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Regulatory effects of mussel (*Aulacomya maoriana* Iredale 1915) larval settlement by neuroactive compounds, amino acids and bacterial biofilms

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ABSTRACT

Larval settlement responses of the ribbed mussel, *Aulacomya maoriana* Iredale 1915, were investigated after exposure to various chemicals and mono-species bacteria. Identification of settlement inductive compounds assists in the elucidation of intermediary biochemical mechanisms involved in the neuronal control of settlement behaviour downstream from primary cue reception. Neuroactive compounds and amino acids (potassium ions, GABA, acetylcholine, L-Phenylalanine, L-Tyrosine, dopamine, epinephrine, L-Tryptophan, and 5-HTP) and planktonic bacteria, biofilms and biofilm exudates of *Macrococcus* sp. AMGM1, *Bacillus* sp. AMGB1, and *Pseudoalteromonas* sp. AMGP1 were tested for their abilities to induce larval settlement. Toxicity effects of each treatment also were simultaneously identified by recording larval mortalities. Results indicate that all chemicals used induced larvae to settle, with acetylcholine being the most effective (~24% at 10^{-6} M compared to <2% in control assays). Toxicities of treatment compounds were low at optimal settlement inducing concentrations, except for L-Tryptophan (~32%) and GABA (~59%). Our data suggest that catecholamines (and their precursors) play an important role in the biochemical mechanisms of settlement for *A. maoriana*. While serotonin precursors did induce low levels of larval settlement at some concentrations, high toxicity responses to 5-HTP at 10^{-5} M, combined with complete settlement inhibition indicate that the mechanism of action may be more complex than can be elucidated in this study. Larval settlement responses to bacterial treatments were low for planktonic and biofilm phases across all three strains, and settlement inhibition was observed when larvae were exposed to biofilm exudates of all bacterial strains. Comparisons of *A. maoriana* responses to other endemic and worldwide distributed mussel species are provided as a means to highlight potential evolutionary differences in chemoreception mechanisms.

1. Introduction

A great majority of marine invertebrates has planktonic larvae, which after a period of minutes to months (Hadfield and Paul, 2001) settle onto benthic substrates. A wide range of environmental and biological stimuli or cues mediate this settlement process (Hadfield, 2011; Harder et al., 2002; Pawlik, 1992; Steinberg et al., 2002; Wikstrom and Pavia, 2004). Chemoreception involves the binding of chemicals to receptors in the neural tissues of larvae, which activate neuronal networks (Hay, 2009). Factors which regulate larval settlement behaviour have been investigated extensively for many marine taxa (reviewed by Hadfield and Paul, 2001; Steinberg and De Nys, 2002; Murthy et al., 2009), but the complex chemoreception process has yet to be elucidated. For example, within the Class Bivalvia, many cues have been found to induce larval settlement in oysters (Tamburri et al., 2008; Yu et al., 2008; Yu et al., 2010a), scallops (Leyton and Riquelme, 2008; Mesías-Gansbiller et al., 2008), clams (García-Lavandeira et al., 2005; Neo et al., 2009; Sumin et al., 2006), and mussels (Alfaro et al., 2006; Bao et al., 2007; Dobretsov and Qian, 2003; Ganesan et al., 2010; Young, 2009). Settlement responses to different cues appear to be genus-, species- and even intraspecies-specific (Ritson-Williams et al., 2010; Rodríguez et al., 1993; Williams et al., 2008). These differences suggest evolutionary variations in cue-binding receptors, endogenous biochemical processes, and the metabolites produced during the settlement process.

Chemical compounds that mediate larval settlement often are produced by bacteria or the biofilms they form on just about every surface in the marine environment (Hadfield and Paul, 2001). The chemical cues generated by bacteria may be surface-bound (bound to bacterial cells or exopolymeric substances) or water-soluble (produced by free-swimming planktonic bacteria or released by their biofilms) (Hadfield, 2011). The surface-bound cues induce larval settlement only when larvae come into contact with the bacteria (Hadfield, 2011). On the other hand, water-soluble cues (e.g., low and high molecular weight polyshaccharides [Dobretsov and Qian, 2004; Harder et al., 2004], low molecular weight peptides [Tamburri et al., 1992; Zimmer-Faust and Tamburri, 1994], and even neurotransmitters [Mountfort and Pybus, 1992]) may regulate larval settlement without the need for them to contact the substrate (Browne and Zimmer, 2001; Tamburri et al., 1996). For example, studies on the green-lipped

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mussel, *Perna canaliculus*, showed that water-soluble chemical cues produced by the biofilm of two bacterial strains (*Macrococcus* sp. [AMGM1] and *Bacillus* sp. [AMGB1]) induced larval settlement (Ganesan et al., 2010).

While settlement cues for various taxa include those derived from marine biofilms, the specific molecular characteristics of these inductors remain elusive. A commonly applied method for gaining insight into the endogenous mechanisms of cue reception is to agonise or antagonise particular endogenous receptor classes with pharmacologically active compounds. These chemicals are likely to act directly at some intermediate site downstream from primary chemoreceptors (Pechenik et al., 1995), or as precursors in the biosynthesis of neuroactive ligands (Young, 2009). Some of these compounds include ions (Yu et al., 2008), amino acids (Kang et al., 2003; Young, 2009), neurotransmitters (Faimali et al., 2003; Young, 2009), choline derivatives (Dobretsov and Qian, 2003; García-Lavandeira et al., 2005), and enzyme inhibitors (Mesías-Gansbiller et al., 2008). For example, potassium is a universal regulator of ion gradients across cell membranes, and is involved in depolarisation of neurons, causing formation of action potentials. Generally, potassium ions have been branded as the universal inducer of metamorphosis and settlement for numerous taxa (Rodríguez et al., 1993; Yool et al., 1986), while GABA is a settlement and metamorphosis inducer for many gastropods (reviewed by Roberts, 2001) and a few bivalves (García-Lavandeira et al., 2005; Mesías-Gansbiller et al., 2008).

The role of cholinergic neurotransmission in modulating larval behaviours and important life-history events, such as settlement and metamorphosis, are poorly understood. The ability of the neurotransmitter acetylcholine to induce settlement and metamorphosis has been shown to have highly variable results across marine invertebrate taxa (Coniglio et al., 1998; Dobretsov and Qian, 2003; Young, 2009; Yu et al., 2007, 2008, 2010b). L-Phenylalanine, L-Tyrosine and dopamine are members of the epinephrine biosynthesis pathway (Fig. 1A). While some of these compounds have been investigated for their ability to induce settlement of bivalve larvae, the role of dissolved amino acids as precursors for epinephrine induction has yet to be identified.

The importance of catecholamines in bivalve life-history events is well recognised (Pani and Croll, 2000). For these taxa, the biochemical pathways involved in catecholamine synthesis and catabolism are proving to be similar to those operating in vertebrate nervous systems (Pani and Croll, 1995, 1998), and may highlight the conserved and critical role they play in early evolutionary development. In bivalves, these neurotransmitters and hormones are known to regulate spawning (Martínez et al., 1996), larval swimming behaviour (Beiras and Widdows, 1995), larval settlement (García-Lavandeira et al., 2005), metamorphosis (O'Connor et al., 2009; Wang et al., 2006), feeding rates (Beiras and Widdows, 1995), muscular activity (Aiello et al., 1981; Gies, 1986), respiration (Catapane, 1983) and digestion (Giard et al., 1995). Since the uptake of epinephrine precursors (L-Tyrosine, L-DOPA, and dopamine) into bivalve tissues from seawater is well documented (Brown et al.,

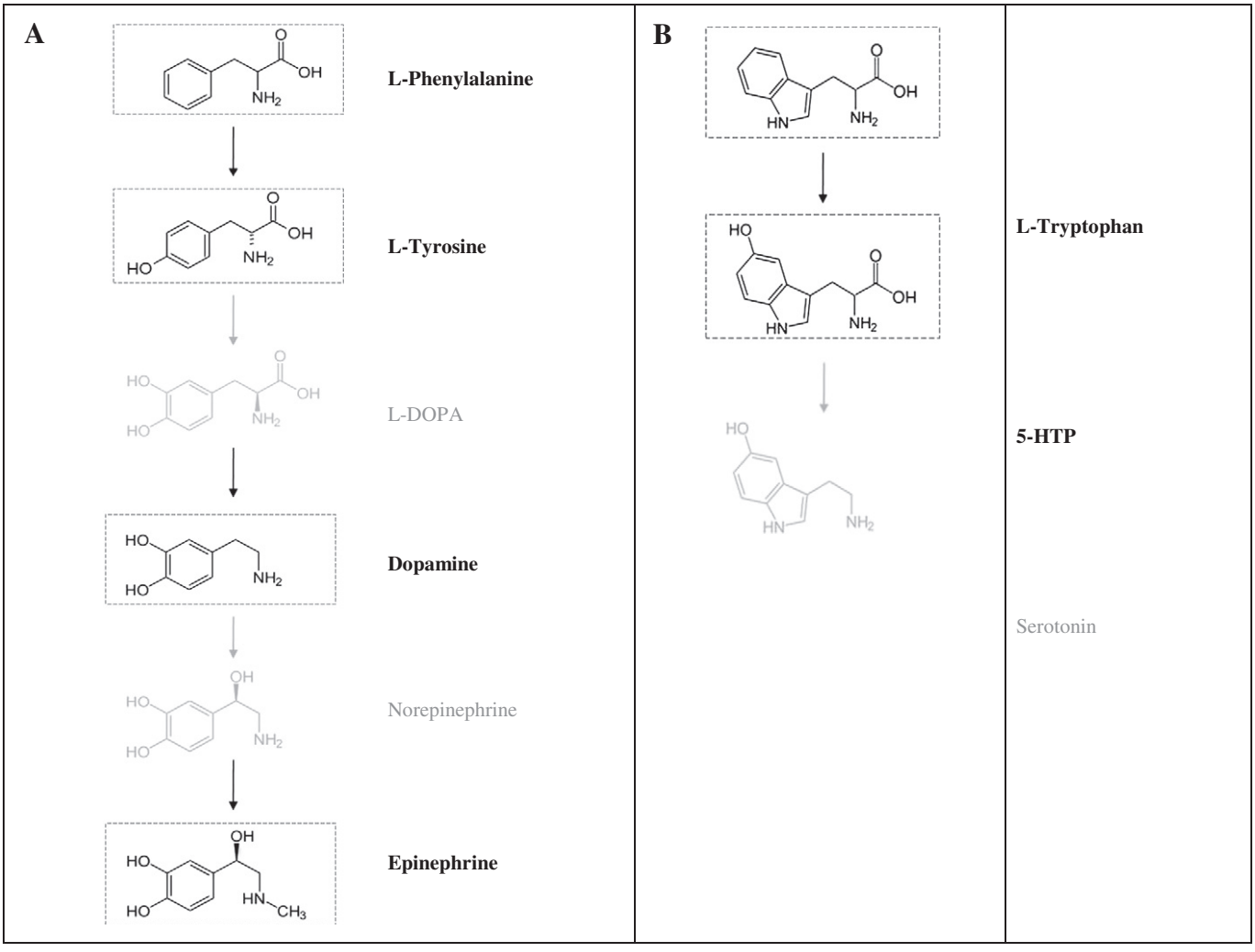

Fig. 1. Chemical structure of treatment compounds used in settlement assays: A) epinephrine biosynthesis pathway; B) serotonin biosynthesis pathway.