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Second Messenger Systems Mediating Sex Pheromone and Amino Acid Sensitivity in Goldfish Olfactory Receptor Neurons

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Introduction

Teleost fish have three morphologically different types of olfactory receptor neurons (ORNs), ciliated, microvillous and crypt cells, which employ either adenylate cyclase (AC)/cyclic-AMP (cAMP) or phospholipase C (PLC)/inistol triphosphate (IP_3) as second messenger systems (Sorensen and Caprio, 1998; Hansen *et al.*, 2003). The chemical sensitivities and functions of these cell types are poorly understood. For example, EOG recording using forskolin and U-71322 as adapting solutions has suggested that sensitivity to amino acids is mediated by at least two classes of neurons, one employing AC/cAMP, another PLC/IP_3 (Rolen *et al.*, 2002; Hansen *et al.*, 2003). The present study used a combination of single-unit and EOG recording together with pharmacological agents to further elucidate the roles of AC and PLC pathways mediating responses to amino acid (food) odors and sex pheromones in goldfish ORNs.

Materials and methods

Sexually mature, male goldfish were anaesthetized, immobilized and their olfactory epithelia exposed. Single-unit activity was simultaneously recorded using metal filled glass electrodes tip plated with a platinum-black ball while EOG was recorded using gelatin-filled electrodes. For odorants we employed a mixture of four representative L-amino acids (AAs; 10^{-4} M arginine, 10^{-4} M methionine, 10^{-4} M alanine, 10^{-4} M glutamic acid), a mixture of sex steroids (SS; 10^{-9} M 17,20β-dihydroxy-4-pregnen-3-one, 10^{-8} M 17,20β-dihydroxy-4-pregnen-3-one-20-sulfate; 10^{-8} M androstenedione) and a mixture of F prostaglandins (PGFs; 10^{-8} M prostaglandin $F_{2\alpha}$, 10^{-8} M 15-keto-prostaglandin $F_{2\alpha}$, 10^{-8} M 13,14-dihydro-prostaglandin $F_{2\alpha}$). Amino acids are feeding cues in goldfish while SS and PGFs function as different sex pheromones (Rolen *et al.*, 2002; Stacey and Sorensen, 2002). We also examined the effects of five pharmacological modulators of signal transduction on ORN activity: forskolin (AC activator), MDL-12,330A (AC inhibitor), IBMX (phosphodiesterase inhibitor), *m*-3M3FBS (PLC activator; Bae *et al.*, 2003), imipramine (putative PLC and IP_3-gated channel activator; Cadiou and Molle, 2003), U-73122 (PLC inhibitor) and niflumic acid (Cl^- channel blocker; Sato and Suzuki, 2000).

Results and discussion

Amino acids and pheromones detection by distinct ORNs

We screened the olfactory responsiveness of 108 individual ORNs to amino acid and sex pheromones using single-unit recording. Amino acids were detected by different ORNs than those which detected sex pheromones (Sato and Sorensen, 2003).

The AC/cAMP pathway in ORNs

Although forskolin is a potent and useful AC activator, it has also been shown to activate ion channels and other signal cascades. Hence, we used single unit recording to confirm that forskolin activates AC/cAMP pathways. Forskolin evoked excitation in 48 of the 90 goldfish ORNs we tested. IBMX was without effect on these forskolin-sensitive neurons except when tested immediately after forskolin application ($n = 3$; Figure 1), thereby demonstrating both production and accumulation of cAMP. We found about one-third (16/48) of all forskolin-sensitive ORNs responded to amino acids and 10% to SS or PGFs (Sato and Sorensen, 2003). Confirming the role of AC/cAMP in goldfish ORNs, we found that although 2 min prior exposure to MDL-12,330A had no effect on EOG responses to AAs, responses to both SS and PGFs were greatly reduced ($P < 0.05$), in a concentration-dependent manner (Figure 2).

The PLC/IP_3 pathway and imipramine in ORNs

Although *m*-3M3FBS did not elicit an EOG response (data not shown), imipramine was a potent activator of the EOG (Figure 3). Confirming the role of PLC/IP_3 in ORNs, we found EOG responses to imipramine and all three test odors were strongly suppressed when the epithelium was exposed to niflumic acid for 2 min (Figure 3).

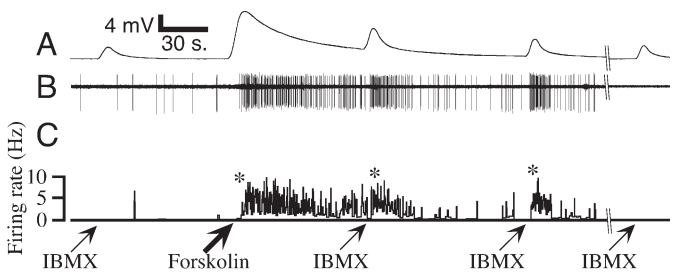

Figure 1 Effects of forskolin and IBMX on a representative ORN. **(A)** EOG responses. Top bars indicate time and magnitude. **(B)** Single-unit activity. **(C)** ORN firing rates. Five second pulses of 10^{-5} M forskolin and 10^{-4} M IBMX are noted. Asterisks denote increased firing rate ($P < 0.05$).

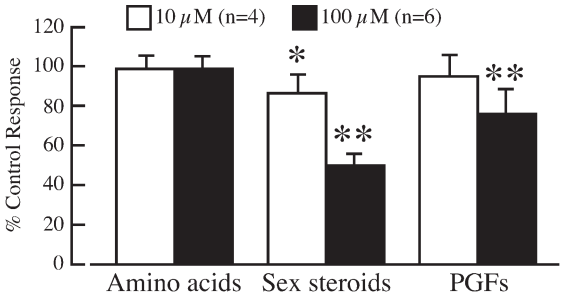

Figure 2 Effects of MDL-12,3330A on EOG responses to odors. Effects of this inhibitor are expressed as mean percentage inhibition ± standard error ($n = 10$). *$P < 0.05$; **$P < 0.01$.

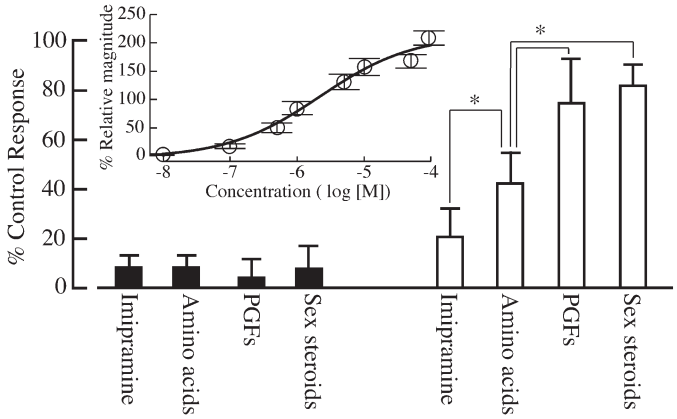

Figure 3 Summary of the effects of 1 mM niflumic acid (black bars) and 10 μM U-73122 (white bars) on EOG responses. The concentration–response curve of EOG to imipramine (relative to 10^{-4} M L-serine) is shown (inset). The inhibitory effect of these compounds is expressed as mean percentage inhibition ± standard error. The effect of U-73122 on imipramine and amino acids was much stronger than that to other odors (ANOVA; $*P < 0.01$).

Further, while EOG recording also showed U-73122 to strongly suppress responses to imipramine and AAs ($P < 0.01$), it had only very weak effects on SS and PGFs ($P < 0.05$; Figure 3); apparently some of those ORNs which detect AAs employ PLC.

Conclusions

These results provide further evidence that individual goldfish ORNs employ either AC or PLC/IP_3 pathways. Our data also demonstrate that responses to the PGF and SS pheromones are mediated by different classes of ORNs, both of which employ AC/cyclic-AMP. Responses to AAs (feeding stimuli) appear to be mediated by at least two other types of ORNs, one using AC/ cAMP, the other PLC/IP_3. The latter finding is supported by previous studies which suggest that both microvillar and ciliated ORNs mediate responses to AAs (Rolen *et al.*, 2002; Hansen *et al.*, 2003).

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