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# Metamorphic-Signal Transduction in *Hydroides elegans* (Polychaeta: Serpulidae) Is Not Mediated by a G Protein

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**Abstract.** Evidence from larvae of hydrozoans, gastropods, and barnacles suggests that G protein-coupled receptors mediate induction of settlement and metamorphosis in response to environmental cues. We examined responses of larvae of the serpulid polychaete *Hydroides elegans* to neuropharmacological agents to determine if G protein-coupled receptors or their associated signal-transduction pathways regulated induction of metamorphosis by bacterial cues. Larvae of *Hydroides elegans* metamorphose rapidly and in high proportions when exposed to bacterial biofilms. Neither the G-protein activator Gpp[NH]p nor the inhibitor GDP-$\beta$-S affected metamorphosis. Although the nonspecific phosphodiesterase inhibitors IBMX, theophylline, and papaverine induced larvae to metamorphose, RO-20-1724 (an inhibitor selective for cAMP-specific phosphodiesterase IV) and the cyclic nucleotide analogs db-cAMP and db-cGMP had no effect on metamorphosis. The adenylate cyclase activator forskolin inhibited responses of larvae to inductive bacterial biofilms. These apparently conflicting results may be due to side effects of IBMX, theophylline, papaverine, and forskolin on ion transport. The phorbol ester TPA, an activator of protein kinase C, also had no effect on larval metamorphosis. These experiments indicate that G protein-coupled receptors and signal transduction by the adenylate cyclase/cyclic AMP or phosphatidyl-inositol/diacylglycerol/protein kinase C pathways are not components of the morphogenetic pathway that is directly responsible for processing metamorphic cues in *H. elegans*.

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*Abbreviations*: AC/cAMP, adenylate cyclase/cyclic AMP; db-cAMP, dibutyryl-cyclic AMP; db-cGMP, dibutyryl-cyclic GMP; DMSO, dimethyl sulfoxide; FSW, filtered seawater; GDP-$\beta$-S, guanosine 5′-*O*-(2-thiodiphosphate); Gpp[NH]p, 5′-guanylylimidodiphosphate; IBMX, 3-isobutyl-1-methylxanthine; PI/DAG/PKC, phosphatidylinositol/diacylglycerol/protein kinase C; TPA, phorbol-12-myristate-13-acetate.

## Introduction

Several decades of research have demonstrated the importance of biochemical and physical cues in determining, on small spatial scales, where larvae of marine invertebrates settle and metamorphose (see Scheltema, 1974; Crisp, 1984; Svane and Young, 1989, for reviews). Identification of the exact nature and ecological role of exogenous cues remains an active field of study. Over the last 20 years, however, increased attention has focused on the physiological mechanisms underlying detection of these cues and the initiation of metamorphosis. The approach generally taken in this work consists of exposing larvae to solutions of potentially neuroactive compounds, in the presence or absence of a cue, and noting the responses. Although such experiments may be difficult to interpret (Pawlik, 1990; Leitz, 1997), a common theme is developing from the results.

In the marine invertebrate larvae that have been most extensively studied, it appears that G protein-coupled receptors and their associated signal-transduction pathways play an important role in regulating metamorphosis. In the hydrozoans *Hydractinia echinata* (Müller, 1985; Leitz and Müller, 1987; Leitz and Klingmann, 1990; Schneider and Leitz, 1994) and *Mitrocomella polydiademata* (Freeman and Ridgway, 1990), the phosphatidylinositol/diacylglycerol/protein kinase C (PI/DAG/PKC) pathway transduces the metamorphic signal provided by bacterial cues. In addition, Leitz and Wirth (1991) found that *H. echinata*

metamorphosed in response to the G-protein activators ortho- and metavanadate, suggesting that G protein-coupled receptors are involved in the process. Schneider and Leitz (1994) proposed that the bacterial inducer of metamorphosis acted by first binding to a G protein-coupled receptor. In the barnacle *Balanus amphitrite*, two signal-transduction systems, the adenylate cyclase/cyclic AMP (AC/cAMP) pathway (Rittschof *et al.*, 1986; Clare *et al.*, 1995) and the PI/DAG/PKC pathway (Yamamoto *et al.*, 1995; Holm *et al.*, unpubl. data), appear to regulate metamorphosis. Clare (1996) proposed a model linking the two pathways; in this model the exogenous metamorphic cue was bound to a G protein-coupled receptor. Finally, in the abalone *Haliotis rufescens*, the AC/cAMP pathway transduces the metamorphic signal provided by compounds found on the surface of crustose coralline algae, while the PI/DAG/PKC pathway facilitates the response to these compounds (Morse *et al.*, 1980; Trapido-Rosenthal and Morse, 1985, 1986; Baxter and Morse, 1987; Morse, 1990, 1991). The receptor of the metamorphic cue did not appear to be associated with a G protein, but evidence suggested that the separate, facilitating pathway was activated by binding of amino acids to a G protein-coupled receptor (Baxter and Morse, 1987, 1992; Wodicka and Morse, 1991).

G protein-coupled receptors and their associated signal-transduction pathways present a compelling general model for detection of a variety of stimuli in an exceptionally broad range of organisms (Carr, 1992). It is not clear, however, whether the regulation of metamorphosis by G proteins is a general feature of marine invertebrate larvae (Hadfield, 1998). For larvae of *Haliotis rufescens*, at least, a G protein-coupled receptor does not bind the metamorphic signal (Baxter and Morse, 1987). There are several other classes of receptors (Féger *et al.*, 1994) that could conceivably function in marine larvae to detect exogenous cues for settlement and metamorphosis. These include lectins, which have been implicated in the metamorphosis of larvae of the spirorbid polychaete *Janua brasiliensis* (Kirchman *et al.*, 1982; Maki and Mitchell, 1985), and ligand-gated ion channels.

To test the hypothesis that G protein-coupled receptors or the AC/cAMP and PI/DAG/PKC signal-transduction pathways regulate metamorphosis in the serpulid polychaete *Hydroides elegans*, we examined the response of larvae of this species to various neuropharmacological agents. *Hydroides elegans* (Haswell, 1883) is a common member of the shallow subtidal fouling community throughout tropical and warm temperate seas (Hadfield *et al.*, 1994). In the laboratory, competent larvae settle and metamorphose after as little as 15 min of exposure to bacterial biofilms (Hadfield *et al.*, 1994; Carpizo-Ituarte and Hadfield, 1998). Experiments with larvae of other polychaetes, including *Capitella capitata* Sp. I (Biggers and Laufer, 1992), *Phragmatopoma lapidosa californica* (Jensen and Morse, 1990; Pawlik, 1990), and *Hydroides elegans* from Hong Kong (Bryan *et al.*, 1997), indicate that the AC/cAMP pathway may be involved in metamorphosis. Our results, however, suggest that G protein-coupled receptors and their associated signal-transduction pathways *do not* play a direct role in regulating metamorphosis in *Hydroides elegans*.

## Materials and Methods

### *Obtaining and culturing larvae*

We collected sexually mature *Hydroides elegans* from the surface of plastic screens previously placed in the water in Pearl Harbor, Hawaii, to serve as a substratum for settlement of larvae and subsequent growth of recruits. Clumps of worms were removed from the screens and placed into dishes of seawater filtered through a 0.22-$\mu$m filter (hereafter, FSW). When we broke open their tubes, ripe male and female worms released gametes, and fertilization took place within minutes (see also Wisely, 1958; Hadfield *et al.*, 1994).

After several hours we separated developing larvae from adult worms and debris by sieving, and transferred the larvae to beakers of FSW so as to realize a density in culture of 5–10 larvae $ml^{-1}$. Larvae were reared at room temperature (25° ± 2° C) and fed daily with *Isochrysis galbana* (Tahitian strain) at a density of about $6 \times 10^4$ cells $ml^{-1}$. Every 2 days, larvae were transferred to fresh FSW. Under these conditions we obtained metamorphically competent larvae 4–6 days after fertilization.

### *Assays for metamorphosis*

We tested for metamorphosis of larvae in response to potentially neuroactive compounds by conducting assays in still water. In these assays larvae were introduced to polystyrene petri dishes (Falcon 1006, 50 × 9 mm; Fisher, 60 × 15 mm) containing 4–5 ml of a solution of the agent of interest. Larvae were added in a volume of FSW between 15 and 25 $\mu$l, yielding from 2 to 113 larvae per dish. Four or five replicate assays for each experimental treatment were conducted at room temperature and, when compounds were light labile, in darkness.

For each experiment, appropriate control treatments were also tested. Controls for metamorphic competence ('Biofilm') consisted of petri dishes containing FSW and a biofilmed surface, either a 1.6-$cm^2$ chip of polystyrene or a piece of plastic screen. We obtained biofilms by soaking these substrata in flowing seawater for at least 5 d. Petri dishes containing only FSW served as negative controls. For experiments in which we examined inhibitory effects, larvae were added to dishes containing both the test solution and a biofilmed surface. Test solutions

were made by diluting stock solutions of the compound of interest in FSW. Stock solutions were prepared by dissolving the compound in either FSW or dimethyl sulfoxide (DMSO). When the stock solution included DMSO, we ran controls for the potential effects of DMSO on metamorphosis.

We scored metamorphosis of larvae after 24 h of continuous exposure to the test compounds. Larvae were considered to have metamorphosed if they had permanently attached to the surface of the petri dish (or the biofilmed substratum); constructed either a primary (proteinaceous) tube or both a primary and a calcareous, secondary tube; and exhibited differentiation of the head region into the branchial crown. Some compounds evoked abnormal metamorphosis in a small proportion of larvae. Such larvae did not construct primary or secondary tubes but did show evidence of metamorphosis, including loss of the prototroch and development of the branchial crown. Except for assays with the phosphodiesterase inhibitor IBMX (see Results section below), we counted these larvae as metamorphosed.

*Neuropharmacological agents*

We tested 10 compounds for their effects on metamorphosis. We chose these compounds to differentiate the roles that G proteins, the AC/cAMP signal-transduction pathway, or the PI/DAG/PKC signal-transduction pathway might play in metamorphosis (Table I). Neuropharmacological agents tested included 5′-guanylylimidodiphosphate (Gpp[NH]p), guanosine 5′-*O*-(2-thiodiphosphate) (GDP-$\beta$-S), 3-isobutyl-1-methylxanthine (IBMX), theophylline, papaverine, RO-20-1724, dibutyryl-cAMP (db-cAMP), dibutyryl-cGMP (db-cGMP), forskolin, and phorbol-12-myristate-13-acetate (TPA). Gpp[NH]p is a nonhydrolyzable GTP analog that activates G proteins. Provocation of a response by Gpp[NH]p or other GTP analogs has been proposed as a criterion for involvement of G proteins in a given process (Gilman, 1987). GDP-$\beta$-S is a nonhydrolyzable GDP analog that inhibits activation of G proteins. IBMX, theophylline, and papaverine inhibit phosphodiesterases that hydrolyze cAMP and cGMP. Whereas these compounds inhibit phosphodiesterases in general, RO-20-1724 is a selective inhibitor of the mammalian cAMP-specific phosphodiesterase IV (Thompson, 1993). Dibutyryl-cAMP and -cGMP are analogs of cAMP and cGMP, respectively, that may penetrate cell membranes more freely than cAMP and cGMP (Posternak and Weimann, 1974) and may also be resistant to hydrolysis by phosphodiesterases (Cheung and Lin, 1974; Posternak and Weimann, 1974). Forskolin activates adenylate cyclase (Seamon *et al.*, 1981), the enzyme that catalyzes generation of cAMP. TPA activates protein kinase C. Müller (1985) and Freeman and Ridgway (1990) found TPA, among the phorbol esters, to be the most effective inducer of metamorphosis in the hydrozoans *Hydractinia echinata* and *Mitrocomella polydiademata*, respectively. Table I provides a brief description of the activity of each of the compounds tested and their predicted effect on metamorphosis of larvae of *Hydroides elegans*.

Except for Gpp[NH]p and RO-20-1724, the concentration ranges we tested for these compounds spanned effective concentrations previously determined to induce metamorphosis in larvae of other marine invertebrates. We could find no comparable data for Gpp[NH]p and RO-20-1724. RO-20-1724 was obtained from Calbiochem, San Diego, California; all other compounds were purchased from Sigma, St. Louis, Missouri.

*Statistical analysis*

The results of the experiments were analyzed using either two-sample *t*-tests or Wilcoxon rank-sum tests. The percentage of larvae metamorphosing in each replicate petri dish was subjected to the angular transformation, and the means and variances for each treatment were calculated. Variances for the two treatments to be compared were then tested for homogeneity using the *F*-max test (Sokal and Rohlf, 1981). If variances were homogeneous, the transformed data were compared by two-sample *t*-test. Otherwise the untransformed data were analyzed by the nonparametric Wilcoxon rank-sum test. All *t*-tests were calculated by hand; the nonparametric tests were conducted using the NPAR1WAY procedure in SAS (SAS Institute Inc., 1989). Significant results were identified using the sequentially rejective Bonferroni procedure (Holm, 1979; Rice, 1989) to correct for multiple tests.

## Results

*Compounds affecting the activity of G proteins*

Neither the G-protein activator Gpp[NH]p nor the inhibitor GDP-$\beta$-S affected metamorphosis of the larvae of *Hydroides elegans* (Fig. 1A, B). In the absence of a stimulatory bacterial biofilm, Gpp[NH]p did not induce metamorphosis (Fig. 1A), and GDP-$\beta$-S failed to inhibit metamorphosis in response to a biofilmed surface (Fig. 1B).

*Compounds affecting the AC/cAMP transduction pathway*

In two separate trials, the phosphodiesterase inhibitors IBMX, theophylline, and papaverine all induced metamorphosis in the absence of a biofilm (Fig. 2A, B). Concentrations at which we observed maximal responses were the same in both trials; $10^{-4}$ *M* for IBMX (*t*-test, $P < 0.001$, both trials), $10^{-3}$ *M* for theophylline (*t*-test, $P <$

**Table I**

*Compounds tested for effects on metamorphosis of larvae of* Hydroides elegans

| | Activity | Predicted effect on metamorphosis |
|---|---|---|
| Compounds affecting G proteins | | |
| Gpp [NH] p | Activates G proteins | Induction |
| GDP-$\beta$-S | Inhibits activation of G proteins | Inhibition |
| Compounds affecting the AC/cAMP signal-transduction pathway | | |
| IBMX | Inhibits phosphodiesterases* | Induction |
| Theophylline | Inhibits phosphodiesterases* | Induction |
| Papaverine | Inhibits phosphodiesterases* | Induction |
| RO-20-1724 | Inhibits cAMP-specific phosphodiesterase IV* | Induction |
| db-cAMP | Increases intracellular cAMP | Induction |
| db-cGMP | Increases intracellular cGMP | Induction |
| Forskolin | Activates adenylate cyclase* | Induction |
| Compounds affecting the PI/DAG/PKC signal-transduction pathway | | |
| TPA | Activates protein kinase C | Induction |

A brief description of the activity of each compound is provided (Activity). Predicted Effect describes the result one would expect if G proteins, or the affected signal-transduction pathway, mediated responses of larvae to metamorphic cues. See text for details.

* Activity increases intracellular cAMP or cGMP.

0.001, both trials), and $10^{-5}$ *M* for papaverine (*t*-test, $P < 0.001$, trial 1; *t*-test, $P < 0.005$, trial 2). IBMX at $10^{-3}$ *M* caused a large number of larvae to metamorphose without constructing a primary or secondary tube. Figure 2 shows only the percentage of larvae that had undergone *normal* metamorphosis (characterized by permanent attachment to the substratum and construction of either a primary or secondary tube, see Materials and Methods) when exposed to IBMX at $10^{-3}$ *M*. Papaverine was a much less effective inducer of metamorphosis than either IBMX or theophylline, with a maximum mean percentage of metamorphosis < 20%, as compared to > 80% for both IBMX and theophylline (Fig. 2A, B). Exposure of larvae to theophylline at $10^{-2}$ *M* was fatal. In the second trial (Fig. 2B) both IBMX (rank-sum test, $P < 0.05$) and papaverine (rank-sum test, $P < 0.05$) inhibited normal metamorphosis at their highest concentrations, although the effects were not strong. These compounds may have been toxic at the highest concentrations we tested. The selective phosphodiesterase inhibitor RO-20-1724 exhibited no inductive effects (Fig. 3).

Neither the adenylate cyclase activator forskolin, the cAMP analog db-cAMP, nor the cGMP analog db-cGMP induced metamorphosis in the absence of biofilm (Fig. 4). In the presence of a stimulatory biofilm, however, $10^{-4}$ *M* forskolin strongly inhibited metamorphosis (Fig. 5A, *t*-test, forskolin *vs.* DMSO control, $P < 0.001$). Inhibition did not appear to be the result of toxicity, as larvae exposed to $10^{-4}$ *M* forskolin in this experiment and in the absence of biofilm swam and behaved normally. We observed no mortality in these trials. Neither db-cAMP nor db-cGMP inhibited responses of larvae to biofilms (Fig. 5B).

### *Compounds affecting the PI/DAG/PKC transduction pathway*

Exposure of larvae to the phorbol ester TPA caused no significant induction of metamorphosis as compared to the appropriate DMSO controls (Fig. 6).

Table II lists the compounds tested, their predicted effect on metamorphosis of larvae of *Hydroides elegans* if G protein-coupled receptors or the AC/cAMP or PI/DAG/PKC signal-transduction pathways mediated responses of larvae to metamorphic cues, and the effect we observed in our experiments.

## Discussion

A growing body of research on the effects of neuropharmacological agents on marine invertebrate larvae suggests that G protein-coupled receptors and their associated signal-transduction pathways are important regulators of larval settlement and metamorphosis, either directly through sensation and processing of exogenous metamorphic cues, or indirectly by modifying responses to these cues. It is not clear, however, whether this role for G proteins in metamorphosis is a characteristic of all marine invertebrate larvae (Hadfield, 1998). Our experiments indicate that G protein-coupled receptors or signal transduction by the AC/cAMP or PI/DAG/PKC pathways

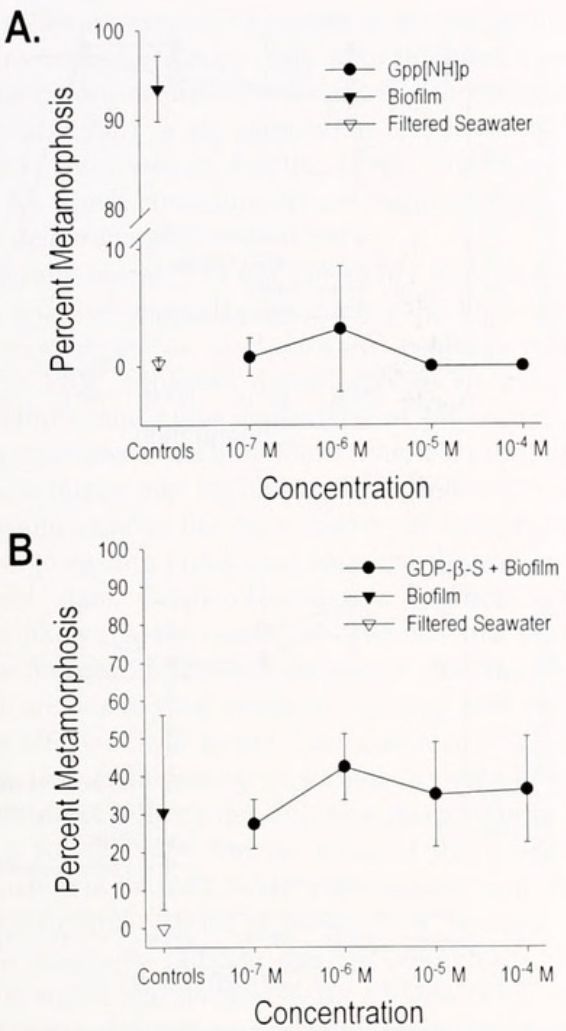


**Figure 1.** Metamorphosis of larvae of *Hydroides elegans* exposed to activators and inhibitors of G proteins. (A) Response to the G-protein activator Gpp [NH]p. (B) Effect of the G-protein inhibitor GDP-$\beta$-S on metamorphosis in response to bacterial biofilms. Points represent means of 4 replicates; error bars are standard deviations.

do not mediate responses of larvae of the serpulid polychaete *Hydroides elegans* to metamorphic cues.

If cues for settlement and metamorphosis of larvae of *H. elegans* were bound by a G protein-coupled receptor, we would have expected competent larvae to (1) metamorphose when exposed to the GTP analog Gpp[NH]p, and (2) fail to respond to inductive bacterial biofilms when those biofilms were presented in combination with GDP-$\beta$-S, a competitive inhibitor of G protein activation by GTP. We observed no increase in metamorphosis over FSW control treatments in the presence of Gpp[NH]p (Fig. 1A), and no inhibition of response of larvae to biofilmed surfaces when exposed to GDP-$\beta$-S (Fig. 1B). These results imply that the receptors responsible for detecting the metamorphic cue in *H. elegans* are not associated with G proteins.

The absence of evidence for the involvement of G protein-coupled receptors does not exclude the possibility that the metamorphic signal is transduced by either the AC/cAMP or PI/DAG/PKC pathways. Baxter and Morse (1987) found that Gpp[NH]p and GDP-$\beta$-S did not directly affect metamorphosis of larvae of *Haliotis rufescens*, but larvae were induced to metamorphose by application of forskolin, IBMX, and theophylline, suggesting that the metamorphic signal was likely to be transduced by the AC/cAMP pathway, although not by a G protein-coupled receptor (Baxter and Morse, 1987; Morse, 1990).

If signal transduction by the AC/cAMP pathway were a necessary event in the metamorphic sequence in *Hydroides elegans*, then competent larvae should have metamorphosed when exposed to the phosphodiesterase inhibitors, db-cAMP, and the adenylate cyclase activator forskolin. The nonspecific phosphodiesterase inhibitors IBMX, theophylline, and papaverine induced significant levels of metamorphosis (Fig. 2); however, we observed no effect of the selective inhibitor RO-20-1724 (Fig. 3). Additionally, neither forskolin nor db-cAMP caused larvae to metamorphose (Fig. 4). Forskolin had the opposite

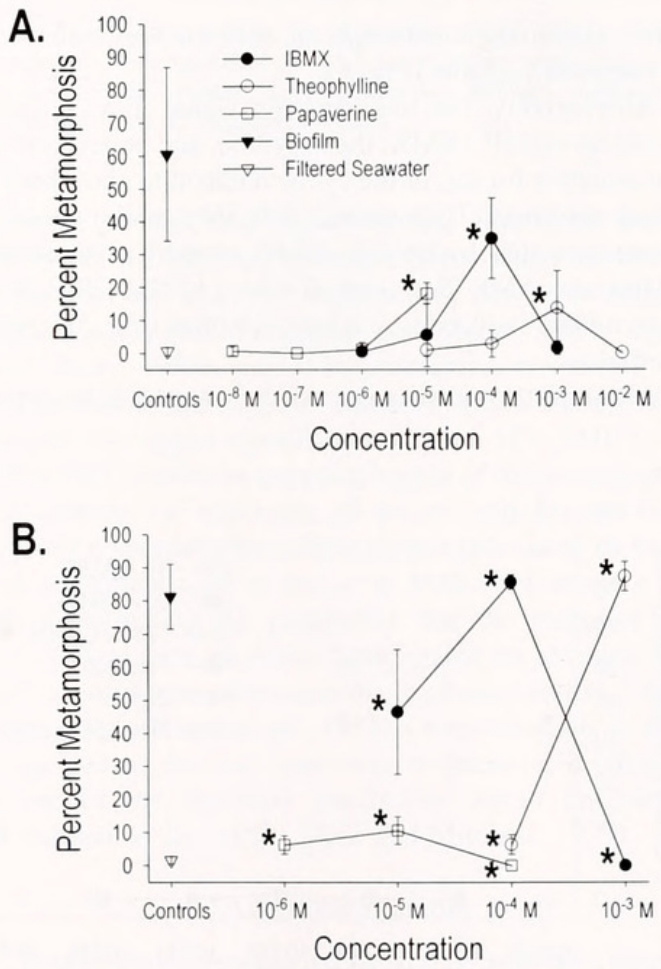


**Figure 2.** Metamorphosis of larvae of *Hydroides elegans* exposed to the phosphodiesterase inhibitors IBMX, theophylline, and papaverine. (A) and (B) present results from two separate trials. Note that the concentration ranges ($x$ axes) differ between trials. Points represent means of 5 replicates in (A), 4 replicates in (B); error bars are standard deviations. * - significantly different from the FSW control treatment at $P \leq 0.05$.

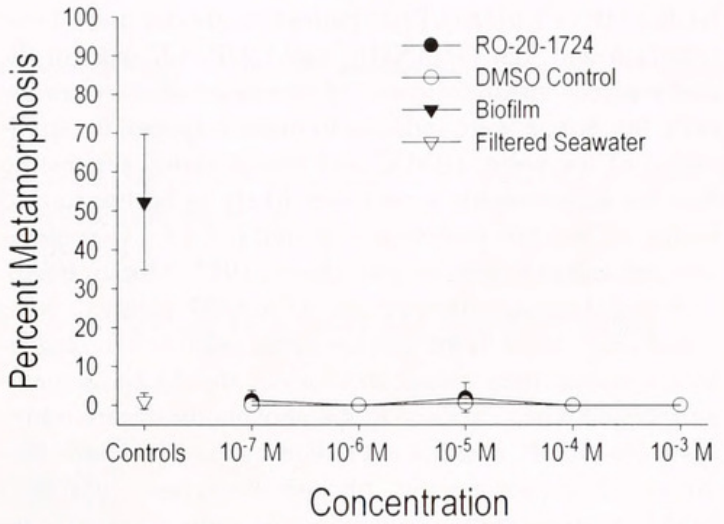


**Figure 3.** Metamorphosis of larvae of *Hydroides elegans* exposed to RO-20-1724, a selective inhibitor of the mammalian cAMP-specific phosphodiesterase IV. Exposure of larvae to DMSO served as the control for the RO-20-1724 treatment. Points represent means of 4 replicates; error bars are standard deviations.

effect, inhibiting metamorphosis of *H. elegans* in response to bacterial biofilms (Fig. 5).

Alternatively, the metamorphic signal may be transduced by cGMP. IBMX, theophylline, and papaverine are not selective for any of the cyclic nucleotide phosphodiesterase isozymes (Thompson, 1993); they inhibit phosphodiesterases that hydrolyze cGMP as well as those that hydrolyze cAMP. Exposure of larvae to db-cGMP, however, neither induced (Fig. 4) nor inhibited (Fig. 5B) metamorphosis.

Although the results presented here appear to be contradictory, they may be explained by the effects on ion transport of some of the compounds used. IBMX (Kopf *et al.*, 1983; Simasko and Yan, 1993; Usachev and Verkhratsky, 1995), theophylline (Kopf *et al.*, 1983), and papaverine (Fujioka, 1984; Iguchi *et al.*, 1992) all have effects on calcium transport that can be either independent of or stronger than their effects on intracellular cAMP levels. Forskolin can block both potassium (Watanabe and Gola, 1987; Coombs and Thompson, 1987; Hoshi *et al.*, 1988; Garber *et al.*, 1990) and calcium (Park and Kim, 1996) channels, and desensitize acetylcholine receptors (Wagoner and Pallotta, 1988; White, 1988), independently of its effects on cAMP. Calcium ions are important second messengers in the metamorphic pathway of some hydrozoan larvae (Freeman and Ridgway, 1987, 1990), and may serve the same function in larvae of polychaetes (Ilan *et al.*, 1993) and barnacles (Rittschof *et al.*, 1986; Clare,

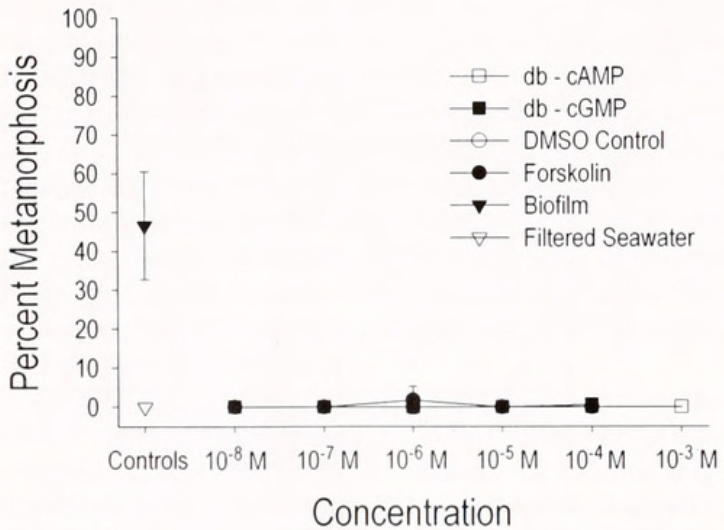


**Figure 4.** Metamorphosis of larvae of *Hydroides elegans* exposed to the cyclic nucleotide analogs db-cAMP and db-cGMP, and the adenylate cyclase activator forskolin. Exposure of larvae to DMSO served as the control for the forskolin treatment. Points represent means of 4 replicates; error bars are standard deviations.

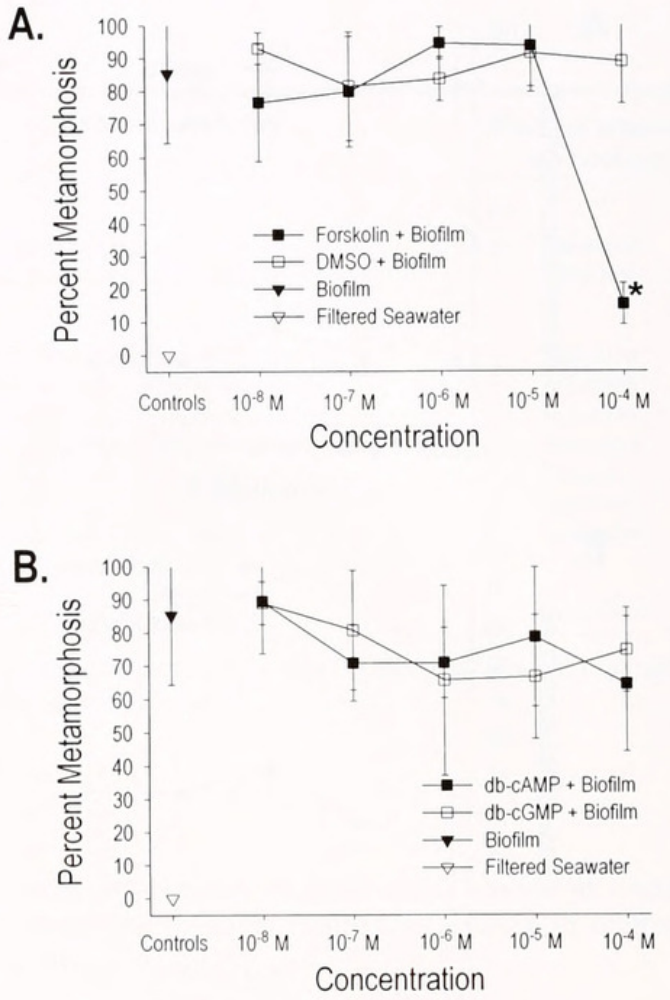


**Figure 5.** Metamorphosis of larvae of *Hydroides elegans* exposed to bacterial biofilms in combination with forskolin, db-cAMP, and db-cGMP. (A) Response to biofilmed surfaces in the presence of forskolin. The DMSO + Biofilm treatment served as the control for larvae exposed to forskolin. (B) Response to biofilmed surfaces in the presence of db-cAMP and db-cGMP. Points represent means of 4 replicates; error bars are standard deviations. * - significantly different from the control treatment at $P \leq 0.05$.

1996). Metamorphosis of larvae of several phyla of marine invertebrates can be induced or inhibited by elevated concentrations of potassium (reviewed in Woollacott and Hadfield, 1996) or by application of potassium-channel blockers (reviewed in Pawlik, 1990). The exact mechanism by which potassium affects metamorphosis has yet to be determined.

Ongoing research in our laboratory indicates that calcium and potassium play important roles in the metamorphic morphogenesis of *H. elegans*. Addition of calcium ions to FSW enhances metamorphosis in the presence of biofilms, and pulse application of potassium induces metamorphosis in the absence of other cues (unpubl. data; Carpizo-Ituarte and Hadfield, 1996, 1998). Calcium and potassium channel blockers inhibit metamorphosis in response to cesium pulses and bacterial films, respectively (unpubl. data; Carpizo-Ituarte and Hadfield, 1998). It seems likely that the results we observed after application of the nonspecific phosphodiesterase inhibitors and forskolin are due to their effects on ion transport, rather than on cAMP or cGMP levels. This conclusion is supported by the lack of inhibitory or stimulatory effects from db-cAMP, db-cGMP, or the selective phosphodiesterase inhibitor RO-20-1724. On the basis of this evidence, we conclude that the AC/cAMP pathway does not transduce the metamorphic signal in larvae of *H. elegans*.

Our data also indicate that metamorphosis does not require signal transduction by the PI/DAG/PKC pathway. We attempted to induce metamorphosis with the protein kinase C activator TPA, the phorbol ester found to be the most effective stimulator of metamorphosis in two species of hydrozoans (Müller, 1985; Freeman and Ridgway, 1990). No effect was observed, implying that the PI/DAG/PKC pathway does not directly participate in the metamorphic process. The possibility that G proteins and the PI/DAG/PKC pathway transduce signals that modify responses of larvae to the metamorphic cue, as in *Haliotis rufescens* (Trapido-Rosenthal and Morse, 1985; Baxter and Morse, 1987), cannot be eliminated, however.

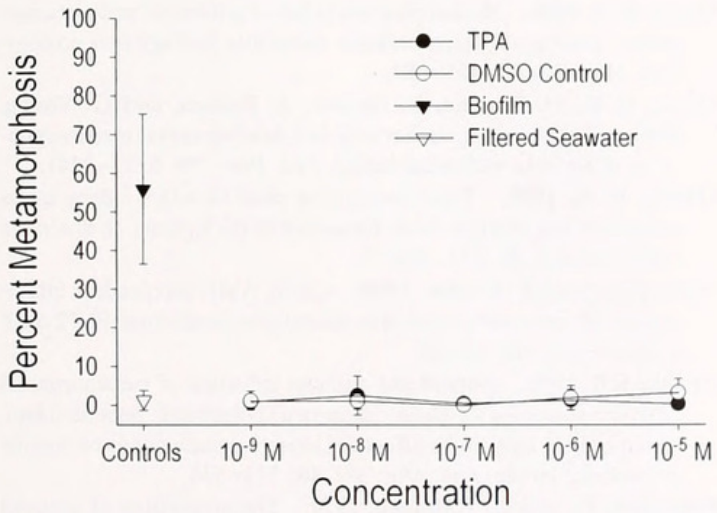


**Figure 6.** Metamorphosis of larvae of *Hydroides elegans* exposed to the protein kinase C activator TPA. Exposure of larvae to DMSO served as the control for the TPA treatment. Points represent means of 4 replicates; error bars are standard deviations.

**Table II**

*Predicted and observed effects of compounds tested on larvae of* Hydroides elegans

| | Effect on metamorphosis | |
|---|---|---|
| | Predicted | Observed |
| Compounds affecting G proteins | | |
| Gpp [NH] p | Induction | None |
| GDP-$\beta$-S | Inhibition | None |
| Compounds affecting the AC/cAMP signal-transduction pathway | | |
| IBMX | Induction | Induction |
| Theophylline | Induction | Induction |
| Papaverine | Induction | Induction |
| RO-20-1724 | Induction | None |
| db-cAMP | Induction | None |
| db-cGMP | Induction | None |
| Forskolin | Induction | Inhibition |
| Compounds affecting the PI/DAG/PKC signal-transduction pathway | | |
| TPA | Induction | None |

See Table I and the text for descriptions of the activity of each compound. Predicted Effect describes the result one would expect if G proteins, or the affected signal-transduction pathway, mediated responses of larvae to metamorphic cues.

Our results indicate that neither G protein-coupled receptors nor signal transduction by the AC/cAMP or PI/DAG/PKC pathways are components of the morphogenetic pathway (in the sense of Baxter and Morse, 1987) directly responsible for sensation and processing of bacterial settlement cues in larvae of *Hydroides elegans*. We are investigating the possibility that the receptors that detect these cues are either ligand-gated ion channels similar to amino acid taste receptors in channel catfish (Teeter *et al.*, 1990; Brand *et al.*, 1991), or lectins such as those suggested to mediate responses to bacterial biofilms in larvae of the spirorbid polychaete *Janua brasiliensis* (Kirchman *et al.*, 1982; Maki and Mitchell, 1985).

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