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GABA from *Hypnea valentiae* (Turn.) Mont. and its effect on larval settlement of *Perna viridis* Linnaeus

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ABSTRACT

The crude GABA (ã- amino butyric acid) extracted from *Hypnea valentiae* collected from Vizhinjam coast was tested for its activity of favouring settlement of pediveliger larvae of *Perna viridis* by comparing the similar activity of standard commercial grade GABA. The results showed better rate of settlement in 10 ppm followed by 15 ppm standard GABA (100% in 10 ppm and 83.3 % in 15 ppm standard GABA) against only 55 % in both 10 and 15 ppm of crude GABA extracted from the seaweed, whereas the control showed 20.73 % of larval settlement at the end of 48 h. The results are discussed in the light of its advantages and improving its activity through purification of the crude extract.

Introduction

The ã- amino butyric acid, popularly known as GABA is a natural brain calming agent and is used in the treatment of epilepsy, hypertension and anxiety (Asada *et al*., 1997). GABA, a functional analogue of the natural inducer of larval settlement induces behavioural and developmental metamorphosis in planktonic molluscan larvae (Morse *et al*., 1979; Leise and Hadfield, 2000). *Haliotis* larvae require exogenous GABA or its homologs for induction of their genetically programmed behavioural and developmental metamorphosis to the adult form (Morse *et al.*, 1980) and settlement occurred even under condition in which most protein synthesis was inhibited as expected for a chemosensory system response (Fenteany and Morse, 1993). Compounds capable of inducing larval metamorphosis in molluscs are given off by their alga food (Scheltema, 1961; Kriegstein *et al.*,1974; Morse *et al.*,1979; Boettcher and Targett, 1996).The presence of GABA in a red seaweed species and comparison of its activity with that of a pure as well as commercial analogue are given in this paper.

Materials and Methods

The healthy plants of *Hypnea valentiae* (Turn.) Mont. (500 gm) collected from Vizhinjam-Mulloor coast (Trivandrum District, Kerala) was ground with equal volume of Chloroform – methanol mixture (1:3). The mixture was centrifuged at 7000 rpm for 10 minutes. The dissolved pigments were separated with petroleum ether as upper layer. The supernatant was vacuum dried in a rotary evaporator (Kaladharan and Sridhar, 1999). The crude GABA collected from the evaporator was dissolved in ethanol and made into 5, 10, 15 and 20ppm solutions in 30 ppt seawater

(filtered through 0.45µ). Similar concentrations of commercial grade GABA (Sigma Ltd., USA) was also prepared to compare the activity of crude GABA obtained from seaweed. A set of control (0 ppm) was also maintained .

A batch of 150 pediveliger larvae of *Perna viridis* Linn. each was kept in a glass beaker of two litre capacity in triplicate with one litre each of 5 ppm, 10ppm and 15ppm solution of crude GABA extracted from seaweed and a triplicate jars of similar concentrations of Sigma grade GABA separately for 48 h. At every 6th hour, the larvae found settled to the sides of the beaker were counted and expressed in percentage.

Results and Discussion

For triggering metamorphosis in most of the molluscan larvae, certain chemosensory cues are required. GABA is one of the chemosensors capable of inducing the settlement of larvae of gastropod and bivalves (Leise and Hadfield, 2000). In the present study, the settlement rate of pediveliger larvae of *P.viridis* was observed to be 100% and 83.3% in 10 ppm and 15 ppm concentrations of Sigma grade GABA respectively against only 55% in both the 10 ppm and 15 ppm of crude GABA extracted from the seaweed species, whereas the control showed only 20-73% of larval settlement at the end of 48 hours (Table 1).

It is evident that many complex factors contribute to the successful settling of bivalve larvae in the hatchery and in the field (Bonnar *et al.,* 1990). Spat settlement in mussels is broadly classified into primary and secondary settlement (Bayne, 1984). Primary settlement of mussel spats is noted always on the seaweeds attached to the inter-tidal rocks or other harbour structures. Thick carpet like settlement of brown mussel in granite stones at Vizhinjam and the density was higher towards substrata with luxuriant growth of red seaweeds (Appukuttan *et al.,*1989). The phenomenon of natural bivalve settlement involves a complex of behavioural, physiological and morphogenetic events. *Haliotis* larvae require exogenous GABA or its homologues (10^{-6} M) for induction of their genetically programmed behavioural and developmental metamorphosis to the adult form (Morse *et al.,* 1980). Larvae of queen conch *Strombus gigas* are induced to metamorphose by cues associated with red alga *Laurencia poitei* (Boettcher and Targett, 1996).

The impaired rate of settlement of mussel larvae in the presence of crude GABA extracted from *H. valentiae* over the commercial grade analogue (Table 1) is believed mainly due to the crude nature of GABA with some impurities and insists the need to further concentrate the

Table 1. *Effect of crude GABA from* H. valentiae *on mussel larvae*

Time	Source	Rate of larval settlement (%)						
		Std. GABA			GABA from *H. valentiae*			Control
	ppm	5	10	15	5	10	15	0
0 hr		0	0	0	0	0	0	0
6		0	0	0	0	0	0	0
12		25	0	0	0	0	0	0
18		25	10	0	16.7	50	50	11.8
24		25	20	66.7	16.7	50	55	15.6
36		25	35	66.6	16.7	55	55	17.2
48		50	100	83.3	33.4	55	55	21.4

crude extract. The advantages of GABA extract obtained from *H. valentiae* are cost effective preparation, easy availability of raw material, induces rapid settlement of mussel larvae, improves the rate of survival, the water quality is not affected and reduces the cost involved for prolonged water exchange in mussel hatcheries.

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