Nikon Diaphot or an Olympus BX) and imaged through a ×40 objective. The chamber was continuously washed with APS during recording. Stimuli were dissolved in APS and delivered as a 10–30 s pulse with a Warner fast step perfusion system. A cooled CCD camera (Astrocam Model TE3/A/S) was used to collect 200 ms paired images at 350 and 380 nm wavelengths at 4 s intervals. Differential imaging was controlled with a Spectramaster imaging system using Merlin software (Life Science Resources, Cambridge, UK) to calculate image ratios, calibrate and measure calcium concentration and display images both on- and off-line. The absolute Ca^{2+} concentrations are estimates; all conclusions about odor responses are based on relative changes.

Necturus OSNs respond to amino acids, bile salts and various other soluble compounds as odorants. Twelve amino acids were used as chemical stimuli in these experiments (Table 1). All amino acids were the L-form, dissolved in APS and applied at concentrations from 10 μM to 10 mM. Cells were also stimulated with the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX), several analogs of cAMP [8-bromo-adenosine 3′,5′-cyclic monophosphate (8-br-cAMP); 8-(4-chlorophenylthio)-adenosine 3′,5′-cyclic monophosphate (8-cpt-cAMP); N^6,2′-O-dibutyryladenosine 3′,5′-cyclic monophosphate (db-cAMP)] and the forskolin analog L-858051 [7-deacetyl-7-[O-(*N*-methylpiperazino)-γ-butyryl]-forskolin] to activate adenylyl cyclase. In some experiments cells were exposed to an adenylyl cyclase inhibitor, *cis*-*N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine (MDL-12,330A), or a phospholipase inhibitor, neomycin. The pH of all solutions was adjusted to 7.2 with NaOH, except for choline-APS where Tris base was used. Fura-2AM and pluronic F-127 were purchased from Molecular Probes. IBMX, MDL-12,330A and L-858051 were purchased from Calbiochem-Novabiochem Corp., as were the cAMP analogs. All other chemicals including neomycin sulfate and the amino acids were purchased from Sigma Chemical Corp.

Table 1 Numbers of cells (*n*) showing a Ca^{2+} response to the indicated stimulus, together with the number tested (*N*)

Stimulus	Percentage of responsive OSNs (*n*/*N*)
Arginine* (Arg)	10 (4/62)
Asparagine* (Asn)	18 (11/62)
Glutamine (Gln)	12 (2/12)
Histidine* (His)	18 (11/62)
Leucine (Leu)	7 (2/28)
Lysine* (Lys)	4 (1/28)
Proline* (Pro)	15 (9/62)
Taurine (Tau)	29 (22/76)

Most responses involved an increase in [Ca^{2+}]. Stimuli which also elicited decreases in [Ca^{2+}] are indicated by an asterisk. Only one cell responded to lysine, and it showed a decrease [Ca^{2+}]. Also tested were alanine, methionine, valine, serine, and crude minnow extract, but no responses to these stimuli were observed.

Extracellular bath solutions were as follows. APS contained (in mM): NaCl, 112; HEPES [*N*-(2-hydroxyethyl)-piperazine-*N*′(2-ethanesulfonic acid)], 3; KCl, 2; $CaCl_2$, 8; glucose 5. K-APS contained (in mM): NaCl, 12; HEPES, 3; KCl, 102; $CaCl_2$, 8; glucose, 5.

Results

The dissociated OSNs selected for testing appeared to be intact except for their axons, which had been severed; the somata were ovoid and the dendritic endings each held a tuft of cilia. Dendrites varied in length from ~15 to 100 μm. OSNs were deemed viable if their resting intracellular [Ca^{2+}] was in the range of 20–100 nM before testing. Cells were tested first with a puff of K-APS to depolarize them and verify an ability to generate a transient rise in intracellular [Ca^{2+}]; unresponsive cells were discarded. Chemosensitivity was tested by applying one or more amino acids individually. Although 12 amino acids were used in this study, not all cells were tested with all stimuli. When a cell was seen to respond to one or more odorants, it was treated with and/or tested with pharmacological agents to examine the properties of the intracellular pathways that mediated the odor responses. These results are based on recordings from >330 OSNs.

Seventy-six OSNs responded to at least one amino acid with an increase in intracellular [Ca^{2+}] (Figure 1). Typically, responses rose quickly to a peak concentration, returned to baseline with washing and were reproducible (Figure 2A). For most cells used in this study, only one amino acid was identified that elicited a response; this is unlikely to reflect the full range of the cells' chemosensitivity because most testing was stopped once an adequate stimulus was found. Fourteen of 41 OSNs that were tested with multiple amino

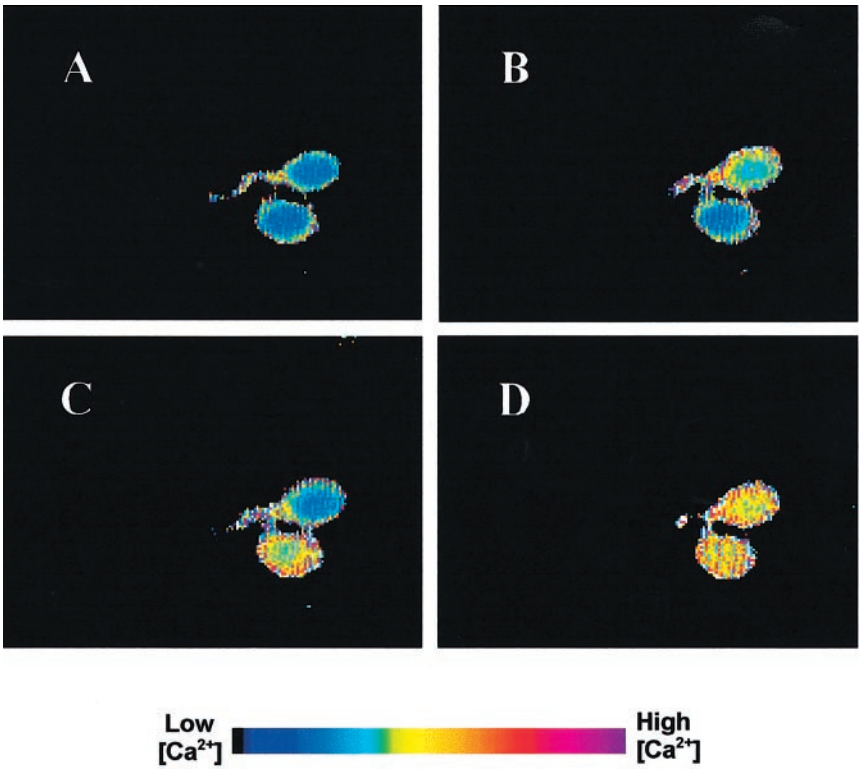

Figure 1 Calcium responses as pseudo-color images recorded from a pair of OSNs that were exposed successively to several amino acids. Although the somata and dendrites are apparent, the fine structure of the cells was not resolved because of the large pixel size. The images in panels B, C and D were recorded 12–16 s after the beginning of the stimulus puff. Additional data from these cells are shown in Figure 5. **(A)** Baseline responses recorded in the absence of stimulus. **(B)** The upper cell responded to proline. **(C)** The lower cell responded to asparagine. **(D)** Both cells responded to taurine.

acids responded to two or more compounds, but no cell tested with multiple amino acids responded to all the test stimuli. Eight OSNs showed a decrease in $[Ca^{2+}]$ (Figure 2B) rather than an increase in response to a stimulus; most of these cells (7/8) also showed an increase in $[Ca^{2+}]$ in response to other stimuli. Five different amino acids elicited $[Ca^{2+}]$ decreases (Table 1) and all but one (lysine) also elicited $[Ca^{2+}]$ increases in other cells. $[Ca^{2+}]$ decreases were difficult to characterize because of their small headroom (the resting $[Ca^{2+}]$ was already low) and their low incidence; they will not be discussed further. Finally, many apparently healthy cells responded only to K-APS but not to any of the test stimuli, suggesting that an adequate stimulus for those cells had not been included or that their responses did not involve changes in intracellular Ca^{2+}.

Involvement of the AC/cAMP pathway

OSNs that responded to one or more amino acids were used to test the involvement of the AC/cAMP pathway in the response.

Increased [cAMP]

In 37 of 60 cells (62%) that responded with a $[Ca^{2+}]$ increase to one or more amino acids, the Ca^{2+} response was mimicked by elevating intracellular cAMP or by applying a

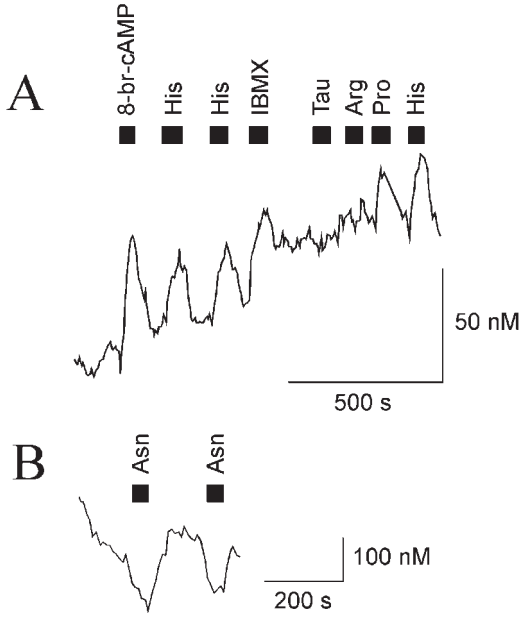

Figure 2 Calcium changes elicited by amino acids in OSNs. **(A)** The typical chemosensory response was an increase in intracellular $[Ca^{2+}]$. This cell responded to histidine and proline, but not taurine or arginine. The responses were mimicked by the cAMP analog 8-br-cAMP and the phosphodiesterase antagonist IBMX. **(B)** Rare chemosensory responses showed a decrease in intracellular $[Ca^{2+}]$. This is a different cell from that in panel A.

cAMP analog (Figure 2A); however, the responses in 23 of the 60 cells (38%) could not be mimicked this way.

Three membrane-permeant analogs of cAMP along with IBMX were used to stimulate cAMP-dependent responses (Firestein *et al.*, 1991). Db-cAMP, 8-br-cAMP and 8-cpt-cAMP were tested at concentrations of 10–1000 μM. All three compounds were able to elicit Ca^{2+} responses, but none were as effective as IBMX. IBMX was also tested at concentrations of 10–1000 μM. In most cells the Ca^{2+} responses elicited by IBMX did not increase monotonically with concentration. Intermediate concentrations of IBMX (50 and 100 μM) frequently elicited the largest increases in [Ca^{2+}] (Figure 3A), but the most potent concentration of IBMX varied among individual OSNs. The cause of the non-linear dose–response relation was unclear. Although the magnitude of the Ca^{2+} response showed adaptation by decreasing with repeated applications of IBMX, the non-monotonic dose–response relation did not appear to be a consequence of adaptation. The Ca^{2+} responses induced by IBMX required bath Ca^{2+}, indicating that they were mediated by an increase in the Ca^{2+} permeability of the membrane (Figure 3B). Similarly, the amino acid-induced Ca^{2+} responses required extracellular Ca^{2+} (not shown).

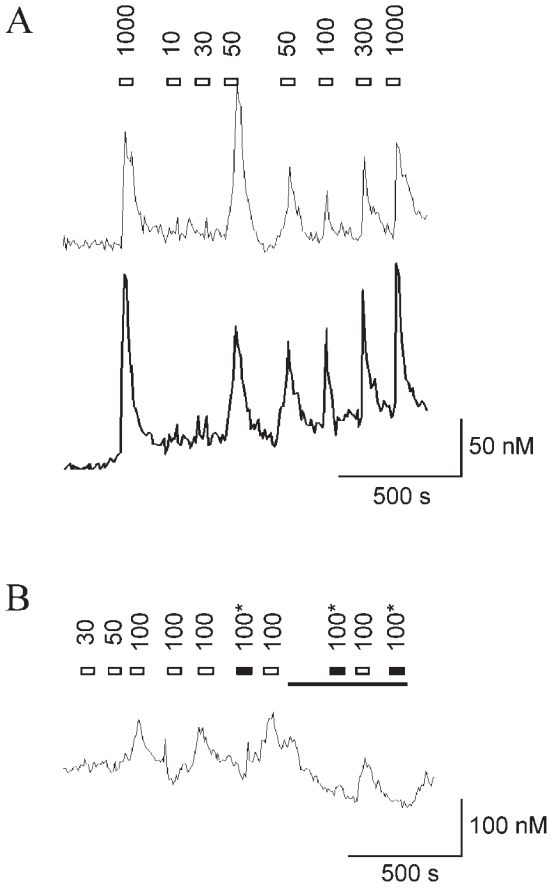

Figure 3 Ca^{2+} responses elicited by IBMX (concentrations are micromolar). **(A)** Responses elicited by IBMX from two OSNs recorded simultaneously. The traces were offset for clarity. Some cells responded in a relatively graded way (bottom trace), but the responses from most cells were not monotonically dose-dependent (top trace). This suggests either that IBMX may have effects in addition to blocking phosphodiesterase, or that intracellular Ca^{2+} may affect the response, possibly through AC activity or the Ca^{2+} transport mechanism. **(B)** Extracellular Ca^{2+} was required for the Ca^{2+} response to IBMX. The cell was tested with IBMX dissolved either in normal APS or Ca^{2+}-free APS (indicated by an asterisk); during the marked interval at the end of the record, the APS bath was exchanged for Ca^{2+}-free APS. Ca^{2+} responses were elicited by 100 μM IBMX only when the stimulus medium contained Ca^{2+}.

Inhibition of adenylyl cyclase

A membrane-permeant AC inhibitor was used to test whether OSNs needed the ability to generate cAMP to produce odor-elicited Ca^{2+} responses. As described below, cells that initially responded to one or more amino acid were treated with MDL-12,330A (Guellaen *et al.*, 1977) to inhibit AC, then retested. Of 14 OSNs tested in this way, inhibition of AC blocked the Ca^{2+} responses in nine cells (64%), but was ineffective in five (36%), indicating that cAMP is necessary for transducing some but not all odor responses.

An example of an OSN whose amino acid-induced Ca^{2+} response was blocked following inhibition of AC is shown in Figure 4. During initial testing, the cell responded to histid-

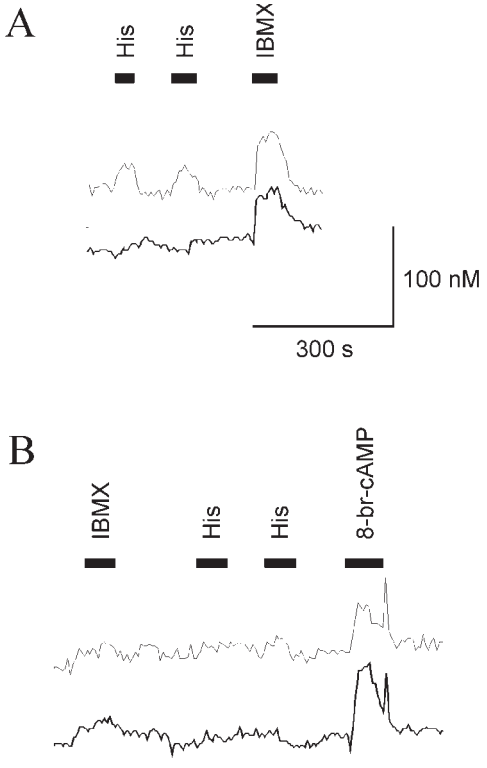

Figure 4 Block of amino acid induced responses by inhibition of AC. The Ca^{2+} responses from two cells are shown before and following treatment with MDL-12,330A to inhibit AC. **(A)** Cell 1 (lighter line), but not cell 2 (heavier line), responded to histidine (100 μM); both cells responded to the phosphodiesterase inhibitor IBMX (400 μM). **(B)** After treatment with MDL-12,330A (50 μM, 12 min), the histidine and IBMX responses in both cells were blocked. Despite the treatment, both cells remained responsive to 8-br-cAMP.

ine and the response was mimicked by IBMX; a second cell tested simultaneously responded to IBMX only. Following incubation in MDL-12,330A, the responses to histidine and IBMX were eliminated in both cells, indicating their dependence on cAMP. Nevertheless, 8-br-cAMP was still able to elicit an increase in [Ca^{2+}] in both cells, demonstrating that the elements of the AC/cAMP transduction pathway downstream of the cyclase remained functional.

In some OSNs that responded to multiple amino acids, inhibition of AC by MDL-12,330A blocked some but not all responses. Two such cells that were recorded from simultaneously are shown in Figure 5. The initial chemosensitivity of these cells differed, with one cell responding to histidine, proline and taurine and the other responding to asparagine and taurine. Following treatment with MDL-12,330A, only the taurine responses remained. This suggests that the taurine responses were mediated by an intracellular pathway different from the AC/cAMP pathway.

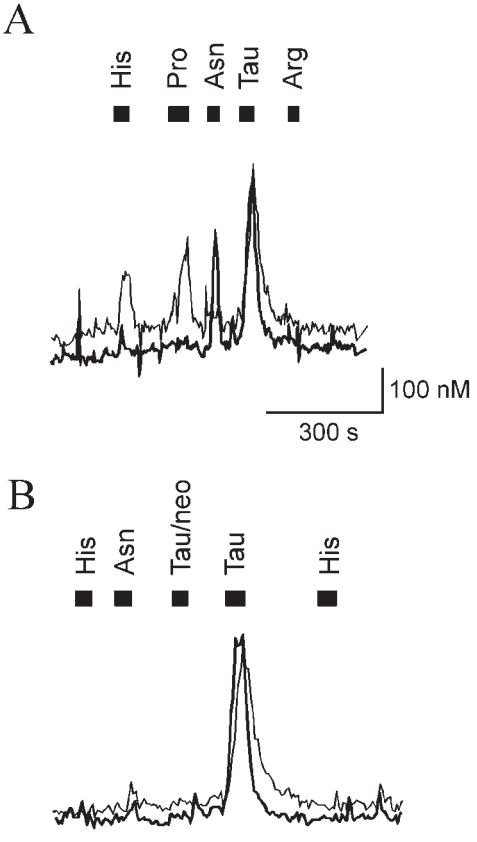

Figure 5 Block of amino acid responses by inhibitors of AC and PLC. The Ca^{2+} responses from two cells are shown before and following treatment with the AC inhibitor MDL-12,330A. **(A)** The cells were screened initially with five amino acids. Cell 1 (lighter line) responded to histidine, proline and taurine, while cell 2 (heavier line) responded to asparagine and taurine. **(B)** Following treatment with MDL-12,330A, the responses to histidine and asparagine were blocked (proline not tested), but the responses to taurine in both cells were largely unaffected. When taurine was co-applied with neomycin, a PLC inhibitor, the taurine responses were reversibly blocked.

Involvement of PLC

Inhibition of AC failed to block the Ca^{2+} responses elicited by one or more amino acid in 5 of the 14 cells tested. To determine whether odor responses that were insensitive to AC inhibition might be sensitive to inhibition of the PLC pathway, the PLC inhibitor neomycin was used. Neomycin (1 mM) was dissolved in APS and co-applied at neutral pH with amino acids. Figure 5B shows two cells whose responses to taurine persisted after treatment with MDL-12,330A to inhibit AC. The taurine responses in both cells were reversibly blocked by neomycin. This indicates that the taurine responses in these cells were not transduced by the AC/cAMP pathway and it suggests that the responses may have been transduced by the PLC pathway.

Thirty OSNs gave useful data with neomycin. By itself, neomycin caused no Ca^{2+} responses (not shown), but it blocked Ca^{2+} responses that were regarded as cAMP-independent. Most of the cAMP-independent responses were elicited by taurine; in six of seven taurine-responsive cells, taurine responses were unaffected by the AC inhibitor MDL-12,330A, while one was blocked. In contrast, the taurine responses in 14 of 15 taurine-responsive OSNs were blocked by neomycin; this included six cells pretreated with MDL-12,330A and eight cells not pretreated; the latter group indicate that neomycin's effect was independent of AC inhibition. Neomycin also blocked a response to leucine in one cell. To demonstrate that the blocking action of neomycin was not a non-specific effect, it was tested on cAMP-dependent responses elicited by IBMX and amino acids in cells that had not been treated with MDL-12,330A. Neomycin failed to block the responses to IBMX in 11 cells; in three other cells that individually responded to histidine, arginine or asparagine, the responses were both insensitive to neomycin and mimicked by IBMX or IBMX plus the forskolin analog L-858051 (see below). Taken together, these data suggest that neomycin selectively blocked cAMP-independent responses and failed to block responses mediated by the cAMP/AC pathway. These effects of neomycin are consistent with the idea that some but not all of the amino-acid-induced responses were transduced using the PLC pathway.

Interactions between the AC/cAMP and PLC pathways

Of the three cells noted above in which neomycin failed to block the amino acid responses, the responses in two cells appeared to be potentiated by neomycin. An example is shown in Figure 6; not only was the histidine response in Figure 6A potentiated by neomycin, but it was mimicked by IBMX plus 30 μM forskolin analog L-858051, suggesting that while the response to histidine was transduced by the AC/cAMP pathway, it was modulated by the PLC pathway. To study modulation of the AC/cAMP pathway by PLC, we examined how neomycin affected Ca^{2+} responses elicited directly by IBMX. The use of IBMX preserved the AC/

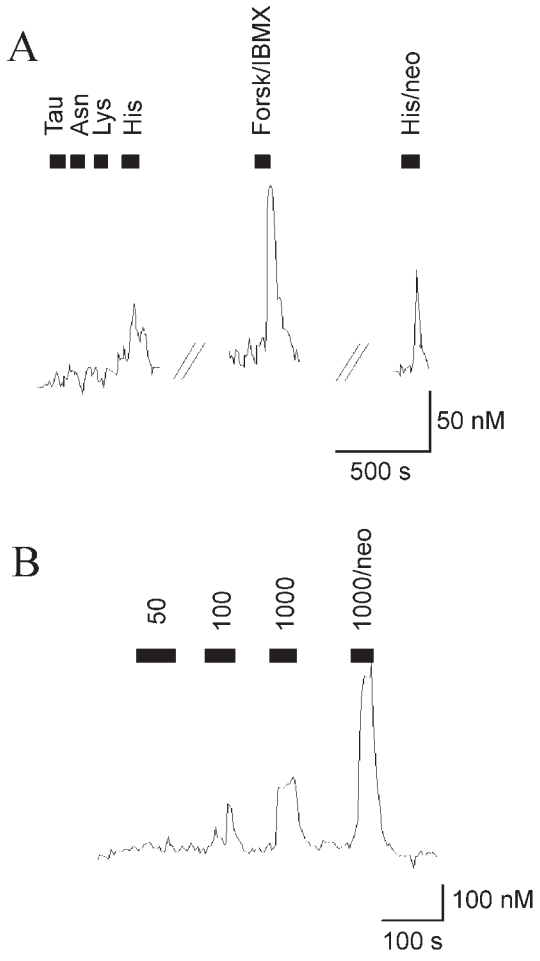

Figure 6 Modulation of AC/cAMP-mediated responses by the PLC pathway. **(A)** Responses from a cell that was sensitive to histidine but not taurine, asparagine or lysine. The forskolin analog L-858051 plus IBMX elicited a large response from the cell. When histidine was applied together with neomycin, the response was larger than for histidine alone. **(B)** Successive responses are shown to 50, 100 and 1000 μM IBMX, followed by a response to 1000 μM IBMX plus neomycin. Neomycin potentiated the IBMX response to >250%.

cAMP pathway intact, but eliminated the need to find amino acids that stimulated it. An example of potentiation of the response to IBMX by neomycin is shown in Figure 6B. Of 11 OSNs studied in this way, neomycin potentiated the IBMX response in eight cells by 2–10-fold and caused a partial inhibition of the response in one cell. These results suggest that the PLC pathway can modulate the AC/cAMP pathway, frequently but not exclusively by suppression. Thus the PLC pathway may serve roles in both the transduction of odor responses and their modulation.

We examined whether the reciprocal form of modulation might also occur by comparing the magnitudes of MDL-12,330A-resistant, amino-acid-induced responses measured before and after treatment with the AC inhibitor. The data were from the five OSNs discussed earlier. Although the responses following treatment with MDL-12,330A were consistently smaller than before treatment, the changes were similar to normal rundown. Thus the data provided no convincing evidence that responses mediated by the PLC pathway were modulated by the AC/cAMP pathway; however, the data are insufficient to definitively rule out this possibility.

Discussion

The importance of the AC/cAMP pathway as a mediator of vertebrate odor transduction is well established; however, the role of the PLC pathway in transduction has been less clear, especially for vertebrates. Some studies have suggested that the PLC pathway can mediate transduction, while others have suggested that it is a modulator of transduction but not a mediator (Zufall and Munger, 2001). This distinction is clear when modulation is caused by a stimulus such as a hormone, but less obvious when both pathways are activated by odor. In this study we have examined the issue using *Necturus* OSNs; the data suggest that both the AC/cAMP and PLC pathways can act in the transduction of odorants and that the PLC pathway also has a role in modulation.

Our approach was to measure changes in intracellular [Ca^{2+}] induced by odorants and use pharmacological tools to detect and distinguish biochemical pathways. While action potentials, not changes in intracellular [Ca^{2+}], are the signals that communicate olfactory information to neurons in the olfactory bulb, Ca^{2+} is a reliable indicator of odor-induced receptor potentials in many if not all OSNs. The Ca^{2+} change begins in the cilia where odors stimulate ORs and it propagates to the cell body as depolarization opens voltage-gated Ca^{2+} channels (Leinders-Zufall *et al.*, 1997, 1998). Thus, while the increase in [Ca^{2+}] throughout the cell is clearly secondary, it reflects the transduction events. The use of drugs to block or stimulate selected pathways allowed us to monitor the differential effects of these changes in individual OSNs, strengthening the evidence of two pharmacologically distinct pathways.

Approximately two-thirds of the amino-acid-induced Ca^{2+} responses in *Necturus* OSNs were blocked by inhibition of the AC/cAMP pathway and a similar portion of responses were mimicked either by a cAMP analog or stimulation of AC. These results must be interpreted together, for mimicry alone, while consistent with transduction by the AC/cAMP pathway, cannot be distinguished from responses mediated by alternate pathways. The combined results support both the necessity and sufficiency of the AC/cAMP pathway in the transduction of the majority of olfactory responses. In addition, one-third of the odor responses were unaffected by AC inhibition or cAMP stimulation, suggesting that at least one other intracellular biochemical pathway is used for odor transduction. While these latter results might be expected if the concentrations of antagonists/agonists were too low to elicit effects in the unaffected cells, the observation of OSNs

with heterogeneous responses makes this an unlikely explanation. OSNs with heterogeneous responses had one or more amino acid responses blocked by inhibition of AC, while the responses to other amino acids were unaffected. This provided evidence of the efficacy of the AC inhibitor, despite its inability to block all responses. The results from OSNs with heterogeneous responses not only support the main conclusion that multiple biochemical pathways are used for odor transduction, but also suggest that more than one transduction pathway operates in some OSNs. These data do not, however, allow us to determine whether multiple transduction pathways occur in all OSNs.

Our data suggest that some, if not all, of the amino acid responses not mediated by the AC/cAMP pathway may be transduced through the PLC pathway. Neomycin was an effective blocker of responses that were insensitive to inhibition of the AC/cAMP pathway and neomycin is a potent blocker of PLC (Striggow and Bohnensack, 1994). Taurine responses, in particular, were blocked by neomycin. In agreement with earlier reports (Dubin and Dionne, 1993), taurine was the most effective of the amino acids tested in eliciting responses from *Necturus* OSNs (29% of cells, Table 1). While it may be tempting to think that the amino acids taurine and leucine, whose responses were blocked by neomycin, might be transduced only through a PLC pathway, that seems unlikely; both amino acids gave responses in other cells that were insensitive to neomycin, blocked by inhibition of AC, or mimicked by cAMP analogs. These odorants and possibly others may be transduced by activating different ORs, one type linked to the AC/cAMP pathway, the other to the PLC pathway. Alternatively, their receptors may couple to both pathways. This latter possibility, however, seems inconsistent with data from several taurine-responsive cells in which both pathways were present and mediated the transduction of different amino acids, but neomycin and MDL-12,330A appeared to affect separate amino acid responses and were not additive. This apparent diversity of odor response mechanisms could explain an earlier report (Chen *et al*., 2000) that blocking AC in dissociated salamander OSNs inhibited responses to odors that were assumed to activate only the PLC pathway.

Besides being a non-specific PLC inhibitor, neomycin can also block some types of Ca^{2+} channels, especially in mammalian muscle and brain preparations (Perrier *et al.*, 1992; Langton *et al.*, 1996). If neomycin blocked voltage-dependent Ca^{2+} channels in *Necturus* OSNs, that could explain the disruption of odor-elicited Ca^{2+} responses that we observed. However, several observations suggest that the action of neomycin in these experiments was not produced by channel block. First, neomycin inhibited only a subset of the Ca^{2+} responses elicited by amino acids. If neomycin were blocking Ca^{2+} channels, it should inhibit all odorant-elicited responses. Second, rather than inhibiting some Ca^{2+} responses, neomycin potentiated them. This is inconsistent with channel block, but could be explained if inhibition of PLC relieved suppression of the AC/cAMP pathway. Finally, Ca^{2+} is reported to compete with neomycin in the block of Ca^{2+} channels (Parsons *et al.*, 1992; Langton *et al.*, 1996); block is rapidly reduced as the $[Ca^{2+}]$ is increased above 1 mM. In the experiments described here, we used 8 mM Ca^{2+}, a concentration that effectively minimizes neomycin block in the preparations where it occurs. It should also be noted that the mechanism of Ca^{2+} channel block by neomycin is unclear. Most of the reports on this subject are consistent with either a direct action in which neomycin might occlude the channel pore, or an indirect action mediated by inhibition of PLC.

It has been demonstrated (Frings, 1993) that factors affecting protein kinase C, one of the enzymes regulated by the PLC pathway, can enhance or attenuate the AC/cAMP pathway by affecting AC performance. More recently, Gomez *et al*. (Gomez *et al*., 2000a,b) reported modulation of odor-induced Ca^{2+} responses in rat and human OSNs by protein kinases, where an inhibitor of protein kinase C potentiated AC/cAMP-mediated odor responses and an inhibitor of cAMP-dependent protein kinase A potentiated PLC-mediated responses. Our data suggest that the PLC pathway can modulate responses transduced by AC/cAMP in *Necturus* OSNs, similar to the results of Gomez *et al*., but we saw no evidence that the AC/cAMP pathway modulated PLC-mediated responses elicited by amino acids in these cells. Inhibition of the PLC pathway led to larger amino-acid-elicited Ca^{2+} responses in several cells. When this effect was examined using IBMX to stimulate responses, it appeared that the Ca^{2+} responses in most OSNs were potentiated after PLC was inhibited, suggesting that this form of modulation is widespread if not ubiquitous. Interactions between these pathways may underlie the non-monotonic dose–response relations seen using IBMX as a stimulus. Modulation of olfactory responses *in vivo* may be produced by neuroactive compounds such as hormones or neuropeptides. Nevertheless, the observations that the PLC pathway may also be involved in the transduction of certain odorants and that transduction events mediated by this and the AC/cAMP pathway can be observed in the same OSNs, suggest that complex interactions between the pathways during odor transduction may occur in some cells. In these cells, stimulation of the PLC pathway by an odorant could modulate transduction events mediated by the AC/cAMP pathway. The consequences would affect odor perception if the affected cells were sensitive to certain stimuli, for then the sensitivity of the olfactory system to particular odors could be selectively enhanced or suppressed.

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