NATURAL AND ARTIFICIAL INDUCTION OF METAMORPHOSIS OF *PHRAGMATOPOMA LAPIDOSA CALIFORNICA* (POLYCHAETA: SABELLARIIDAE), WITH A CRITICAL LOOK AT THE EFFECTS OF BIOACTIVE COMPOUNDS ON MARINE INVERTEBRATE LARVAE

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ABSTRACT

Recent studies of the effects of bioactive compounds on the settlement and metamorphosis of marine invertebrate larvae prompted the present study of the larval responses of the tube building polychaete *Phragmatopoma lapidosa californica* to these compounds. Choline, succinylcholine and serotonin induced variable levels of abnormal metamorphosis of *P. l. californica*. Larvae did not respond to γ-aminobutyric acid (GABA). Both D- and L-3,4-dihydroxyphenylalanine (DOPA) induced 10–30% normal metamorphosis, but larvae did not respond to dopamine, norepinephrine or epinephrine. L-DOPA underwent rapid oxidation in seawater, and solutions left standing for longer than 2 h lost the capacity to induce metamorphosis. Whereas metamorphosis in response to palmitoleic acid (a naturally occurring inducer) was rapid, larval response to L-DOPA was delayed for several hours, suggesting that L-DOPA does not act on an external epithelial chemoreceptor. Four compounds that alter the transport of ions across membranes were tested and three of these, tetraethylammonium (TEA), sulfonyl isothiocyanostilbene (SITS) and picrotoxin, had no apparent effect on larvae. Ouabain at 10^{-5} M induced abnormal metamorphosis in a high percentage of larvae exposed to sand coated with palmitoleic acid, but had no effect on larvae exposed to control sand. Ouabain also had no effect at lower concentrations, and was toxic at higher concentrations. Three compounds that alter intracellular concentrations of cyclic AMP (cAMP) in some cell systems were tested and two of these, dibutyryl cAMP (db-cAMP) and cholera toxin, had no effect on larvae of *P. l. californica*. Isobutylmethylxanthine (IBMX) induced a high percentage of normal metamorphosis at 10^{-5}–10^{-4} M.

Available evidence does not support the hypotheses that (1) natural inducers of marine invertebrate larval settlement and metamorphosis are structurally related to neurotransmitters, and (2) metamorphic activation among different invertebrate larvae occurs by one general pathway. The compounds used in this and previous studies frequently have multiple or variable effects on different cell lines or tissues. Assays of the effects of bioactive compounds on whole invertebrate larvae are not specific, neither in terms of the cells that are affected nor the responses that are observed. Therefore, it is presently untenable to propose detailed molecular pathways controlling the activation of invertebrate larval settlement and metamorphosis based on the responses of whole larvae to these compounds.

Although the specificity of larval settlement of many benthic marine invertebrates is widely believed to be chemically mediated, very few natural inducers of invertebrate larval settlement and metamorphosis have been identified (Kato et al., 1975; Pawlik, 1986). In contrast, a plethora of bioactive compounds and neuropharmacological agents have been demonstrated to have varying effects on mature larvae, ranging from normal settlement and metamorphosis to abnormal metamorphosis and death. Molluscan larvae have been studied most intensively, and three groups of compounds have been found to effect larval metamorphosis in separate systems: choline derivatives (Hadfield, 1978), catecholamines (Coon et al., 1985), and other amino acid derivatives (Morse et al., 1979). Experimentation with bioactive substances often appears to have commenced after natural

inducers of larval metamorphosis frustratingly eluded isolation or identification. Structural and functional relationships often have been implied between bioactive compounds that were found to induce metamorphosis and the unknown, naturally occurring inducers.

The planktonic larvae of the reef-building sabellariid polychaete worms *Phragmatopoma lapidosa californica* and *P. l. lapidosa,* from the eastern Pacific and western Atlantic, respectively, settle and metamorphose in response to specific free fatty acids (FFAs) isolated from the sand/cement matrix of adult tubes (Pawlik, 1986; 1988b). Larvae of an ecologically equivalent species of sabellariid from European waters, *Sabellaria alveolata,* did not settle in response to FFAs, indicating that the larvae of the two subspecies of *Phragmatopoma* possess a specific mechanism for recognizing FFAs at the time of settlement (Pawlik, 1988a). Moreover, there was greater than 10 times the concentration of FFAs present in the tube sand from reefs of *P. l. californica* and *P. l. lapidosa* than from reefs of *S. alveolata,* suggesting that adults of the two subspecies of *Phragmatopoma* produce the settlement cue (Pawlik, 1988a; 1988b).

Jensen and Morse (1984) surmised that quinone-tanned proteins (or a precursor or enzyme involved in their formation) were responsible for inducing settlement of *Phragmatopoma lapidosa californica,* an hypothesis previously advanced by Wilson (1968) to explain the specific settlement of another reef-forming sabellariid, *Sabellaria alveolata.* They reported that analogs of L-3,4-dihydroxyphenylalanine (L-DOPA) were effective to varying degrees in stimulating larval metamorphosis. This corroborated the hypothesis of Morse and colleagues (Morse et al., 1980b) that amino acid-derived neurotransmitters induced settlement and metamorphosis of a wide range of invertebrate larvae.

The present study was prompted by the work of Jensen and Morse (1984) and by investigations of the effects of bioactive compounds on the settlement and metamorphosis of a variety of invertebrate larvae (Hadfield, 1978; Coon et al., 1985; Rittschof et al., 1986). It was initiated to determine whether there were any similarities in larval responses to these compounds across species lines, with new data presented for larvae of *Phragmatopoma lapidosa californica.* Because of the diversity of compounds that have been investigated, this paper is divided into sections that describe larval responses to compounds that have similar structures or pharmacological effects. Each section contains a short introduction to the type of bioactive compound, followed by results of experiments with these compounds on larvae of *P. l. californica,* and then a comparison of their effects among other invertebrate larvae. Four of the 22 compounds assayed in this study have been previously employed in experiments with larvae of *P. l. californica* (TEA: Yool et al., 1986; L-DOPA, db-cAMP, IBMX: Jensen, 1987); the experiments presented herein repeat and extend some of this previous work. All of the data presented in the tables and figures are original.

Materials and Methods

Techniques for the collection of adult *Phragmatopoma lapidosa californica* and the rearing of their larvae have been described previously (Pawlik, 1986; 1988a). Larval assays were performed with only minor adjustments to earlier methods. Twenty (±2) larvae were added to each assay dish, with three replicate dishes per experimental treatment. Sand was not added to assay dishes except for experiments designed to test the effect of a compound on larval response to palmitoleic acid (16:1), an inducer found in extracts of natural tube sand. For these assays, 150 μl of a diethyl ether solution containing 1 mg of palmitoleic acid (treated) or 150 μl of diethyl ether alone (control) was spread onto 1 g of clean sand. The solvent was evaporated under vacuum, and the treated and control sands were added to separate assay dishes. Unless stated otherwise, assays were scored after 24 h.

All compounds were purchased from Sigma Chemical Co. (St. Louis, Missouri) at their highest level

of purity. Compounds were dissolved in 1-μm filtered, natural seawater immediately prior to use. No adjustments were made to the pH of these solutions, as most were within 1 pH unit of seawater (pH = 8). There was no effect of pH alone (adjusted with 1 N HCl or NaOH) on larval response, except toxicity at very low (<4) and very high (>10) levels.

Results and Discussion

Larval Responses to Choline Derivatives

Choline is a bound constituent of the membranes surrounding all cells. It is also a precursor of acetylcholine. Acetylcholine is perhaps the best characterized of neurotransmitters, and plays an important role in bridging neuron-neuron and neuron-muscle synapses in both vertebrates and invertebrates (Kuffler et al., 1984). Bonar (1976) was the first to discover that succinylcholine chloride stimulated metamorphosis of the nudibranch *Phestilla sibogae.* Succinylcholine was also found to induce metamorphosis of the ascoglossan mollusc *Elysia chlorotica* (Harrigan and Alkon, 1978). Subsequent studies by Hadfield (1978; 1984) revealed that the choline moiety alone was responsible for metamorphic induction of *P. sibogae,* as several choline derivatives similarly affected the response. However, larvae of *P. sibogae* did not respond to acetylcholine.

Phestilla sibogae is a specific predator on hard corals of the genus *Porites*; larvae of this nudibranch metamorphose rapidly (<1 day) in response to an unidentified, water-soluble substance derived from extracts of coral tissue or mucus, or from extracts of seawater collected from the vicinity of coral heads (Hadfield and Karlson, 1969; Hadfield and Scheuer, 1985). Larvae responded differently to the natural inducer than to choline derivatives. The concentrations at which choline compounds induced metamorphosis of *Phestilla sibogae* (10^{-3}–10^{-2} M) were several orders of magnitude greater than similarly effective concentrations of the coral-derived inducer. Also, larval metamorphosis in response to choline compounds required 3 to 4 days of exposure, whereas response to the natural inducer occurred in less than 1 day.

Larvae of the polychaete *Phragmatopoma lapidosa californica* also responded to choline and succinylcholine at concentrations similar to those reported by Hadfield for *Phestilla sibogae* (Table 1, Fig. 1). Unlike *Phestilla sibogae,* metamorphosis of *Phragmatopoma lapidosa californica* was incomplete; larvae failed to attach to the substrate and did not form mucoid tubes. At concentrations $\geq 4 \times 10^{-2}$ M, succinylcholine induced 100% of the larvae to undergo abnormal metamorphosis, but with some of the larvae subsequently dying. Choline at the same concentrations killed larvae without prior induction of abnormal metamorphosis (Fig. 1).

Choline derivatives have also been assayed for their effects on the larvae of the Pacific oyster, *Crassostrea gigas* (Coon et al., 1985), although the concentrations employed (10^{-6}–10^{-4} M) were much lower than those eliciting a response in either *Phestilla sibogae* or *Phragmatopoma lapidosa californica.* Acetylcholine induced low or inconsistent levels of metamorphosis of *C. gigas* at 10^{-4} M, whereas succinylcholine had no effect on larvae at this concentration. Choline and acetylcholine did not induce metamorphosis of the abalone *Haliotis rufescens* (Morse et al., 1979) or *H. discus hannai* (Akashige et al., 1981). Levantine and Bonar (1986) reported that choline, acetylcholine and succinylcholine induced low levels of metamorphosis of the mud snail *Ilyanassa obsoleta,* while methacholine (acetyl-β-methylcholine) induced 80–100% metamorphosis. Methacholine had no effect on larvae of *Phragmatopoma californica lapidosa,* except toxicity at the highest concentration (10^{-1} M, Table 1).

Table 1. Responses of larvae of *Phragmatopoma lapidosa californica* to compounds tested in studies of other invertebrate larvae. The numbers in parentheses are the approximate percentage of larvae that had undergone normal or abnormal metamorphosis (the remainder were unmetamorphosed). — = not assayed; O = no effect; N = normal metamorphosis; A = abnormal metamorphosis; X = dead

	Concentration (M)						
	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}
Choline derivatives							
choline Cl	—	—	O	O	O	A (50)	X
acetylcholine Cl	—	—	—	O	O	O	X
succinylcholine Cl	—	—	O	O	A (30)	A (90)	X
phosphorylcholine Cl	—	—	—	O	O	O	X
methacholine Cl	—	—	—	O	O	O	X
carbamylcholine Cl	—	—	—	O	O	O	X
Catecholamines							
catechol	—	—	—	O	X	X	X
L-DOPA	O	O	N (15)	X	X	—	—
D-DOPA	—	O	N (15)	X	—	—	—
dopamine	O	O	O	X	X	—	—
norepinephrine	—	O	O	O	X	—	—
epinephrine	O	O	O	O	X	—	—
Other compounds							
GABA	—	—	O	O	O	O	—
serotonin HCl	—	—	O	A (10)	O	X	—
serotonin creatinine SO_4	—	—	O	A (20)	O	X	—

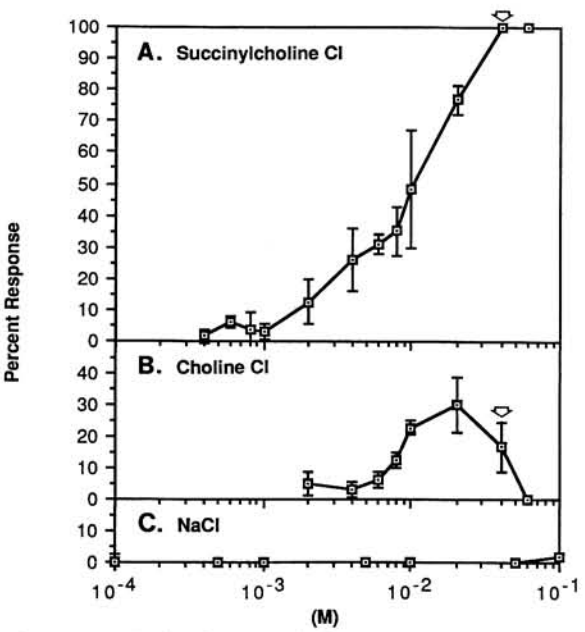


Figure 1. Abnormal metamorphosis of larvae of *Phragmatopoma lapidosa californica* in response to seawater containing (A) succinylcholine Cl, (B) choline Cl, and (C) NaCl (±SD, N = 3). Toxic effects were observed at concentrations equal to and greater than those indicated by the open arrow. Metamorphosis occurred without attachment and tube formation in response to both succinylcholine and choline.

Choline was never postulated to be the naturally occurring inducer of larval metamorphosis for *Phestilla sibogae* (Hadfield, 1978; 1984). Indeed, recent work has demonstrated that the sites of action of choline and the coral-derived inducer are different (Hirata and Hadfield, 1986): (1) Larvae of *Phestilla sibogae* metamorphose in response to the coral inducer within 24 h, whereas choline induction requires ~3 days. (2) Precompetent larvae exposed to coral inducer habituate to it (i.e., they do not metamorphose in response to it when fully mature), but habituation does not occur in response to choline. (3) Habituation to coral inducer does not interfere with induction by choline, and conversely, exposure of precompetent larvae to choline does not interfere with induction by coral inducer. (4) There were no synergistic effects observed between the two inducers at low concentrations. Hirata and Hadfield (1986) concluded that choline was somehow involved in the neurological events leading to metamorphosis of *Phestilla sibogae.* For *Phragmatopoma lapidosa californica,* choline derivatives similarly do not appear to act on external receptors, but by influencing the larval nervous system.

Larvae of *Phragmatopoma lapidosa californica* showed a delayed reaction to succinylcholine of between 12 and 24 h, similar to *Phestilla sibogae* in response to choline, but began metamorphosing immediately on exposure to palmitoleic acid, a natural inducer (Pawlik, 1986; see later discussion). In addition, larval response by *Phragmatopoma lapidosa californica* to choline derivatives lacked the attachment and tube building behaviors found in normal metamorphosis, suggesting that a step in the normal sequence of metamorphic events had been omitted. Unfortunately, the lipophilic nature of the natural inducers of *Phragmatopoma lapidosa californica* settlement precluded studies of larval habituation similar to those performed on *Phestilla sibogae* by Hirata and Hadfield (1986).

If choline derivatives influence the neural pathways involved in metamorphosis of the aforementioned larvae, their mechanism of action is not clear. In cholinergic neurons, free choline is the precursor of the neurotransmitter acetylcholine (Blusztajn and Wurtman, 1983). Yet, larvae of *Phragmatopoma lapidosa californica* did not respond to acetylcholine at concentrations that brought about abnormal metamorphosis by choline and succinylcholine. For larvae of *Phestilla sibogae,* acetylcholine was toxic at concentrations equal to those that brought about induction by choline (Hadfield, 1978). Hirata and Hadfield (1986) discuss several nonexclusive hypotheses regarding the mode of action of choline on the larval nervous system of *Phestilla sibogae*; they are: (1) that choline acts on internal receptor sites usually specific for acetylcholine; (2) that choline serves as a precursor in acetylcholine biosynthesis; and (3) that choline stimulates the synthesis and release of catecholamine neurotransmitters. The results of the present study lend little support to any one of these hypotheses over the others.

Larval Responses to DOPA and Catecholamines

L-β-3,4-dihydroxyphenylalanine (L-DOPA) and the catecholamines (dopamine, norepinephrine and epinephrine) are derivatives of tyrosine that have diverse biological functions, including their use as systemic hormones and neurotransmitters (Darnell et al., 1986) and in the formation of pigments, adhesives and structural proteins (Lindner and Dooley, 1976; Hopkins et al., 1982; Waite, 1983). In his studies of the gregarious settlement of the barnacle *Semibalanus balanoides,* Knight-Jones (1953) was the first to make a connection between invertebrate larval settlement and tyrosine derivatives by hypothesizing that polymers containing quinone-tanned (-oxidized) proteins induced barnacle settlement. Tyrosine derivatives were also implicated in the settlement of the oyster, *Cras-*

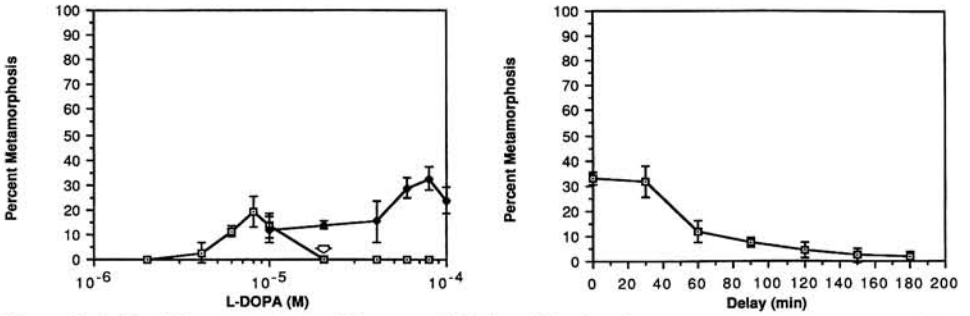


Figure 2 (left). Metamorphosis of larvae of *P. l. californica* in response to seawater containing L-DOPA (±SD, N = 3). Open squares indicate larval response to 24-h continuous exposure to L-DOPA; toxic effects were observed at concentrations equal to and greater than those indicated by the open arrow. Solid diamonds indicate larval response to a 1-h exposure to L-DOPA; larvae were returned to filtered seawater and scored after 23 h.

Figure 3 (right). Metamorphosis of larvae of *P. l. californica* in response to a freshly prepared seawater solution of 10^{-5} M L-DOPA that was allowed to stand for the indicated length of time prior to addition of larvae (±SD, N = 3). Metamorphosis in control dishes was 1.9 ± 1.7% (N = 3).

sostrea virginica (Veitch and Hidu, 1971). Cooper (1982; 1983) reported that L-DOPA induced metamorphosis of the mussel *Mytilus edulis* and the oyster *Crassostrea gigas*. Subsequent work on *C. gigas* by Coon et al. (1985) demonstrated that L-DOPA induced both settlement and metamorphosis, that epinephrine and norepinephrine induced metamorphosis without settlement, and that dopamine exhibited little inductive activity. In contrast, Hadfield (1984) found that larvae of *Phestilla sibogae* underwent a low percentage of partial metamorphosis in response to dopamine and, to a lesser extent, to epinephrine, but that larval response to norepinephrine was negligible. The scallop *Pecten maximus* metamorphosed in response to L-DOPA and epinephrine, but not to norepinephrine (Cochard et al., 1989). Larvae of the mud snail *Ilyanassa obsoleta* did not respond to L-DOPA, norepinephrine or epinephrine, but exhibited low levels of metamorphosis in response to dopamine (Levantine and Bonar, 1986). Abalone larvae did not settle in response to epinephrine or norepinephrine (Morse et al., 1979; Akashige et al., 1981).

Responses of larvae of *Phragmatopoma lapidosa californica* to DOPA and catecholamines were different from those reported for molluscan larvae. Continuous exposure to L-DOPA at $\sim 10^{-5}$ M consistently induced low levels (~10–20%) of normal metamorphosis, but larvae were unresponsive to epinephrine, norepinephrine and dopamine when these compounds were assayed at sub-toxic concentrations (Table 1, Fig. 2). Whereas D-DOPA, the isomer of L-DOPA, was much less effective at inducing metamorphosis of *Crassostrea gigas* (Coon et al., 1985), there was no difference in larval response to the two isomers by larvae of *P. l. californica* (Table 1).

Catecholamines and DOPA are subject to oxidation and polymerization in solution. A 10^{-5} M solution of L-DOPA left standing for longer than one hour induced less than half the level of metamorphosis of *Phragmatopoma lapidosa californica* than a solution used immediately upon preparation (Fig. 3). After 2 h, larval response to the solution was no different than to control seawater. DOPA auto-oxidizes to form heterogenous polymeric melanin pigments (Lindner and Dooley, 1976). After 24 h, black precipitates of these pigments covered the bottoms of dishes in assays of L-DOPA at concentrations $\geq 2 \times 10^{-5}$ M. Continuous exposure at these concentrations was toxic to larvae (Fig. 2). However, this toxicity

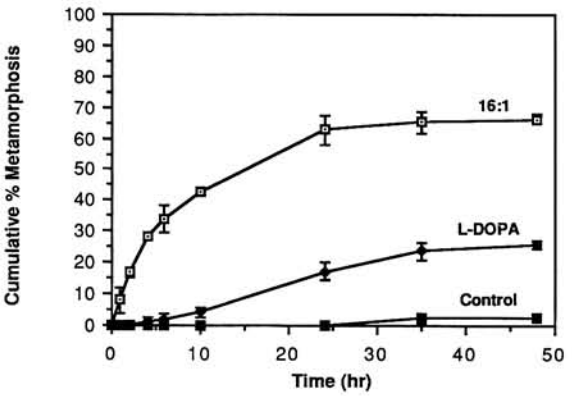


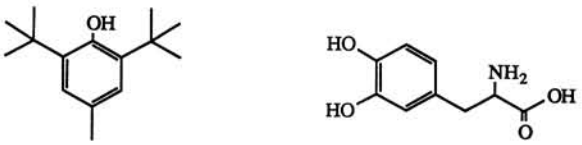
Figure 4. Cumulative percentage metamorphosis of larvae of *P. l. californica* in dishes containing seawater and sand coated with 1 $mg \cdot g^{-1}$ palmitoleic acid (16:1), dishes containing seawater with 10^{-5} M L-DOPA and clean sand (L-DOPA), and dishes containing seawater and clean sand (Control) (±SD, N = 3).

did not mask a greater response to higher L-DOPA concentrations, because larvae exposed to $\geq 2 \times 10^{-5}$ M L-DOPA for 1 h only (prior to oxidation), and then returned to filtered seawater, still metamorphosed at low levels (<35%; Fig. 2). For *Crassostrea gigas,* prolonged exposure of larvae to high concentrations of L-DOPA was also toxic (Coon et al., 1985).

There was a considerable delay between administration of L-DOPA and larval metamorphosis (Fig. 4). Whereas larvae exposed to palmitoleic acid commenced metamorphosis rapidly, with half of the cumulative mean total metamorphosing within 6 h of the start of the assay, substantive metamorphosis was not observed in response to L-DOPA until several hours later (Fig. 4). Unlike *Phragmatopoma lapidosa californica,* larvae of *Crassostrea gigas* initiated settlement behavior within 5–10 min of exposure to L-DOPA (Coon et al., 1985). L-DOPA and palmitoleic acid appear to induce metamorphosis of *P. l. californica* in different ways. The delay observed for induction by L-DOPA suggests that it does not act on an external chemoreceptor, but by affecting some other pathway of metamorphic activation.

Because both epinephrine and norepinephrine induced metamorphosis, but not settlement, of *Crassostrea gigas,* Coon et al. (1985) postulated that these compounds, or analogs, did not induce metamorphosis by way of a surface chemoreceptor, but by influencing an endogenous sequence of metamorphic events. In support of this hypothesis, they isolated norepinephrine from homogenates of whole larvae and juveniles of *C. gigas* (Coon and Bonar, 1986). Experiments employing a variety of adrenergic agonists and antagonists provided evidence for $alpha_1$-adrenoceptors in these larvae (Coon and Bonar, 1987a). The lack of larval responses of *Phragmatopoma lapidosa californica* to catecholamines, but not to DOPA, would indicate a difference in the mechanism of metamorphic activation relative to that of *C. gigas.*

Unlike the aforementioned catecholamines, L-DOPA induced both settlement and metamorphosis of *Crassostrea gigas,* originally prompting Coon et al. (1985) to hypothesize that L-DOPA, or a similar molecule, was the stimulus perceived by larvae in nature. This hypothesis was well corroborated by Weiner and colleagues (Weiner et al., 1985; Bonar et al., 1985), who found that larvae of *C. gigas* preferred to settle and metamorphose on substrates fouled by a bacterium that produced an exopolymeric melanin-like pigment under culture conditions. How-



A B

Figure 5. A. 2,6-di-tert-butyl-4-methylphenol, or butylated hydroxytoluene (BHT). B. L-β-3,4-dihydroxyphenylalanine (L-DOPA).

ever, subsequent research by Coon and Bonar (1987a; 1987b), employing decarboxylase inhibitors and selective DOPA agonists and antagonists, revealed that exogenously applied L-DOPA is decarboxylated to dopamine within the larva, and that dopamine then affects dopaminergic receptors. It is unclear why exogenously applied dopamine had little effect on *C. gigas* larvae. Coon and Bonar (1987b) point out, however, that their recent evidence is not consistent with the hypothesis that the exogenous application of L-DOPA mimics natural induction by a DOPA-like moiety bound to a macromolecule (*cf.* Morse, 1985). Evidence now indicates that the bacterial inducer of *C. gigas* settlement is ammonia (Coon et al., 1988).

In their studies of the larval settlement of *Phragmatopoma lapidosa californica,* Jensen and Morse (1984) hypothesized that quinone-tanned proteins (or precursors or enzymes involved in their formation) present in the tubes of adult worms were responsible for the induction of larval settlement, a postulate previously advanced by Wilson (1968) to explain the induction of settlement of a European sabellariid polychaete, *Sabellaria alveolata.* Jensen and Morse (1984) reported that analogs of the amino acid DOPA showed variable effectiveness at inducing larval settlement of *Phragmatopoma lapidosa californica,* but these compounds were not identified. They found that only ~10% of the larvae metamorphosed in response to L-DOPA at 10^{-5} M, that L-DOPA was toxic at higher concentrations, and that it was subject to oxidation (Jensen and Morse, 1984; Jensen, 1987), results supported by the present study (Table 1, Figs. 2, 3). Jensen (1987) again stated that "many aromatic analogs" of L-DOPA induced larvae to begin metamorphosis, often with variable results, partial metamorphosis and toxic effects. Only one compound was identified that induced metamorphosis consistently: 2,6-di-tert-butyl-4-methylphenol (DBMP), more commonly known as butylated hydroxytoluene (BHT; Fig. 5A), a lipophilic anti-oxidant (preservative) widely used in foods, petroleum products, plastics, oils and soaps (Merck Index, 1983). Jensen (1987) considered it likely that BHT acts as a structural analog of a cross-linked component in the quinone-tanned protein of tube cement. However, BHT is a derivative of cresol and bears little structural similarity to the amino acid L-DOPA (Fig. 5B). Nothing structurally similar to BHT has been reported from degradative chemical analyses of sabellariid tube cement (Mitterer, 1971; Jensen, 1987), and the free fatty acid fraction was the only naturally-occurring lipophilic inducer isolated from the tube sand of adult *P. l. californica* (Pawlik, 1986).

Larval Responses to Other Amino Acid Derivatives

GABA.—γ-Aminobutyric acid (GABA), a decarboxylation product of glutamic acid, is most commonly an inhibitory neurotransmitter that hyperpolarizes postsynaptic membranes by increasing membrane permeability to Cl^- ions (Kuffler et al., 1984; but cf. Baloun and Morse, 1984). Morse et al. (1979) found that GABA induced settlement and metamorphosis of the red abalone, *Haliotis ru-*

fescens, at 10^{-6} M, with toxic effects observed at higher concentrations. GABA was subsequently reported to induce settlement in several other molluscan species (Morse et al., 1984), and in the echinoid *Strongylocentrotus droebachiensis* (Pearce and Scheibling, 1988).

Larvae of *Phragmatopoma lapidosa californica* did not respond to GABA, and there were no toxic effects of the compound observed at 10^{-2} M, the highest concentration assayed (Table 1). GABA was similarly ineffective or inconsistent at inducing larval metamorphosis of the cnidarians, *Alcyonium siderium* (Sebens, 1983) and three species of *Agaricia* (Morse et al., 1988), and the molluscs, *Phestilla sibogae* (Hadfield, 1984), *Crassostrea gigas* (Coon et al., 1985), *Mytilus edulis* (Cooper, 1982; Eyster and Pechenik, 1987) and *Crepidula fornicata* (Pechenik and Heyman, 1987). GABA induced cessation of ciliary activity in larvae of the chiton, *Katharina tunicata,* causing them to fall out of the water column and settle on the bottom, but subsequent metamorphosis occurred only on encrusting red algae (Rumrill and Cameron, 1983).

Settlement and metamorphosis of the abalone *Haliotis rufescens* in response to GABA was first attributed to the presence of GABA molecules "covalently-linked" to proteins, and to phycoerythrobilin (a "GABA homolog") both of which were isolated from the tissues of encrusting red algae (Morse et al., 1979). Abalone larvae settled preferentially on these algae in laboratory experiments (Morse et al., 1980c). Further research, however, limited the inductive activity to a macromolecular fraction isolated from several species of red algae and cyanobacteria, but available for larval detection only on the surface of encrusting red algae (Morse and Morse, 1984a; 1984b; Morse et al., 1984). Proteolytic digestion (Morse and Morse, 1984a) or gel-filtration (Morse et al., 1984) of the macromolecular fraction resulted in the isolation of a group of small, "peptide-associated" inducer molecules. These molecules, which have yet to be fully identified, are referred to as "GABA-mimetic" (Morse, 1985) only in that they induce settlement, as does GABA; there are no published data supporting any structural similarity between the isolated fraction and GABA.

However, the effectiveness of GABA and the absolute requirement of encrusting red algae in the natural induction of settlement and metamorphosis of abalone remains unclear. Akashige et al. (1981) recorded high levels of normal settlement and metamorphosis of the Japanese abalone, *Haliotis discus hannai,* in response to plates coated with a film of diatoms or the mucoid trail of adult animals. Working on the same species studied by Morse and co-workers (*H. rufescens*), Slattery (1987) also recorded preferential larval settlement on mucus secreted from juvenile abalone. He designed a factorial experiment to test the effect of three variables on settlement: substrate (clean, diatom film, diatom film plus 24-h old juvenile abalone mucus, and diatom film plus 72-h old mucus), water treatment (with or without 1 mM GABA) and seasonality. Significantly more larvae settled on mucus than on the other substrates, although high levels of settlement occurred on all surfaces, with means ranging from 43% on clean plastic to 64% on 72-h-old mucus. Leighton (1988) also reported that films of diatoms and flagellates induced nearly 100% normal settlement and metamorphosis of *H. rufescens* under laboratory conditions, and that abundant postlarvae of *H. rufescens* could be found on the primary stipe and holdfast of the giant kelp, *Macrocystis pyrifera,* in the field. These results are in marked disagreement with those of Morse and co-workers, who reported a near absence of metamorphosis of *H. rufescens* on natural substrates other than encrusting red algae (Morse et al., 1980c; Morse and Morse, 1984a; Morse, 1988).

Akashige et al. (1981) reported that exposing larvae of *Haliotis discus hannai*

to GABA (10^{-6}, 10^{-4} M) resulted in them falling to the bottom of the culture vessel, crawling momentarily, and then dying. Morse (1984) speculated that this mortality resulted from the deleterious effects of high populations of microbes, organic molecules, inorganic ions, or the pH of the seawater used in cultivation; he successfully induced settlement of the same species with GABA. Slattery (1987) found no significant treatment effect of GABA at 1 mM on larvae of *H. rufescens,* although he observed the same narcotic effect as Akashige et al. (1981). Larvae of *H. rufescens* settled whether GABA was present or not; survival on mucus-coated substrates was highest over an 11-week period (Slattery, 1987). These variable results may explain why GABA is not generally used in the commercial cultivation of abalone, either in the U.S. or Japan (Grant, 1981; Ebert and Houk, 1984; Hahn, 1989).

Larvae of the hydroid *Coryne uchidai* cease swimming upon exposure to extracts of brown algae (Nishihira, 1968), and the swimming cilia of larvae of the polychaete *Spirobranchus giganteus* have been demonstrated to arrest in response to a variety of pharmacological agents (Marsden and Hassessian, 1986). Similar responses of molluscan larvae to GABA have been observed (Akashige et al., 1981; Rumrill and Cameron, 1983; Barlow, 1988). In these cases, "settlement" results from larvae falling out of suspension. Although Morse and coworkers maintain that GABA acts on an epithelial chemoreceptor to induce larval attachment to the substrate (Morse, 1985; Trapido-Rosenthal and Morse, 1986b), an equally plausible scenario is that GABA simply arrests the velar cilia of these larvae (Barlow, 1988). Unable to swim, and in a stressed condition, larval attachment, crawling and metamorphosis may follow, but not necessarily as a result of any natural sequence of events.

In addition to GABA, Morse and colleagues have reported that several other amino acid derivatives influence the settlement of *Haliotis rufescens* in laboratory assays. Although they induce only low levels of settlement directly, some diamino acids facilitate settlement in response to GABA, i.e., higher percentages of larvae settle in response to low GABA concentrations in the presence of diamino acids than in their absence (Trapido-Rosenthal and Morse, 1985). These results led to the hypothesis that abalone larvae might be primed to settle when encountering higher levels of diamino acids in coastal seawater (where favorable adult habitats occur), than far from shore (Morse, 1985; Trapido-Rosenthal and Morse, 1986a). Larval responses to a variety of bioactive compound led the authors to propose a complex regulatory pathway involving a GTP-binding protein (G protein)-diacylglycerol cascade (the "regulatory" or "amplifier" pathway), whereby larvae might perceive higher levels of coastal dissolved organic material and thus be poised to settle (Baxter and Morse, 1987; Morse, 1988; Morse and Morse, 1988). This would be an attractive hypothesis, particularly if abalone larvae dispersed over great distances. However, abalone larvae do not feed in the plankton and are competent to settle and metamorphose after a very short time (~1 week). Moreover, field studies by Prince et al. (1987; 1988) and McShane et al. (1988) on *H. rubra* indicate that abalone larvae develop on or near the bottom and have very limited dispersal capacities, on the order of 0–50 m. It seems unlikely, then, that abalone larvae would be exposed to sufficient variability in the concentration of dissolved organic molecules to provide the selective advantage that would drive the evolution of a complex regulatory pathway.

Serotonin.—Serotonin (5-hydroxytryptamine), a derivative of tryptophan, is a neurotransmitter and modulator of vertebrate and invertebrate nervous systems (Kuffler et al., 1984). Serotonin has not been observed to stimulate any response

in the larvae of the molluscs *Haliotis rufescens* (Morse et al., 1979), *H. discus hannai* (Akashige et al., 1981), *Phestilla sibogae* (Hadfield, 1984) or *Crassostrea gigas* (Coon et al., 1985), but has been reported to induce 80–100% metamorphosis of the mud snail *Ilyanassa obsoleta* (Levantine and Bonar, 1986).

The responses of larvae of *Phragmatopoma lapidosa californica* to serotonin were difficult to interpret. Both the creatinine sulfate and HCl salts of the compound induced low levels of abnormal metamorphosis (10–20%) at 10^{-4} M (Table 1); however, neither lower nor higher concentrations of the compounds had any effect on larvae. Abnormal metamorphosis in response to serotonin was similar to that previously described for choline derivatives: larvae underwent metamorphosis without attachment or tube formation. Serotonin was toxic at very high concentrations ($\geq 10^{-2}$ M). Further assays at intermediate concentrations revealed that the creatinine sulfate salt was a more effective inducer of abnormal metamorphosis (~30% at 2×10^{-4} M); again, larval response tailed-off at both lower and higher concentrations (Pawlik, 1988c). Inasmuch as serotonin did not produce effects similar to those seen in studies of molluscan larvae, these results again suggest that the mechanisms of metamorphic activation may differ between *P. l. californica* and these molluscs.

Larval Responses to Compounds that Influence the Transport of Ions Across Membranes

Conduction of electrical impulses in nervous tissue depends on the maintenance of an electrical potential across the cell membrane; this potential is a function of the differential permeability of the membrane to Na^+, K^+ and Cl^- (Kuffler et al., 1984). Direct electrical stimulation has been shown to induce metamorphosis of echinoid larvae (Cameron and Hinegardner, 1974; Burke, 1983), and electrical activity increases when echinoplutei are brought into contact with a substrate that induces metamorphosis (Satterlie and Cameron, 1985). Müller and coworkers were the first to identify systematically the monovalent cations that induced larval metamorphosis at elevated concentrations (Spindler and Müller, 1972; Müller and Buchal, 1973; for earlier work on ionic effects, see Lynch, 1952). A pulsed exposure to seawater containing elevated concentrations of Cs^+, Rb^+, Li^+ or K^+ induced metamorphosis of the cnidarian, *Hydractinia echinata,* in a dose-dependent fashion. Induction by Cs^+, Rb^+ and Li^+ (but not K^+) was inhibited by addition of ouabain, a cardiac glycoside that blocks active transport of Na^+ and K^+, suggesting that the increased concentration of monovalent ions affect the Na^+/K^+-transport system. Elevating the K^+ concentration has proven to be an effective means of inducing metamorphosis of several invertebrate species, including the molluscs *Haliotis rufescens, Phestilla sibogae, Astrea undosa* and *Crepidula fornicata* (Baloun and Morse, 1984; Yool et al., 1986; Pechenik and Heyman, 1987), and the polychaete *Phragmatopoma lapidosa californica* (Yool et al., 1986), but not corals of the genus *Agaricia* (Morse et al., 1988). Excess K^+ inhibited settlement of the barnacle, *Balanus amphitrite* (Rittschof et al., 1986).

Ouabain.—Various compounds are known to block ion transport across membranes. As already mentioned, ouabain blocks the function of the Na^+/K^+-ATPase that maintains an electrical potential across cell membranes (Kuffler et al., 1984). Metamorphosis of *Hydractinia echinata* larvae in response to a bacterial inducer was inhibited in the presence of ouabain (10^{-3} M), implicating ion transport in the process of metamorphic activation (Müller, 1973).

Ouabain was toxic to larvae of *Phragmatopoma lapidosa californica* at concentrations $\geq 10^{-4}$ M, but had no effect on larvae at 10^{-6} M (Table 2). At 10^{-5}

Table 2. Metamorphosis of larvae of *Phragmatopoma lapidosa californica* in response to compounds that affect transmembrane ion transport. Mean percentage metamorphosis ± one SD is indicated (N = 3) for larvae exposed to clean sand (control) and to sand coated with palmitoleic acid (16:1) at 1 $mg \cdot g^{-1}$ sand. For comparison, in assays of larvae on clean sand and in the absence of any compound, the mean of the means of 11 experiments (each with 3–5 replicates) was 1.5 ± 1.7% metamorphosis (Pawlik, 1986; 1988a). * = toxic; abnormal behavior or death; ** = remainder underwent abnormal metamorphosis

Compound	Conc. (M)	% Metamorphosis	
		Control	With 16:1
ouabain	10^{-4}	0.0 ± 0.0*	0.0 ± 0.0*
	10^{-5}	0.0 ± 0.0	24.8 ± 8.9**
	10^{-6}	1.7 ± 2.4	63.9 ± 3.5
TEA	10^{-3}	5.2 ± 3.3	55.0 ± 9.2
	10^{-4}	4.4 ± 2.8	52.5 ± 12.0
	10^{-5}	0.0 ± 0.0	43.0 ± 25.0
SITS	10^{-4}	2.0 ± 3.5	47.9 ± 11.5
	10^{-5}	2.0 ± 2.0	33.9 ± 14.0
	10^{-6}	1.0 ± 1.8	54.2 ± 14.4
picrotoxin	10^{-4}	0.0 ± 0.0	44.2 ± 19.3
	10^{-5}	0.0 ± 0.0	34.7 ± 19.2
	10^{-6}	0.0 ± 0.0	23.9 ± 20.8

M, ouabain had no effect on larvae exposed to control sand, but 25% of the larvae exposed to ouabain plus sand treated with palmitoleic acid underwent normal metamorphosis, and the remainder underwent abnormal metamorphosis (without attachment or tube formation). At this concentration, ouabain may have some effect on metamorphic activation of *Phragmatopoma lapidosa californica,* but not on larval behavior prior to metamorphosis.

TEA.—Tetraethylammonium chloride (TEA) selectively blocks voltage-dependent K^+ channels in nerve and muscle cells (Hermann and Gorman, 1981; Darnell et al., 1986). TEA inhibited metamorphosis of abalone larvae in response to K^+ but not in response to GABA (Baloun and Morse, 1984). Larvae of *Phestilla sibogae, Phragmatopoma lapidosa californica,* and *Balanus amphitrite* were insensitive to TEA (Yool et al., 1986; Rittschof et al., 1986); this has been confirmed for larvae of *Phragmatopoma lapidosa californica* in the present study (Table 2). In neurophysiological experiments on isolated molluscan neurons, both outward K^+ currents and Ca^{2+}-activated K^+ currents were differentially sensitive to TEA, depending on external or internal application of the compound (Hermann and Gorman, 1981). Without employing similar techniques, the effects of TEA on invertebrate larvae remain ambiguous.

SITS.—Sulfonyl isothiocyanostilbene (SITS; 4-acetamido-4′-isothiocyanato-stilbene-2,2′-disulfonic acid) blocks anion transport across the membranes of human red blood cells (Cabantchik and Rothstein, 1972). In more recent studies of the membranes of other tissues, it has been observed to inhibit ATP-ase activity (Pazoles et al., 1980), glucose-6-phosphatase activity (Zoccoli and Karnovsky, 1980), Na^+ transport (Lee et al., 1983) and ATP-dependent Ca^{2+} uptake (Rangachari et al., 1984). SITS inhibited the larval metamorphosis of *Haliotis rufescens* in response to GABA, but not in response to excess K^+ (Baloun and Morse, 1984). For larvae of *Balanus amphitrite,* SITS also inhibited larval metamorphosis in the presence of a soluble inducing factor (Rittschof et al., 1986). In contrast, larvae of *Phragmatopoma lapidosa californica* were insensitive to SITS; it had no ap-

parent effect on larval response to sand treated with palmitoleic acid or to control sand (Table 2). Given the wide range of effects of this compound reported from studies of various isolated tissue types, any conclusions regarding the action of SITS on whole invertebrate larvae would seem premature.

Picrotoxin.—Picrotoxin, a mixture of the compounds picrotoxinin and picrotin, blocks inhibitory synapses in vertebrates and invertebrates by interfering with Cl^- transport (Gallagher et al., 1978; Yarowsky and Carpenter, 1978). Morse et al. (1980a) found that picrotoxin induced metamorphosis of *Haliotis rufescens* at 10^{-4} M, but had no effect at lower concentrations. In contrast, Rittschof et al. (1986) found that picrotoxin strongly inhibited metamorphosis of *Balanus amphitrite* at 10^{-6}–10^{-5} M. Picrotoxin had no apparent effect on larvae of *Phragmatopoma lapidosa californica* at 10^{-6}–10^{-4} M, whether they were metamorphosing in response to sand treated with palmitoleic acid or swimming in dishes containing control sand (Table 2). The observed variability in larval response between species suggests that different routes to metamorphic activation may be involved.

Responses of Larvae to Compounds that Influence Intracellular cAMP Concentrations

The nucleotide, adenosine-5′-cyclic monophosphate (cAMP), is an important mediator of cellular metabolism and cell-to-cell signalling (Darnell et al., 1986). For β-adrenergic receptors, perhaps the best understood cell surface receptor system, cAMP acts as a signal transducer in modifying the rates of several different enzyme catalyzed reactions.

db-cAMP.—Dibutyryl cAMP (db-cAMP; N^6-2′-O-dibutyryladenosine-3′,5′-cyclic monophosphate) is a derivative of cAMP that is resistant to phosphodiesterase activity; it is capable of passing through membranes and effecting a rise in intracellular cAMP. Administration of db-cAMP has resulted in a variety of effects in assays of specific cell and tissue types, including Ca^{2+} and Na^+ fluxes (Auld et al., 1987; Hwang and Van Breemen, 1987; Lohrmann and Kamemoto, 1987), increases in enzymatic activity (Montiel et al., 1986) and increases in lipid biosynthesis (Vaidya and Khuller, 1988).

Morse et al. (1980b) exposed larvae of *Haliotis rufescens* to db-cAMP at 1 and 5 mM in the presence and absence of 1 mM GABA. There was no apparent effect of db-cAMP at 1 mM after 30 min and 19 h, and at 5 mM after 30 min. After 19 h at 5 mM, however, a mean of 24% (N = 2) of the larvae had undergone "behavioral metamorphosis," although it was also noted that exposure for this period of time was toxic to larvae. Nevertheless, Morse and coworkers concluded that this effect was significant and implicated cAMP in metamorphic activation. More surprisingly, these data were used to support a complex morphogenetic pathway postulated to control settlement and metamorphosis in *H. rufescens* (Trapido-Rosenthal and Morse, 1986b; Baxter and Morse, 1987).

In the present study, larvae of *Phragmatopoma lapidosa californica* did not metamorphose in response to db-cAMP at 1 or 5 mM concentrations ($\bar{x} = 0 \pm 0\%$, N = 3). No mortality or abnormal behavior was noted among larvae exposed to the higher concentration. Although the test concentrations were not mentioned, Jensen (1987) also noted that db-cAMP did not induce metamorphosis of *Phragmatopoma lapidosa californica.* Similarly, db-cAMP was ineffective at stimulating metamorphosis of the mollusc, *Phestilla sibogae* (Hadfield, 1984), and the cnidarian, *Cassiopea andromeda* (Fitt et al., 1987). Rittschof et al. (1986) reported

that db-cAMP induced metamorphosis of *Balanus amphitrite,* but neither assay concentrations nor data were presented.

Cholera Toxin.—The guanine nucleotide-binding proteins (G proteins) are the subject of intense study, as these molecules serve as membrane-bound transducers of receptor-generated signals in the cellular systems in which they have been studied. Cholera toxin acts on the α subunit of one such G protein, the stimulatory regulator of adenylate cyclase (G_s), causing an increase in the intracellular concentration of cAMP. Research on the mechanism of action has revealed an increasingly complex scheme (Gilman, 1987). Cholera toxin, however, is also thought to induce several cellular responses that do not involve cAMP (or G proteins, in some cases; Aksamit et al., 1985; Johnston and Kennedy, 1985), including Ca^{2+} uptake (Maenz et al., 1987) and phosphoinositide phosphodiesterase activity (Xuan et al., 1987; Gilman, 1987).

Larvae of the cnidarian *Cassiopea andromeda,* metamorphosed in response to cholera toxin at 5–40 $\mu g \cdot ml^{-1}$, suggesting cAMP may be involved, although db-cAMP had no effect on *C. andromeda* larvae (Fitt et al., 1987). In the present study, cholera toxin did not induce metamorphosis of *Phragmatopoma lapidosa californica* at 1, 5, 10 or 50 $\mu g \cdot ml^{-1}$. No toxic effects were observed at these concentrations. Fitt et al. (1987) reported highly variable results, depending on the source and preparation of the cholera toxin; with this in mind, assays of larvae of *P. l. californica* were performed at higher concentrations, but with no apparent effects. Baxter and Morse (1987) found that cholera toxin did not induce metamorphosis of *Haliotis rufescens,* although it facilitated larval response to GABA. They considered this further evidence of the involvement of a novel G protein-diacylglycerol cascade regulating metamorphosis induced by GABA. Given the variable effects of the compound on specific cell types, and the complexity of G protein mediated responses (see criteria for involvement of a G protein in Gilman, 1987), it would be premature to propose complex regulatory mechanisms based on the responses of whole invertebrate larvae to cholera toxin.

IBMX.—The xanthine derivative, 3-isobutyl-1-methylxanthine (IBMX), elevates intracellular cAMP levels by inhibiting the phosphodiesterase responsible for the degradation of cAMP. IBMX has also been shown to stimulate Ca^{2+} transport (Deth and Lynch, 1981), particularly in gametes of *Haliotis rufescens* (Kopf et al., 1983), to stimulate adenylate cyclase production of cAMP by directly blocking the inhibitory regulatory protein, G_i (Parsons et al., 1988), and to stimulate enzymatic activity in a mechanism independent of cAMP (Cary and Mendelsohn, 1987).

Morse and co-workers demonstrated that IBMX induced metamorphosis of *Haliotis rufescens* (Trapido-Rosenthal and Morse, 1986b; Baxter and Morse, 1987). They considered this evidence of the involvement of cAMP in metamorphic activation, citing Kopf et al. (1983), but they did not assay for changes in cAMP concentration (*cf.* Fitt et al., 1987) and neglected the confounding effect of IBMX on Ca^{2+} transport. Baloun and Morse (1984) had previously shown that external application of excess Ca^{2+} influenced settlement of *H. rufescens,* although the effect was inhibitory. Two other compounds used by Morse and colleagues to demonstrate the involvement of cAMP, theophylline (Morse et al., 1980b) and forskolin (Baxter and Morse, 1987), also induce concomitant changes in Ca^{2+} transport in abalone sperm (Kopf et al., 1983), and in other systems (Henquin et al., 1983; Guild et al., 1986). In addition, forskolin has been shown to effect cAMP-independent responses in specific cell lines and tissues (Hoshi et al., 1988; Wagoner and Pallotta, 1988).

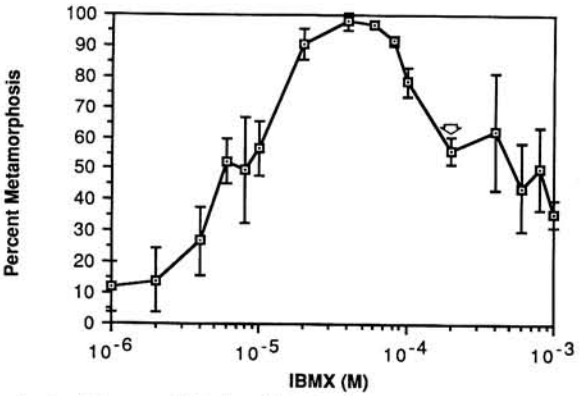


Figure 6. Metamorphosis of larvae of *P. l. californica* in response to seawater containing isobutylmethylxanthine (IBMX) (±SD, N = 3). Toxic effects were observed at concentrations equal to and greater than those indicated by the open arrow.

IBMX was an effective inducer of normal metamorphosis of *Phragmatopoma lapidosa californica* at 10^{-5}–10^{-4} M (Fig. 6). Similar results were obtained by Jensen (1987) for both IBMX and forskolin. Again, she attributed the effects of these compounds to increased internal cAMP levels, even though she also found that db-cAMP had no effect on larvae. Although citing relevant references (Kopf et al., 1983), she similarly neglected the confounding effects of IBMX and forskolin, particularly their effects on Ca^{2+} transport. Her own experiments demonstrated that excess Ca^{2+} induced 10–50% metamorphosis of *P. l. californica* within 48 h (Jensen, 1987).

Like cAMP, Ca^{2+} is an important second messenger, mediating many cellular functions; in some processes the effects of the two are inextricably linked (Darnell et al., 1986). These mediators are very likely involved in the activation of invertebrate larval metamorphosis; indeed, they appear to be involved in most cellular functions. The effects of compounds such as IBMX, cholera toxin and db-cAMP have been rigorously examined on defined cell types. It would not be reasonable to assume that these compounds exert the same effects specifically on the epithelial chemoreceptor cells of the complex neuromuscular system found in an invertebrate larva.

Conclusions

Protein- or peptide-associated neurotransmitter-mimetic molecules derived from amino acids, particularly GABA and DOPA, have been proposed to control the natural induction of settlement and metamorphosis in a wide variety of marine invertebrate larvae (Morse, 1985). Moreover, the common function of these amino acids as potent neurotransmitters has led to the hypothesis that external epithelial chemoreceptors responsible for initiating settlement and metamorphosis are ontogenetically derived from synaptic receptors specific for these compounds (Cooper, 1983). Most theories concerning the origin and evolution of receptor sites and neurotransmitters propose just the opposite: that neurotransmitter (synaptic) receptors evolved from ion-coupled, nutrient transport sites (chemoreceptors) of single-celled organisms (Lenhoff and Heagy, 1977; Boyd, 1979).

Besides the evolutionary incongruity of the concept, three kinds of evidence weaken the neurotransmitter-mimetic hypothesis of invertebrate larval settlement

and metamorphosis. First, for the two cases in which naturally occurring inducers of larval settlement have been isolated and identified, the compounds were unrelated to neurotransmitters: diterpenoid chromanols for the cnidarian, *Coryne uchidai* (Kato et al., 1975) and free fatty acids for the polychaetes *Phragmatopomoa lapidosa californica* and *P. l. lapidosa* (Pawlik, 1986; 1988b). Oyster settlement, originally attributed to DOPA derivatives produced by marine bacteria (Coon et al., 1985; Weiner et al., 1985), is now believed to be triggered by ammonia (Coon et al., 1988). Settlement of the spirorbid polychaete *Janua brasiliensis* is thought to result from the specificity of larval lectins for glycoconjugates produced by film-forming bacteria (Maki and Mitchell, 1985).

Second, although there is considerably more known about chemoreception among terrestrial invertebrates, the literature does not provide any examples of chemical cues structurally related to neurotransmitters (Wilson, 1970; O'Connell, 1986). Insect chemoreception, perhaps the best analogy to invertebrate larval chemoreception, relies primarily on substituted fatty acids, alcohols, ketones and aldehydes (Prestwich, 1985; O'Connell, 1986). The analogy between pheromonal communication in insects and substrate selection by the larvae of gregarious marine invertebrates has been raised by some authors (Burke, 1984; 1986; Pawlik and Faulkner, 1986; 1988). Synthetic analogs of juvenile hormones, sesquiterpenes that regulate metamorphosis in insects, have been shown to induce metamorphosis without attachment of barnacle larvae (Gomez et al., 1973), and similar endogenous compounds have been identified in extracts of crustaceans (Laufer et al., 1987).

Finally, few similarities in the responses of larvae to neuroactive compounds have been found (Table 3). Within the Mollusca alone, larvae respond to choline, but not GABA or DOPA derivatives (*Phestilla sibogae*; Hadfield, 1978), DOPA, but not GABA or choline derivatives (*Crassostrea gigas*; Coon et al., 1985), and GABA, but not choline derivatives or DOPA derivatives (*Haliotis rufescens*; Morse et al., 1979). If these compounds affect external chemoreceptors (and available evidence indicates they do not), the data argue against a common origin of larval chemoreceptors from synaptic receptors for a specific neurotransmitter. On the other hand, if these compounds induce a larval response by circumventing a chemoreceptor and influencing the larval nervous system directly, the data suggest that the neurological basis for metamorphic activation may differ from one species to the next, even within a given phylum. But, because experiments such as those described herein usually involve a behavioral response (settlement or attachment) of whole larvae to bioactive compounds, the site and mechanism of action remains unknown.

Although specific criticisms of the interpretations of the effects of bioactive substances on invertebrate larvae have already been discussed, there is a more general point at issue. Biochemists and neurophysiologists assess *very specific* responses of *very specific* tissues in determining the effects of a given compound. Examples include the application of bioactive compounds to specific crustacean neurons while electrophysiologically monitoring changes in nervous conductance (Hori et al., 1978), to rat liver microsomes while monitoring the enzymatic activity of glucose-6-phosphatase (Zoccoli and Karnovsky, 1980), or to the sperm of *Haliotis rufescens* while monitoring the well-characterized enzymatic and ion-transport changes associated with the acrosome reaction (Kopf et al., 1983). In these studies, models devised to explain the biochemical processes underlying the observed responses are proposed only after exhaustive, controlled experimentation; even then, the models are put forward cautiously, as further research, particularly on different cell lines or tissue types, often brings unexpected effects and

Table 3. Summary of the effects of some of the compounds used in this study on the settlement and metamorphosis of invertebrate larvae. Full reference lists, descriptions of experiments and larval responses are in the text. O = no effect; N = normal settlement and metamorphosis; A = abnormal settlement or metamorphosis; I = inhibition of settlement or metamorphosis. * = low level response; ** = metamorphosis without settlement; *** = settlement without metamorphosis

Compound	Species	Response	Reference
Choline derivatives			
choline Cl	*Haliotis rufescens* (gastropod)	O	Morse et al., 1979
	Phestilla sibogae (gastropod)	N	Hadfield, 1978
	Ilyanassa obsoleta (gastropod)	N*	Levantine and Bonar, 1986
	Phragmatopoma lapidosa californica (polychaete)	A	present study
acetylcholine Cl	*Crassostrea gigas* (bivalve)	O (?)	Coon et al., 1985
	Haliotis rufescens (gastropod)	O	Morse et al., 1979
	Phestilla sibogae (gastropod)	O	Hadfield, 1978
	Ilyanassa obsoleta (gastropod)	N*	Levantine and Bonar, 1986
	Phragmatopoma lapidosa californica (polychaete)	O	present study
succinylcholine Cl	*Crassostrea gigas* (bivalve)	O	Coon et al., 1985
	Phestilla sibogae (gastropod)	N	Bonar, 1976
	Elysia chlorotica (gastropod)	N	Harrigan and Alkon, 1978
	Ilyanassa obsoleta (gastropod)	N*	Levantine and Bonar, 1986
	Phragmatopoma lapidosa californica (polychaete)	A	present study
DOPA and catecholamines			
L-DOPA	*Crassostrea gigas* (bivalve)	N	Cooper, 1983
	Mytilus edulis (bivalve)	N	Cooper, 1982
	Pecten maximus (bivalve)	N	Cochard et al., 1989
	Ilyanassa obsoleta (gastropod)	O	Levantine and Bonar, 1986
	Phragmatopoma lapidosa californica (polychaete)	N*	Jensen, 1987; present study
D-DOPA	*Crassostrea gigas* (bivalve)	O	Coon et al., 1985
	Phragmatopoma lapidosa californica (polychaete)	N*	present study
dopamine	*Crassostrea gigas* (bivalve)	O	Coon et al., 1985
	Phestilla sibogae (gastropod)	A*	Hadfield, 1984
	Ilyanassa obsoleta (gastropod)	N*	Levantine and Bonar, 1986
	Phragmatopoma lapidosa californica (polychaete)	O	present study
norepinephrine	*Crassostrea gigas* (bivalve)	A**	Coon et al., 1985
	Pecten maximus (bivalve)	O	Cochard et al., 1989
	Phestilla sibogae (gastropod)	O	Hadfield, 1984
	Ilyanassa obsoleta (gastropod)	O	Levantine and Bonar, 1986

Table 3. Continued

Compound	Species	Response	Reference
	Haliotis rufescens (gastropod)	O	Morse et al., 1979
	Phragmatopoma lapidosa californica (polychaete)	O	present study
epinephrine	*Crassostrea gigas* (bivalve)	A**	Coon et al., 1985
	Pecten maximus (bivalve)	N	Cochard et al., 1989
	Phestilla sibogae (gastropod)	A*	Hadfield, 1984
	Ilyanassa obsoleta (gastropod)	O	Levantine and Bonar, 1986
	Haliotis rufescens (gastropod)	O	Morse et al., 1979
	Phragmatopoma lapidosa californica (polychaete)	O	present study
Other amino acid derivatives			
GABA	*Agaricia* spp. (coral)	O	Morse et al., 1988
	Alcyonium siderium (coral)	O	Sebens, 1983
	Katharina tunicata (chiton)	A***	Rumrill and Cameron, 1983
	Crassostrea gigas (bivalve)	O	Coon et al., 1985
	Mytilus edulis (bivalve)	O	Cooper, 1982
	Phestilla sibogae (gastropod)	O	Hadfield, 1984
	Haliotis rufescens (gastropod)	N	Morse et al., 1979
	Haliotis discus hannai (gastropod)	A***	Akashigi et al., 1981
	Crepidula fornicata (gastropod)	O	Pechenik and Heyman, 1987
	Phragmatopoma lapidosa californica (polychaete)	O	present study
	Strongylocentrotus droebachiensis (urchin)	N	Pearce and Scheibling, 1988
serotonin	*Crassostrea gigas* (bivalve)	O	Coon et al., 1985
	Phestilla sibogae (gastropod)	O	Hadfield, 1984
	Ilyanassa obsoleta (gastropod)	N	Levantine and Bonar, 1986
	Haliotis rufescens (gastropod)	O	Morse et al., 1979
	Phragmatopoma lapidosa californica (polychaete)	A*	present study
Compounds that affect transmembrane ion transport			
ouabain	*Hydractinia echinata* (hydroid)	I	Müller, 1973
	Phragmatopoma lapidosa californica (polychaete)	O	present study
TEA	*Haliotis rufescens* (gastropod)	I	Baloun and Morse, 1984
	Phestilla sibogae (gastropod)	O	Yool et al., 1986

Table 3. Continued

Compound	Species	Response	Reference
	Phragmatopoma lapidosa californica (polychaete)	O	Yool et al., 1986
	Balanus amphitrite (barnacle)	O	Rittschof et al., 1986
SITS	*Haliotis rufescens* (gastropod)	I	Baloun and Morse, 1984
	Phragmatopoma lapidosa californica (polychaete)	O	present study
	Balanus amphitrite (barnacle)	I	Rittschof et al., 1986
picrotoxin	*Haliotis rufescens* (gastropod)	N	Morse et al., 1980b
	Phragmatopoma lapidosa californica (polychaete)	O	present study
	Balanus amphitrite (barnacle)	I	Rittschof et al., 1986
Compounds that affect intracellular cAMP			
db-cAMP	*Cassiopea andromeda* (medusa)	O	Fitt et al., 1978
	Haliotis rufescens (gastropod)	N* (?)	Morse et al., 1980b
	Phestilla sibogae (gastropod)	O	Hadfield, 1984
	Phragmatopoma lapidosa californica (polychaete)	O	Jensen, 1987; present study
	Balanus amphitrite (barnacle)	N	Rittschof et al., 1986
cholera toxin	*Cassiopea andromeda* (medusa)	N	Fitt et al., 1987
	Haliotis rufescens (gastropod)	O	Baxter and Morse, 1987
	Phragmatopoma lapidosa californica (polychaete)	O	present study
IBMX	*Agaricia* spp. (coral)	O	Morse et al., 1988
	Haliotis rufescens (gastropod)	N	Baxter and Morse, 1987
	Phragmatopoma lapidosa californica (polychaete)	N	Jensen, 1987; present study

greater complexity (Yarowsky and Carpenter, 1978; Chapter 12 in Kuffler et al., 1984; Gilman, 1987).

By comparison, assays of the effects of bioactive compounds on the responses of invertebrate larvae are non-specific, both in the application of the compound and in the assessment of the response. First, because the compounds are being tested on whole animals, all larval surface receptors, controlling a myriad of responses, are exposed to the compound. Inasmuch as the peripheral nervous system of some larvae is intra-epithelial (Lacalli, 1988), these nerves are also likely affected. As most larval assays are run for several hours to days, internal tissues may be influenced after uptake of the compound by ingestion or direct transfer across the larval epithelium. Second, larval assays generally measure the percentage of larvae that have undergone attachment or settlement (a behavioral response) and/or metamorphosis (a morphogenetic response). This is considerably less rigorous than measuring a change in membrane conductance or enzyme activity. For example, normal settlement can be mimicked by narcotizing larvae (Akashigi et al., 1981), settlement can occur without metamorphosis (Rumrill and Cameron, 1983), metamorphosis can occur without settlement (Coon et al., 1985), and various forms of abnormal metamorphosis can occur (this study). With proper caution, well controlled studies of the effects of bioactive compounds on larvae can yield important information (Hirata and Hadfield, 1986). But it is untenable to propose complex pathways detailing the molecular control of metamorphic activation based on the responses of whole larvae to compounds that are known to have multiple effects on specific cells and tissues.

Greater effort might instead be directed toward the isolation and identification of naturally-occurring settlement inducers for a wider range of invertebrate species. Recent advances in chemical isolation techniques and cross-disciplinary collaborations between chemists and biologists may promote new discoveries and reveal general patterns of the classes of compounds perceived by larvae at the time of settlement. Isolation of larval chemosensory organs, cells or receptors by electrophysiological or chemical means would allow for more rigorous assessments of the effects of both natural and artificial inducers.

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Literature cited

Akashigi, S., T. Seki, H. Kan-no and T. Nomura. 1981. Effects of γ-aminobutyric acid and certain neurotransmitters on the settlement and the metamorphosis of the larvae of *Haliotis discus hannai* Ino (Gastropoda). Bull. Tohoku Reg. Fish. Res. Lab. 43: 37–45.

Aksamit, R. R., P. S. Backlund, Jr. and G. L. Cantoni. 1985. Cholera toxin inhibits chemotaxis by a cAMP-independent mechanism. Proc. Natl. Acad. Sci. USA 82: 7475–7479.

Auld, A. M., J. Aspinall and G. J. Barritt. 1987. Effects of verapamil, dibutyryl cyclic AMP, and extracellular calium on intracellular free calcium concentrations in myocytes isolated from rat ventricles. Cardiovascular Research 21: 772–778.

Baloun, A. J. and D. E. Morse. 1984. Ionic control of settlement and metamorphosis in larval *Haliotis rufescens* (Gastropoda). Biol. Bull. 167: 124–138.

Barlow, L. 1988. A comparison of the behavioral and physiological effects of GABA and coralline algae on the larvae of the red abalone. Amer. Zool. 28: 70A (abstract).

Baxter, G. and D. E. Morse. 1987. G protein and diacylglycerol regulate metamorphosis of planktonic molluscan larvae. Proc. Natl. Acad. Sci. USA 84: 1867–1870.
Blusztajn, J. K. and R. J. Wurtman. 1983. Choline and cholinergic neurons. Science 221: 614–620.
Bonar, D. B. 1976. Molluscan metamorphosis: a study in tissue transformation. Amer. Zool. 16: 573–591.
———, S. L. Coon, R. M. Weiner and R. R. Colwell. 1985. Induction of oyster metamorphosis by bacterial products and biogeneic amines. Bull. Mar. Sci. 37: 763 (abstract).
Boyd, C. A. R. 1979. Chemical neurotransmission: an hypothesis concerning the evolution of neurotransmitter substances. J. Theor. Biol. 76: 415–417.
Burke, R. D. 1983. Neural control of metamorphosis in *Dendraster excentricus*. Biol. Bull. 164: 176–188.
———. 1984. Pheromonal control of metamorphosis in the Pacific sand dollar, *Dendraster excentricus*. Science 225: 442–443.
———. 1986. Pheromones and the gregarious settlement of marine invertebrate larvae. Bull. Mar. Sci. 39: 323–331.
Cabantchik, Z. I. and A. Rothstein. 1972. The nature of the membrane sites controlling anion permeability of human red blood cells as determined by studies with disulfonic stilbene derivatives. J. Membrane Biol. 10: 311–330.
Cameron, R. A. and R. T. Hinegardner. 1974. Initiation of metamorphosis in laboratory cultured sea urchins. Biol. Bull. 146: 335–342.
Cary, D. A. and F. A. O. Mendelsohn. 1987. Effect of forskolin, isoproterenol and IBMX on angiotensin converting enzyme and cyclic AMP production by cultured bovine endothelial cells. Mol. Cell. Endocrinology 53: 103–109.
Cochard, J. C., L. Chevolot, J. C. Yvin and A. M. Chevolot-Magueur. 1989. Induction de la metamorphose de la coquille Saint Jacques *Pecten maximus* L. par des derives de la tyrosine extraits de l'algue *Delesseria sanguinea* Lamoroux ou synthetiques. Haliotis 19: 129–154.
Coon, S. L. and D. B. Bonar. 1986. Norepinephrine and dopamine content of larvae and spat of the Pacific oyster, *Crassostrea gigas*. Biol. Bull. 171: 632–639.
——— and ———. 1987a. Pharmacological evidence that alpha$_1$-adrenoceptors mediate metamorphosis of the Pacific oyster, *Crassostrea gigas*. Neuroscience 23: 1169–1174.
——— and ———. 1987b. The role of DOPA and dopamine in oyster settlement behavior. Amer. Zool. 27: 128A (abstract).
———, ——— and R. M. Weiner. 1985. Induction of settlement and metamorphosis of the Pacific oyster, *Crassostrea gigas* (Thunberg) by L-DOPA and catecholamines. J. Exp. Mar. Biol. Ecol. 94: 211–221.
———, M. Walch, W. K. Fitt, D. B. Bonar and R. M. Weiner. 1988. Induction of settlement behavior in oyster larvae by ammonia. Amer. Zool. 28: 70A (abstract).
Cooper, K. 1982. A model to explain the induction of settlement and metamorphosis of planktonic eyed-pediveligers of the blue mussel *Mytilus edulis* L. by chemical and tactile cues. J. Shellfish Res. 2: 117 (abstract).
———. 1983. Potential for application of the chemical DOPA to commercial bivalve setting systems. J. Shellfish Res. 3: 110–111 (abstract).
Darnell, J., H. Lodish and D. Baltimore. 1986. Molecular cell biology. Scientific American Books, New York. 1,187 pp.
Deth, R. C. and C. J. Lynch. 1981. Mobilization of a common source of smooth muscle Ca^{2+} by norepinephrine and methylxanthines. Am. J. Physiol. 240: C239–C247.
Ebert, E. E., and J. L. Houk. 1984. Elements and innovations in the cultivation of red abalone *Haliotis rufescens*. Aquaculture 39: 375–392.
Eyster, L. S. and J. A. Pechenik. 1987. Attachment of *Mytilus edulis* L. larvae on algal and byssal filaments is enhanced by water agitation. J. Exp. Mar. Biol. Ecol. 114: 99–110.
Fitt, W. K., D. K. Hofmann, M. Wolk and M. Rahat. 1987. Requirement of exogenous inducers for metamorphosis of axenic larvae and buds of *Cassiopea andromeda* (Cnidaria: Scyphozoa). Mar. Biol. 94: 415–422.
Gallagher, J. P., H. Higashi and S. Nishi. 1978. Characterization and ionic basis of GABA-induced depolarizations recorded *in vitro* from cat primary afferent neurones. J. Physiol. 275: 263–282.
Gilman, A. 1987. G proteins: transducers of receptor-generated signals. Ann. Rev. Biochem. 56: 615–649.
Gomez, E. D., D. J. Faulkner, W. A. Newman and C. Ireland. 1973. Juvenile hormone mimics: effect on cirriped crustacean metamorphosis. Science 179: 813–814.
Grant, J. F. 1981. Abalone culture in Japan: development and current commercial practice. Tasmanian Fish. Res. 23: 2–17.
Guild, S., Y. Itoh, J. W. Kebabian, A. Luini and T. Reisine. 1986. Forskolin enhances basal and

potassium-evoked hormone release from normal and malignant pituitary tissue: the role of calcium. Endocrinology 118: 268–279.

Hadfield, M. G. 1978. Metamorphosis in marine molluscan larvae: an analysis of stimulus and response. Pages 165–175 in F. S. Chia and M. E. Rice, eds. Settlement and metamorphosis of marine invertebrate larvae. Elsevier, New York.

———. 1984. Settlement requirements of molluscan larvae: new data on chemical and genetic roles. Aquaculture 39: 283–298.

——— and R. H. Karlson. 1969. Externally induced metamorphosis in a marine gastropod. Amer. Zool. 9: 1122 (abstract).

——— and D. Scheuer. 1985. Evidence for a soluble metamorphic inducer in *Phestilla*: ecological, chemical and biological data. Bull. Mar. Sci. 37: 556–566.

Hahn, K. O. 1989. Handbook of culture of abalone and other marine gastropods. CRC Press, Boca Raton, Florida. 348 pp.

Harrigan, J. F. and D. L. Alkon. 1978. Laboratory cultivation of *Haminoea solitaria* (Say, 1822) and *Elysia chlorotica* (Gould, 1870). Veliger 21: 299–305.

Henquin, J.-C., W. Schmeer and H. P. Meissner. 1983. Forskolin, an activator of adenylate cyclase, increases Ca^{2+}-dependent electrical activity induced by glucose in mouse pancreatic B cells. Endocrinology 112: 2218–2220.

Hermann, A. and A. L. F. Gorman. 1981. Effects of tetraethylammonium on potassium currents in a molluscan neuron. J. Gen. Physiol. 78: 87–110.

Hirata, K. Y. and M. G. Hadfield. 1986. The role of choline in metamorphic induction of *Phestilla* (Gastropoda, Nudibranchia). Comp. Biochem. Physiol. 84C: 15–21.

Hopkins, T. L., T. D. Morgan, Y. Aso and K. J. Kramer. 1982. N-β-alanyldopamine: major role in insect cuticle tanning. Science 217: 364–366.

Hori, N., K. Ikeda and E. Roberts. 1978. Muscimol, GABA and picrotoxin: effects on membrane conductance of a crustacean neuron. Brain Research 141: 364–370.

Hoshi, T., S. S. Garber and R. W. Aldrich. 1988. Effect of forskolin on voltage-gated K^+ channels is independent of adenylate cyclase activation. Science 240: 1652–1655.

Hwang, K. S. and C. Van Breemen. 1987. Effect of dB-c-AMP and forskolin on the ^{45}Ca influx, net Ca uptake and tension in rabbit aortic smooth muscle. European J. Pharm. 134: 155–162.

Jensen, R. A. 1987. Factors affecting the settlement, metamorphosis and distribution of larvae of the marine polychaete *Phragmatopoma californica* (Fewkes). Ph.D. Dissertation, University of California, Santa Barbara. 175 pp.

——— and D. E. Morse. 1984. Intraspecific facilitation of larval recruitment: gregarious settlement of the polychaete *Phragmatopoma californica* (Fewkes). J. Exp. Mar. Biol. Ecol. 83: 107–126.

Johnston, M. E. A. and T. G. Kennedy. 1985. Temporal desensitization of rat uteri for the decidual cell reaction is abolished by cholera toxin acting by a mechanism apparently not involving adenosine 3′:5′-cyclic monophosphate. Can. J. Physiol. Pharmacol. 63: 1052–1056.

Kato, T., A. S. Kumanireng, I. Ichinose, Y. Kitahara, Y. Kakinuma, M. Nishihira and M. Kato. 1975. Active components of *Sargassum tortile* effecting the settlement of swimming larvae of *Coryne uchidai*. Experientia 31: 433–434.

Knight-Jones, E. W. 1953. Laboratory experiments on gregariousness during setting in *Balanus balanoides* and other barnacles. J. Exp. Biol. 30: 584–599.

Kopf, G. S., C. A. Lewis and V. D. Vacquier. 1983. Methylxanthines stimulate calcium transport and inhibit cyclic nucleotide phosphodiesterases in abalone sperm. Develop. Biol. 99: 115–120.

Kuffler, S. W., J. G. Nicholls and A. R. Martin. 1984. From neuron to brain. Sinauer Associates, Sunderland, Massachusetts. 651 pp.

Lacalli, T. C. 1988. The suboral complex in the Müller's larva of *Pseudoceros canadensis* (Platyhelminthes, Polycladida). Can. J. Zool. 66: 1893–1895.

Laufer, H., D. Borst, F. C. Baker, C. Carrasco, M. Sinkus, C. C. Reuter, L. W. Tsai and D. A. Schooley. 1987. Identification of a juvenile hormone-like compound in a crustacean. Science 235: 202–205.

Lee, S. M., M. R. An, S. I. Lee and Y. S. Park. 1983. Effects of SITS on sodium transport, oxygen consumption and sodium-potassium-ATPase of the frog skin. Taehan Saengri Hakhoe Chi 17: 55–61.

Leighton, D. L. 1988. Abalone mariculture in California and related San Diego-based research. Ann. Report, Western Soc. of Malacologists 20: 21–25.

Lenhoff, H. M. and W. Heagy. 1977. Aquatic invertebrates: model systems for study of receptor activation and evolution of receptor proteins. Ann. Rev. Pharmacol. Toxicol. 17: 243–258.

Levantine, P. L. and D. B. Bonar. 1986. Metamorphosis of *Ilyanassa obsoleta*: natural and artificial inducers. Amer. Zool. 26: 14A (abstract).

Lindner, E. and C. A. Dooley. 1976. Studies of the reaction mechanism of the adhesive of barnacles.

Proceedings of the 4th International Congress on Marine Corrosion and Fouling, Antibes, Juan-les-Pins, France. pp. 333–344.

Lohrmann, D. M. and F. I. Kamemoto. 1987. The effect of dibutyryl cAMP on sodium uptake by isolated perfused gills of *Callinecties sapidus*. Gen. Comp. Endocrinology 65: 300–305.

Lynch, W. F. 1952. Factors influencing metamorphosis of *Bugula* larvae. Biol. Bull. 103: 369–383.

Maenz, D. D., S. E. Gabriel and G. W. Forsyth. 1987. Calcium transport affinity, ion competition and cholera toxin effects on cytosolic Ca concentration. J. Membrane Biol. 96: 243–249.

Maki, J. S. and R. Mitchell. 1985. Involvement of lectins in the settlement and metamorphosis of marine invertebrate larvae. Bull. Mar. Sci. 37: 675–683.

Marsden, J. R. and H. Hassessian. 1986. Effects of Ca^{2+} and catecholamines of swimming cilia of the trochophore larva of the polychaete *Spirobranchus giganteus* (Pallas). J. Exp. Mar. Biol. Ecol. 95: 245–255.

McShane, P. E., K. P. Black and M. G. Smith. 1988. Recruitment processes in *Haliotis rubra* (Mollusca: Gastropoda) and regional hydrodynamics in southeastern Australia imply localized dispersal of larvae. J. Exp. Mar. Biol. Ecol. 124: 175–203.

Merck Index. 1983. 10th Edition. Merck and Company, Rahway, New Jersey. pp. 215–216.

Mitterer, R. M. 1971. Comparative amino acid composition of calcified and non-calcified polychaete worm tubes. Comp. Biochem. Physiol. 38B: 405–409.

Montiel, F., A. Aranda, A. Villa and A. Pascual. 1986. Regulation of glycerol phosphate dehydrogenase and lactate dehydrogenase activity by forskolin and dibutyryl cyclic AMP in the C6 glial cells. J. Neurochem. 47: 1336–1343.

Morse, A. N. C. 1988. The role of algal metabolites in the recruitment process. Pages 463–473 in M. F. Thompson, R. Sarojini and R. Nagabhushanam, eds. Marine biodeterioration. Advanced techniques applicable to the Indian Ocean. Oxford and IBH, New Delhi.

——— and D. E. Morse. 1984a. Recruitment and metamorphosis of *Haliotis* larvae induced by molecules uniquely available at the surfaces of crustose red algae. J. Exp. Mar. Biol. Ecol. 75: 191–215.

——— and ———. 1984b. GABA-mimetic molecules from *Porphyra* (Rhodophyta) induce metamorphosis of *Haliotis* (Gastropoda) larvae. Hydrobiologia 116: 155–158.

———, C. A. Froyd and D. E. Morse. 1984. Molecules from cyanobacteria and red algae that induce larval settlement and metamorphosis in the mollusc *Haliotis rufescens*. Mar. Biol. 81: 293–298.

Morse, D. E. 1984. Biochemical and genetic engineering for improved production of abalones and other valuable molluscs. Aquaculture 39: 263–282.

———. 1985. Neurotransmitter-mimetic inducers of larval settlement and metamorphosis. Bull. Mar. Sci. 37: 697–706.

———. 1988. Trigger and amplifier pathways: sensory receptors, transducers, and molecular mechanisms controlling larval settlement, adhesion, and metamorphosis in response to environmental chemical signals. Pages 453–462 *in* M. F. Thompson, R. Sarojini and R. Nagabhushanam, eds. Marine biodeterioration. Advanced techniques applicable to the Indian Ocean. Oxford and IBH, New Delhi.

——— and A. N. C. Morse. 1988. Chemical signals and molecular mechanisms: learning from larvae. Oceanus 31: 37–43.

———, N. Hooker and H. Duncan. 1980a. GABA induces metamorphosis in *Haliotis*, V: Stereochemical specificity. Brain Res. Bull. 5(2): 381–387.

———, ———, ——— and L. Jensen. 1979. γ-Aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. Science 204: 407–410.

———, ———, A. N. C. Morse and R. Jensen. 1988. Control of larval metamorphosis and recruitment in sympatric agariciid corals. J. Exp. Mar. Biol. Ecol. 116: 193–217.

———, H. Duncan, N. Hooker, A. Baloun and G. Young. 1980b. GABA induces behavioral and developmental metamorphosis in planktonic molluscan larvae. Fed. Proc. 39: 3237–3241.

———, M. Tegner, H. Duncan, N. Hooker, G. Trevelyan and A. Cameron. 1980c. Induction of settling and metamorphosis of planktonic molluscan (*Haliotis*) larvae. III: Signalling by metabolites of intact algae is dependent on contact. Pages 67–86 *in* D. Müller-Schwarze and R. M. Silverstein, eds. Chemical signals. Plenum Press, New York.

Müller, W. A. 1973. Metamorphose-Induktion bei Planulalarven. I. Der bakterielle Induktor. Wilhelm Roux' Archiv 173: 107–121.

——— and G. Buchal. 1973. Metamorphose-Induktion bei Planulalarven. II. Induktion durch monovalente Kationen: Die Bedeutung des Gibbs-Donnan-Verhaltnisses und der Na^+/K^+-ATPase. Wilhelm Roux' Archiv 173: 122–135.

Nishihira, M. 1968. Brief experiments on the effect of algal extracts in promoting the settlement of the larvae of *Coryne uchidai* Stechow (Hydrozoa). Bull. Mar. Biol. Station Asamushi 13: 91–101.

O'Connell, R. J. 1986. Chemical communication in invertebrates. Experientia 42: 232–241.

Parsons, W. J., V. Ramkumar and G. L. Stiles. 1988. Isobutylmethylxanthine stimulates adenylate cyclase by blocking the inhibitory regulatory protein G_i. Mol. Pharmacol. 34: 37–41.

Pawlik, J. R. 1986. Chemical induction of larval settlement and metamorphosis in the reef-building tube worm *Phragmatopoma californica* (Polychaeta: Sabellariidae). Mar. Biol. 91: 59–68.

———. 1988a. Larval settlement and metamorphosis of two gregarious sabellariid polychaetes: *Sabellaria alveolata* compared with *Phragmatopoma californica*. J. Mar. Biol. Ass. U.K. 68: 101–124.

———. 1988b. Larval settlement and metamorphosis of sabellariid polychaetes, with special reference to *Phragmatopoma lapidosa*, a reef-building species, and *Sabellaria floridensis*, a non-gregarious species. Bull. Mar. Sci. 43: 41–60.

———. 1988c. Chemical induction of the larval settlement of honeycomb worms (Polychaeta, Sabellariidae). Ph.D. Dissertation, University of California, San Diego. 115 pp.

——— and D. J. Faulkner. 1986. Specific free fatty acids induce larval settlement and metamorphosis of the reef-building tube worm *Phragmatopoma californica* (Fewkes). J. Exp. Mar. Biol. Ecol. 102: 301–310.

——— and ———. 1988. The gregarious settlement of sabellariid polychaetes: new perspectives on chemical cues. Pages 475–487 *in* M. F. Thompson, R. Sarojini and R. Nagabhushanam, eds. Marine biodeterioration. Advanced techniques applicable to the Indian Ocean. Oxford and IBH, New Delhi.

Pazoles, C. J., C. E. Creutz, A. Ramu and H. B. Pollard. 1980. Permeant anion activation of MgATPase activity in chromaffin granules: evidence for direct coupling of proton and anion transport. J. Biol. Chem. 255: 7863–7869.

Pearce, C. M. and R. E. Scheibling. 1988. Larval settlement in the green sea urchin, *Strongylocentrotus droebachiensis*. Amer. Zool. 28: 71A (abstract).

Pechenik, J. A. and W. D. Heyman. 1987. Using KCl to determine size at competence for larvae of the marine gastropod *Crepidula fornicata* (L.). J. Exp. Mar. Biol. Ecol. 112: 27–38.

Prestwich, G. D. 1985. Communication in insects II. Molecular communication of insects. Quart. Rev. Biol. 60: 437–456.

Prince, J. D., T. L. Sellers, W. B. Ford and S. R. Talbot. 1987. Experimental evidence for limited dispersal of haliotid larvae (genus *Haliotis*; Mollusca: Gastropoda). J. Exp. Mar. Biol. Ecol. 106: 243–263.

———, ———, ——— and ———. 1988. Confirmation of a relationship between the localized abundance of breeding stock and recruitment for *Haliotis rubra* Leach (Mollusca: Gastropoda). J. Exp. Mar. Biol. Ecol. 122: 91–104.

Rangachari, P. K., A. K. Grover and E. E. Daniel. 1984. Effect of disulfonic stilbenes on Ca^{2+} transport in smooth muscle plasma membranes. Can. J. Physiol. Pharmacol. 62: 1233–1238.

Rittschof, D., J. Maki, R. Mitchell and J. D. Costlow. 1986. Ion and neuropharmacological studies of barnacle settlement. Netherlands J. Sea Research 20: 269–275.

Rumrill, S. S. and R. A. Cameron. 1983. Effects of gamma-aminobutyric acid on the settlement of the black chiton *Katharina tunicata*. Mar. Biol. 72: 243–247.

Satterlie, R. A. and R. A. Cameron. 1985. Electrical activity at metamorphosis in larvae of the sea urchin *Lytechinus pictus* (Echinoidea: Echinodermata). J. Exp. Zool. 235: 197–204.

Sebens, K. P. 1983. Settlement and metamorphosis of a temperate soft-coral larva (*Alcyonium siderium* Verrill): induction by crustose algae. Biol. Bull. 165: 286–304.

Slattery, M. 1987. Settlement and metamorphosis of red abalone (*Haliotis rufescens*) larvae: a critical examination of mucous, diatoms, and γ-aminobutyric acid (GABA) as inductive substrates. M.A. Thesis, San Jose State University, California. 31 pp.

Spindler, K.-D. and W. A. Müller. 1972. Induction of metamorphosis by bacteria and by a lithium-pulse in the larvae of *Hydractinia echinata* (Hydrozoa). Wilhelm Roux' Archiv 169: 271–280.

Trapido-Rosenthal, H. G. and D. E. Morse. 1985. L-α-ω-diamino acids facilitate GABA induction of larval metamorphosis in a gastropod mollusc (*Haliotis rufescens*). J. Comp. Physiol. B 155: 403–414.

——— and ———. 1986a. Regulation of receptor-mediated settlement and metamorphosis in larvae of a gastropod mollusc (*Haliotis rufescens*). Bull. Mar. Sci. 39: 383–392.

——— and ———. 1986b. Availability of chemosensory receptors is down-regulated by habituation of larvae to a morphogenetic signal. Proc. Natl. Acad. Sci. USA 83: 7658–7662.

Vaidya, S. and G. K. Khuller. 1988. Effect of dibutyryl cyclic AMP on lipid synthesis in *Microsporum gypseum*. Biochim. Biophys. Acta 960: 435–440.

Veitch, F. P. and H. Hidu. 1971. Gregarious setting in the American oyster *Crassostrea virginica* Gmelin: I. Properties of a partially purified "setting factor." Chesapeake Sci. 12: 173–178.

Wagoner, P. K. and B. S. Pallotta. 1988. Modulation of acetylcholine receptor desensitization by forskolin is independent of cAMP. Science 240: 1655–1657.

Waite, J. H. 1983. Adhesion in byssally attached bivalves. Biol. Rev. 58: 209–231.

Weiner, R. M., A. M. Segall and R. R. Colwell. 1985. Characterization of a marine bacterium associated with *Crassostrea virginica* (the eastern oyster). Appl. Environ. Microbiology. Jan. 1985, pp. 83–90.

Wilson, D. P. 1968. The settlement behaviour of the larvae of *Sabellaria alveolata* (L.). J. Mar. Biol. Ass. U.K. 48: 387–435.

Wilson, E. O. 1970. Chemical communication within animal species. Pages 133–155 *in* E. Sondheimer and J. B. Simeone, eds. Chemical Ecology. Academic Press, New York.

Xuan, Y.-T., Y.-F. Su, K.-J. Chang and W. D. Watkins. 1987. A pertussis/cholera toxin sensitive G-protein may mediate vasopressin-induced inositol phosphate formation in smooth muscle cell. Biochem. Biophys. Res. Comm. 146: 898–906.

Yarowsky, P. J. and D. O. Carpenter. 1978. A comparison of similar ionic responses to γ-aminobutyric acid and acetylcholine. J. Neurophys. 41: 531–541.

Yool, A. J., S. M. Grau, M. G. Hadfield, R. A. Jensen, D. A. Markell and D. E. Morse. 1986. Excess potassium induces larval metamorphosis in four marine invertebrate species. Biol. Bull. 170: 255–266.

Zoccoli, M. A. and M. L. Karnovsky. 1980. Effect of two inhibitors of anion transport on the hydrolysis of glucose 6-phosphate by rat liver microsomes: covalent modification of the glucose 6-P transport component. J. Biol. Chem. 255: 1113–1119.

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