



Modulation of pumping rate by two species of marine bivalve molluscs in response to neurotransmitters: Comparison of *in vitro* and *in vivo* results



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ABSTRACT

Most studies regarding the neuroanatomy and neurophysiology of molluscan ctenidia have focused on isolated ctenidial tissue preparations. This study investigated how bivalve molluscs modulate their feeding rates by examining the effects of a variety of neurotransmitters, including serotonin, dopamine, and the dopamine agonist apomorphine on both isolated ctenidial tissue and in intact members of two commercially important bivalve species: the blue mussel, *Mytilus edulis*; and the bay scallop *Argopecten irradians*. In particular, we examined the effect of changes in: 1) beat of the lateral cilia (*in vitro*), 2) distance between ctenidial filaments and/or plicae (*in vivo*), and 3) diameter of the siphonal openings (*in vivo*) on alteration of bulk water flow through the mantle cavity. Important differences were found between isolated tissue and whole animals, and between species. Drugs that stimulated ciliary beat *in vitro* did not increase water processing rate *in vivo*. None of the treatments increased water flow through the mantle cavity of intact animals. Results suggest that *A. irradians* was primarily modulating lateral ciliary activity, while *M. edulis* appeared to have a number of ways to control water processing activity, signifying that the two species may have different compensatory and regulatory mechanisms controlling feeding activity.

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1. Introduction

The ciliary tracts of molluscs are involved in a variety of functions, including feeding, gas exchange, digestion, and reproduction. In many species of bivalve mollusc, the ctenidium has evolved from a gas exchange tissue into a complex feeding organ (Cranford et al., 2011). For many temperate, estuarine bivalve species, both ambient oxygen levels and the mix of food and nonfood materials in the water column are highly variable (e.g., Prins et al., 1998). The potential food particles may vary, for example, in concentration, nutritive value, cost of processing, and toxicity (i.e., harmful algae) over both the short and the long term (Shumway et al., 1985, 1987; Lucas et al., 1987; Newell et al., 1989; Shumway, 1990). Ciliary tracts of the ctenidia are involved in suspension feeding, including water processing and the capture and sorting of material suspended in the water column (for full review see Ward and Shumway, 2004). In most bivalves, metachronal beating of the lateral cilia of the ctenidia creates a current used by the animal for both ventilation and feeding. Integration of the respiratory and feeding functions of the ctenidia involves the nervous system. Changes in the

rate of water flow through the mantle cavity of a bivalve can be effected in several ways. Pumping rate can be altered by changes in: 1) the beating of the lateral cilia (either by altering rate or numbers of tracts that are on/off), 2) the functional morphology of the ctenidia (altering distance between ctenidial filaments and/or plicae), and 3) the diameter of the siphonal openings to the mantle cavity. Alterations in ctenidial dimensions are brought about by muscular contractions (Gainey et al., 2003; Gainey and Greenberg, 2005). Changes in filament distances affect both the distance of tracts of lateral cilia on adjacent filaments and the dimensions of the water canals, each of which can affect water processing rates.

The neuroanatomy and neurophysiology of bivalve ctenidia have been examined in considerable detail. Most studies have focused on the lateral ciliated tracts of isolated ctenidial tissue preparations from the marine mollusc *Mytilus edulis* and a handful of other species (Aiello, 1960, 1970, 1990; Aiello and Sleight, 1972; Paparo and Finch, 1972; Stefano and Aiello, 1975; Jørgensen, 1976; Catapane et al., 1978, 1979; Catapane, 1982; Sanderson and Satir, 1982; Paparo, 1985a; Sanderson et al., 1985; Stommel and Stephens, 1985a,b; Stommel, 1986; Murakami, 1987; Riisgård and Larsen, 2007; Carroll and Catapane, 2007). Both catecholaminergic and serotonergic neurons have been found to innervate the ciliated cells of the ctenidial epithelium and stimulation of these nerves mimics the effects of exogenously applied dopamine (3-hydroxytyramine, DA), or serotonin (5-hydroxytryptamine,

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5HT) (Blaschko and Milton, 1960; Paparo and Finch, 1972; Stefano and Aiello, 1975; Catapane et al., 1978, 1979; Catapane, 1982; Stefano et al., 1990; Carroll and Catapane, 2007).

The lateral cilia of the ctenidia are responsible for most, but not all, of the water flow through the mantle cavity (Morton, 1983; Jones and Richards, 1993), and are generally excited by 5HT (Aiello, 1970, 1990; Paparo and Finch, 1972; Jørgensen, 1974, 1976; Motokawa and Satir, 1975; Catapane, 1983). Quiescent lateral cilia on isolated ctenidia of *M. edulis* and *Mercenaria mercenaria* will begin to beat when exposed to 5HT (Aiello, 1960, 1970), and 5HT increases the frequency of metachronal waves produced by active lateral cilia in many other bivalves (Aiello, 1970; Jørgensen, 1976). Dopamine inhibits the lateral cilia on isolated ctenidia of some, but not all, species of bivalves (Catapane, 1983; Paparo, 1985a, 1985b; Gainey and Shumway, 1991). A large body of literature supports the idea that serotonergic innervation regulates initiation and continuation of lateral ciliary beating (Aiello, 1970, 1990; Catapane et al., 1978, 1979; Malanga and Poll, 1979; Murakami and Macheimer, 1982; Carroll and Catapane, 2007). Modulation of beat frequency is suspected to occur through catecholaminergic involvement via inhibitory mechanisms. It remains unclear, however, how this system is regulated or modulated by endogenous chemical messengers. It is also not known whether the ciliated epithelial cells of the lateral ciliary tract are individually innervated (Paparo, 1972; Paparo, 1985a, 1985b; Catapane, 1982). Mechanical and electrical stimulation have been shown to result in local ciliary arrest responses along cells in the lateral tracts of ctenidial filaments, leaving open the possibility that these tracts are intermittently innervated, and that information contained in neuronal signals is passed along linked segments (Murakami and Macheimer, 1982; Stommel, 1986). While the presence of gap junctions among lateral ciliated cells of bivalve ctenidia remains unconfirmed, other mechanisms have been identified to account for the characteristic spread of ciliary arrest seen in electrically coupled cellular systems (Good et al., 1990; Stephens and Good, 1990). This may represent a mechanism by which some sections or tracts of the lateral cilia may be turned off while maintaining the integrity of the metachronal wave on other actively beating sections. Finally, whereas these cilia have been shown to be under the control of the nervous system (Carroll and Catapane, 2007), there is relatively little information on how the regulation of ciliary activity and ctenidial morphology is integrated.

Ctenidial filaments contain muscle fibers and can contract longitudinally (Galtsoff, 1965; Gainey et al., 2003). These muscle contractions can affect the spacing between filaments and the flow of water through the ctenidia (Gardiner et al., 1991; Gainey et al., 2003). In freshwater bivalves, exogenously applied 5HT causes dilation of the water canals between the ctenidial filaments (Gardiner et al., 1991). Musculature of the ctenidia is stimulated by both classical neurotransmitters such as dopamine and 5HT, and by neuropeptides (Gainey et al., 2003). The gaseous neuromodulators nitric oxide and hydrogen sulfide have been associated with seasonal changes in the excitability of the musculature of the ctenidia (Gainey et al., 2003; Gainey and Greenberg, 2005). In addition, neuropeptides may modulate the effects of the classical neurotransmitters on the lateral cilia (Candelario-Martinez et al., 1993; Gainey et al., 1999). The role of the second messenger mechanisms in the regulation of the activity of the cilia of the ctenidia by neuromodulator molecules has not been thoroughly studied.

This study investigated how bivalve molluscs modulate their feeding rates by examining the effects of a variety of neurotransmitters, including the cilioexcitatory amine serotonin (5HT), the cilioinhibitory catecholamine dopamine (DA), and the dopamine agonist apomorphine (APO) on both isolated ctenidial tissue and in intact individuals of two bivalve species: the blue mussel, *M. edulis*, and the bay scallop *Argopecten irradians*. Ctenidia of mytilids are of flat, homorhabdic filibranch construction, whereas the scallop has plicate, heterorhabdic filibranch ctenidia.

A three-pronged approach was taken to examine the effects of these drugs at the level of the organelle, the organ, and the whole animal. Beat

frequency of the lateral cilia was determined on isolated ctenidial tissue. Movements of the ctenidia and the ctenidial filaments and changes in excurrent velocity and cross sectional area of the exhalant aperture over time were recorded in whole animals. Volume flux through the animal as the product of excurrent velocity and cross sectional area of the exhalant aperture was also calculated. The effects of these neurotransmitters at each level were measured to determine if a change in the beat frequency of the lateral cilia would translate into a measurable change in excurrent velocity if the ctenidial dimensions remained relatively constant.

One of the main goals of this work was to explore modulation of water processing activities under “natural” conditions and interspecific differences therein. The drugs used in this study are found endogenously in the animals on which they were tested (Blaschko and Milton, 1960; Welsh and Moorehead, 1960; Aiello, 1962; Paparo and Finch, 1972; Stefano and Aiello, 1975; Stefano et al., 1976; Stefano and Catapane, 1977a, 1977b; Stefano and Catapane, 1977a, b, 1979; Catapane et al., 1978; Deaton, 1990; Gainey et al., 1999, 2003; Stefano et al., 2003). The objectives of this study were to: 1) ensure the efficacy of both the drugs and techniques by recreating experiments performed by other researchers who examined effects on lateral cilia of isolated ctenidial tissue (organelles), 2) explore the effects of these drugs on spacing of ctenidial filaments and expansion/contraction of ctenidial lamellae (organs) in intact animals, and 3) examine effects of these drugs on water processing rates in whole animals (organism).

2. Materials and methods

2.1. Preparation of animals and drugs

Blue mussels, *M. edulis* (4.8–6.8 cm shell length), were collected from Long Island Sound at Groton, CT. Bay scallops, *A. irradians* (2.8–4.6 cm shell height), were obtained from NOAA Fisheries Service Milford Laboratory in Milford, CT. The animals were maintained in flow-through tanks supplied with unfiltered, ambient seawater pumped in from Pine Island Inlet. Supplemental nutrition (monocultured *Tetraselmis chuii* and/or Shellfish Diet (Reed Mariculture)) was added via a gravity drip system. Animals were used within a few days of collection. Experiments were conducted over four consecutive summers (2004–2007) at seawater temperatures between 18 and 21 °C and salinities between 21 and 26 (measured by means of a refractometer and presented as PPT). Water for all experiments was taken from the same facility in which the animals were housed and filtered to 0.45 µm.

The following neurotransmitters were stored in a freezer (−4 °C) until used: 1) the cilioexcitatory amine serotonin (5hydroxytryptamine, 5HT), 2) the cyclic 3',5' adenosine monophosphate agonist forskolin, 3) the protein kinase C agonist phorbol 12,13 diacetate (PDA), 4) the dopamine agonist apomorphine (APO), 5) the D1 subtype agonist (+) 6 chloro PB hydrobromide (Chloro PB), 6) the D2 subtype agonist R(−)-2,10,11-trihydroxy N propyl noraporphine hydrobromide (NPNA), and 7) the cilioinhibitory catecholamine dopamine (3hydroxytyramine, DA).

Just prior to an experiment, a stock solution of the neurotransmitter was prepared by dissolving an appropriate mass in 50 ml of filtered seawater (0.45 µm). Stock solutions were typically made up at 1×10^{-2} M, and kept on ice during the experiment.

2.2. Isolated ctenidia

Bivalves were opened by cutting the adductor muscles and the ctenidia were excised and placed in filtered seawater (0.45 µm). Dissecting pins were used to fasten pieces of ctenidium to strips of rubber glued to the bottom of small plastic dishes (Gainey and Shumway, 1991). The dishes were filled with 5 ml of seawater, and the activity of the cilia observed with the 40× objective of an inverted microscope (Nikon TMS). The frequency of beat of the lateral cilia was determined

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