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PHYSIOLOGICAL EFFECTS OF *PROTOGONYAULAX TAMARENSIS* ON CARDIAC ACTIVITY IN BIVALVE MOLLUSCS*

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Abstract—1. In *Spisula solidissima*, *Mercenaria mercenaria*, *Artica islandica*, and *Placopecten magellanicus*, there was no effect on cardiac activity after exposure to *Protogonyaulax tamarensis* (GT429).

2. In *Mya arenaria*, there was transient cardiac inhibition in 40% of the individuals tested after exposure to GT429.

3. In *Ostrea edulis* there was long term decrease in heart rate in 22% of the animals after exposure to GT429.

4. In *Geukensia demissa*, there was transient cardiac inhibition in 10% of the animals, transient excitation in 40%, and long term inhibition in 10% after exposure to GT429.

5. In *Mytilus edulis*, there was transient inhibition in 25% of the animals, long term inhibition in 21% and long term excitation in 11%, after exposure to GT429.

6. There was no difference in the distribution of responses in between *Mytilus* that had prior exposure to GT429 and *Mytilus* that had no prior exposure.

INTRODUCTION

Although many workers have assumed that red tide has little effect on bivalve molluscs (Quayle, 1969; Prakash *et al.*, 1971), there is increasing evidence to the contrary. In a recent series of papers (Shumway *et al.*, 1985; Cucci *et al.*, 1985; Shumway *et al.*, 1987; Shumway and Cucci, 1987; Shumway and Gainey, 1987), it has been reported that the effects of toxic dinoflagellates on bivalve molluscs are species specific and include shell-valve closure and/or siphon retraction, increased/decreased rates of particle clearance, decreased rates of byssus production, increased/decreased rates of oxygen consumption and mortality.

The heart rate of bivalve molluscs is known to vary in response to a number of factors including aerial exposure, valve-closure, salinity, and hypoxia (reviewed by Hill and Welsh, 1966; Bayne *et al.*, 1976; Jones, 1983). Exposure of bivalves to toxic dinoflagellates could alter cardiac activity through indirect effects such as valve closure or hypoxia caused by reduced filtration. There are no studies of heart rates in bivalve molluscs exposed to toxic dinoflagellates, although Wilkens (1972) and Irisawa *et al.* (1967) found that isolated ventricles of 3 species of bivalves were unaffected by tetrodotoxin, which is pharmacologically similar to saxitoxin. Twarog and Yamaguchi (1972) found varying degrees of neurotoxicity in 8 species of bivalves, with those species that are regularly exposed to red tide being the least sensitive. One could infer from these studies that exposure of bivalve molluscs to toxic dinoflagellates would have little effect on cardiac activity either through direct action on the heart or through inhibition of cardioregulatory nerves. In the present study, we tested the effects of exposure to *Protogonyaulax tamarensis* on cardiac activity in 8 species of marine bivalve molluscs. Preliminary results of this study were presented at the Third International Conference on Toxic Dinoflagellates (Shumway *et al.*, 1985).

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MATERIALS AND METHODS

Specimens of the following bivalve molluscs were collected at various localities in Maine: *Crassostrea virginica* (Gmelin) (Damariscotts River, Walpole), *Mya arenaria* L. (Long Cove, Searsport), *Mytilus edulis* L. (Boothbay Harbor), *Ostrea edulis* L. (Dodge Cove, Damariscotta River), *Placopecten magellanicus* (Gmelin) (lower Damariscotta River), *Spisula solidissima* Dillwyn (Rockland), *Arctica islandica* L. (Jonesport). Specimens of *Mytilus edulis* were also collected from Stony Brook, New York. *Mercenaria mercenaria* L., and *Geukensia demissa* Dillwyn were collected from the Sakonnet River, Tiverton, Rhode Island. Animals were transported to the laboratory immediately and scrubbed to remove all epiphytes. The animals were maintained in sand-filtered, running sea water from Boothbay Harbor at 15°C prior to use in experiments. The sand filter removed all particles larger than 20 μm, i.e. *P. tamarensis*. No supplementary food was given to the animals.

To record heart rates, a pair of small holes were drilled through the shell on either side of the pericardium; platinum electrodes were inserted through the holes and held in place with cyanoacrylate glue and dental cement. The electrodes were connected to an impedance converter (Model 2991, UFI Instruments) with shielded cable, and the output recorded on a chart recorder. Animals were allowed to recover for at least 2 days prior to the beginning of an experiment. Heart rates were measured at least daily for 2 days prior to use in experiments.

Stock cultures of *Protogonyaulax* (= *Gonyaulax*) *tamarensis* (clone GT429), *Thalassiosira pseudonana* (clone 3H), *Chroomonas salina* (clone 3C), and *Prorocentrum minimum* (clone EXUV) were obtained from the Provosolli–Guillard Cutlure Center for Marine Phytoplankton at the Bigelow Laboratory for Ocean Sciences, Boothbay Harbor, Maine. The cultures were diluted so that the final concentration of algae was between 2.5–5.5 × 10^5 cells/l. Heart rates were measured hourly for the first 8 hr after exposure, and at least daily for the duration of the experiment (4–9 days). In all of the experiments, except with *Mya arenaria* and *Mytilus edulis* from Long Island, at least a third of the animals were untreated and served as controls.

Heart rates were calculated for the times when the heart was beating; for those animals that had regular periods of cardiac activity, followed by regular periods of cardiac arrest (burst activity), the rates were calculated for the times when the heart was actually beating, which overestimated total cardiac output. Heart rates were compared before and after exposure to GT429 using repeated measures analysis of variance at the 0.05 level of significance. If the treatment was significant, mean heart rates of individuals before and after exposure to GT429 were compared with *t*-tests, again at the 0.05 level. Control animals were compared using the same statistical methods except that a dummy variable "ORT" was used to code for "No GT429" for the time intervals that the experimental animals were exposed to GT429.

RESULTS

No effects

The presence of GT429 had no effect on either heart rate or the pattern of cardiac activity in *Spisula solidissima* ($N = 4$), *Mercenaria mercenaria* ($N = 16$), *Artica islandica* ($N = 6$), or *Placopecten magellanicus* ($N = 6$) for periods up to 7 days after exposure (Table 1).

Minimal effect

Exposure of *Mya arenaria* to GT429 had no significant, long-term effect upon heart rates in 10 individuals. There was, however, a transient decrease in heart rate between 30 min and 3 hr after exposure in 4 individuals. The rates returned to normal within 24 hr (Fig. 1).

Exposure of *Ostrea edulis* to GT429 affected 2 out of 9 animals: these 2 had a significant decrease in heart rate (Table 2, Fig. 2). Moreover, the animals that had exhibited the decreased rates also experienced cardiac arhythmias (Fig. 3). The heart rates of 6 control animals did not change significantly during the experiment.

Exposure of *Guekensia demissa* to GT429 resulted in a significant decrease in heart rate in 1 out of 10 animals tested (Table 2). In addition, 3 animals had a transient increase in heart rate, which appeared within 1 day after exposure, and disappeared within 2 days after exposure (Figs 4 and 5); after this transient increase, the heart rate returned to normal in 1 animal and was lower in the other 2. Two animals also had a change in the pattern of cardiac activity: one animal exhibited burst activity within 30 min of exposure, which persisted for 1 day; the other one exhibited burst activity prior to exposure, but within 30 min of exposure to GT429 the heart beat became regular for 1 day, and then returned to burst activity. One of the controls also exhibited burst activity throughout the period of the experiment.

Maximal effect

Mytilus edulis (*Maine*). We divided the response of *Mytilus edulis* into 1 of 3 categories: maximal, transient and minimal. Of the 17 *Mytilus* from Maine exposed to GT429, 6 had a maximal response. Two of these animals had a significant decrease in heart rate, while 2 animals had a significant increase in heart rate (Table 2). One animal had a transient increase in rate, followed by a prolonged decrease (Fig. 6). This animal, along with the 2 that had significant decreases in heart rate, died within 6 days of exposure. In addition to the change in heart rate, cardiac arhythmias were present in 5 of the 6 animals.

Table 1. Summary of effects of *Protogonyaulax tamarensis* on cardiac activity in bivalves

Subclass	Species (N)	Response (%)	
Pteriomorpha			
	Mytilus edulis (Maine $N = 17$)	Tran Inh	29%
		Inh	24%
		Exc	12%
		None	35%
	Mytilus edulis (Long Island $N = 11$)	Tran Inh	18%
		Inh	18%
		Exc	9%
		None	55%
	Geukensia demissa ($N = 10$)	Tran Inh	10%
		Inh	10%
		Tran Exc	40%
		None	40%
	Ostrea edulis ($N = 9$)	Inh	22%
		None	73%
	Placopecten magellanicus	None	100%
Heterodonta			
	Mya arenaria ($N = 10$)	Tran Inh	40%
		None	60%
	Spisula solidissima ($N = 4$)	None	100%
	Mercenaria mercenaria ($N = 16$)	None	100%
	Arctica islandica ($N = 6$)	None	100%

Inh, inhibition; Exc, excitation; Tran, transient; None, no effect.

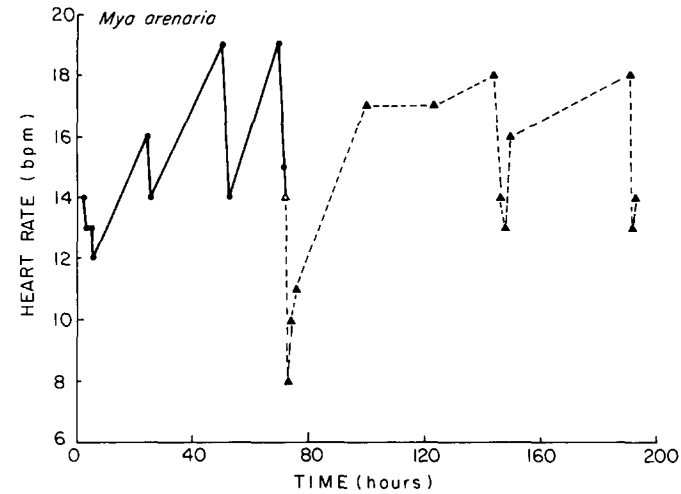

Fig. 1. Heart rate (beats/min) in *Mya arenaria*. Circles, solid line: before exposure to GT429. Triangles, dotted line: after exposure to GT429. The data are repeated measures on the same individual.

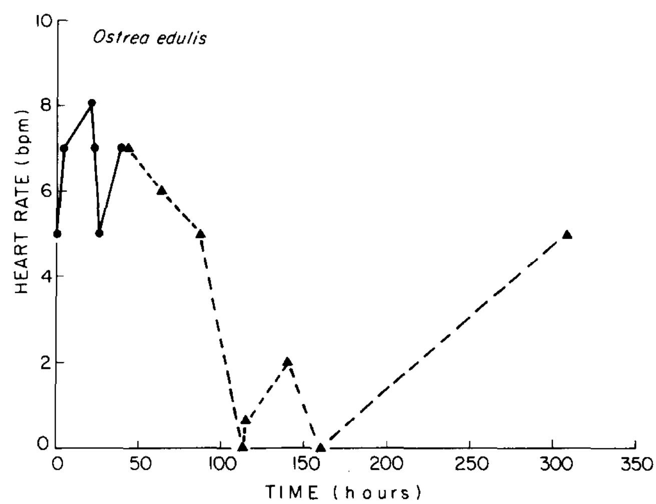

Fig. 2. Heart rate in *Ostrea edulis* before and after exposure to GT429. Symbols are the same as Fig. 1.

These arrhythmias appeared within several hours after exposure and were followed by burst activity that appeared from 1 to 4 days after exposure and persisted for the duration of the experiment (Fig. 7). Five of the 17 *Mytilus* had a transient response to GT429.

Table 2. Heart rates of animals significantly affected by exposure to *Protogonyaulax tamarensis*

Species	Before exposure	After exposure
Ostrea edulis	6.5 ± 0.50 (6)	3.3 ± 0.95 (10)
	6.7 ± 1.2 (6)	2.2 ± 0.73 (10)
Geukensia demissa	10.2 ± 0.58 (5)	5.1 ± 0.85 (14)
Mytilus edulis (Maine)	20.0 ± 1.1 (9)	11.8 ± 0.92 (26)
	16.1 ± 2.1 (9)	11.5 ± 1.3 (26)
	10.2 ± 1.7 (10)	14.3 ± 0.92 (12)
	19.5 ± 2.3 (10)	28.8 ± 1.0 (12)
Mytilus edulis (Long Island)	8.8 ± 0.37 (5)	5.0 ± 1.5 (8)
	8.8 ± 0.87 (5)	5.4 ± 0.99 (8)
	7.0 ± 0.95 (5)	9.5 ± 0.46 (8)

Data are mean heart rates (beats/min) ± SE (*N*) (see text for details).

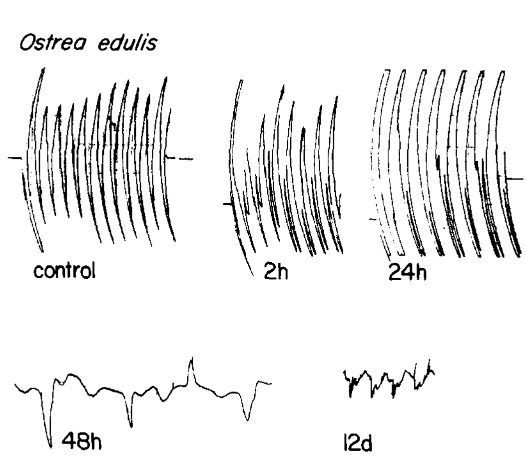

Fig. 3. Traces of cardiac activity of *Ostrea edulis* before (control) and after exposure to GT429. hr, hours after exposure; d, days after exposure.

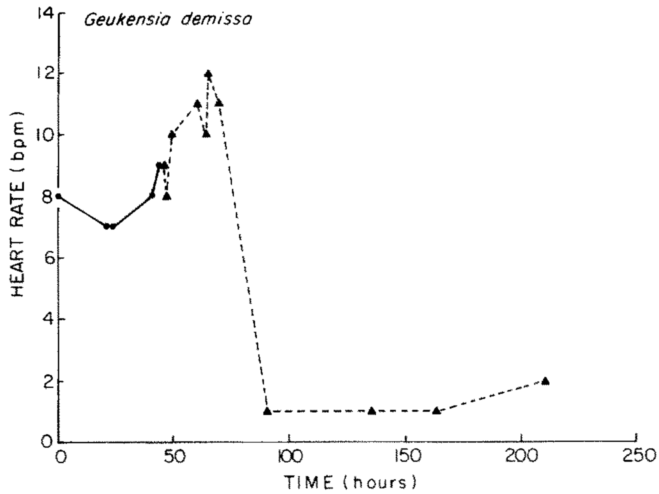

Fig. 4. Heart rate in *Geukensia demissa* before and after exposure to GT429. Symbols are the same as Fig. 1.

The pre- and posttreatment heart rates were not different, but these animals had cardiac arhythmias, burst activity, and depressed rates, but activity returned to normal within 3 days of exposure to GT429.

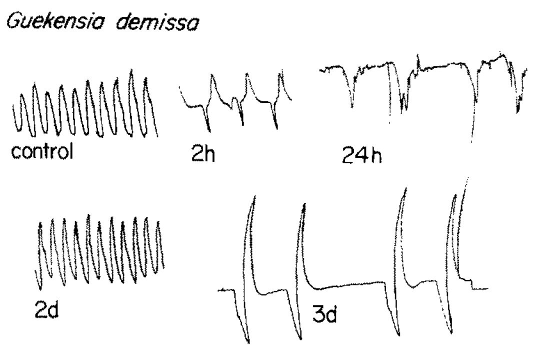

Fig. 5. Traces of cardiac activity of *Geukensia demissa* before and after exposure to GT429.

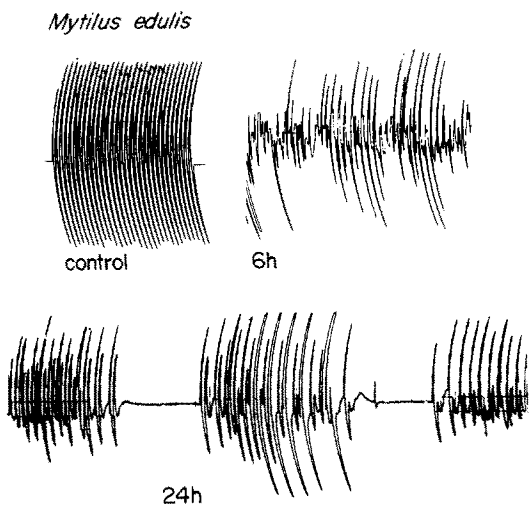

Fig. 7. Traces of cardiac activity of *Mytilus edulis* before and after exposure to GT429.

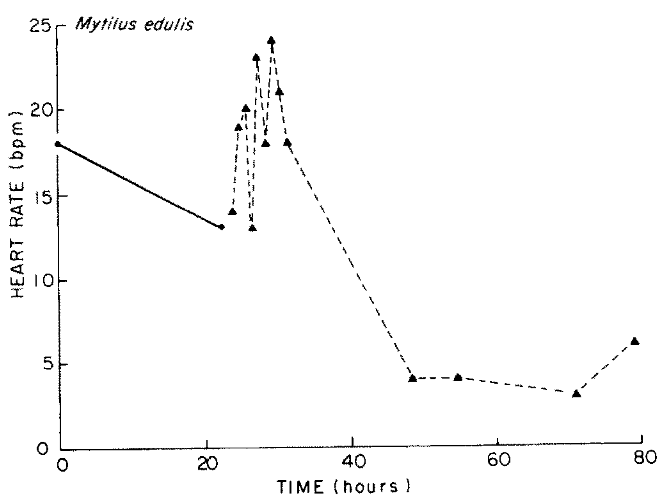

Fig. 6. Heart rate in *Mytilus edulis* before and after exposure to GT429. Symbols are the same as Fig. 1.

Six of the *Mytilus* were unaffected by exposure to GT429, except for the presence of "spikes" superimposed upon the traces of cardiac activity. These spikes only lasted for 1 day after exposure. There were no significant differences in heart rate, nor in the pattern of cardiac activity in any of the 8 control animals. In addition 2 *Mytilus* were exposed to a mixture of algae (3C, 3H, EXUV). These animals had no significant change in heart rate up to 4 days after exposure.

Mytilus edulis (*Long Island*). Eleven *Mytilus* from Long Island were exposed to GT429. Three animals had a maximal response: 2 had a significant decrease in heart rate, and 1 had a significant increase (Table 2). The 2 animals that had depressed heart rates also had pronounced arhythmias within 2 days of exposure. These arhythmias lasted for 8 days. Two of the animals had a transient response consisting of periods of cardiac arhythmia and burst activity that appeared within 4 days after exposure and lasted until 5 days after exposure. Six animals had no response with the exception of spikes superimposed upon the traces of cardiac activity.

DISCUSSION

The 3 species that had an unequivocal response to GT429 are pteriomorphs (Table 1). The only heterodont that had a response to GT429 was *Mya arenaria*: 40% of the individuals tested had a reduced heart rate for several hours after exposure. Shumway and Cucci (1987) found that GT429 reduced clearance rates and induced siphon closure in *Mya*. We observed that siphon closure was accompanied by bradycardia prior to exposure to GT429, and this transient reduction in heart rate probably was due to closing of the siphons. The different response of pteriomorphs and heterodonts to the presence of GT429 is reflected in other aspects of their cardiac physiology. For example, Painter and Greenberg (1979) found that the response of isolated ventricles to 5-hydroxytryptamine (5HT), and the peptide, FMRFamide, varied between the subclasses. Similar differences have also been reported for the ionic basis of cardiac excitability (Deaton and Greenberg, 1980), the sodium dependence of calcium efflux (Plumb and Koch, 1979), and the level of cholinesterase activity (Greenberg *et al.*, 1980).

Within the Mytilidae, cardiac activity in response to GT429 was not uniform: some individuals were inhibited, some were excited, and some were unaffected (Table 1). Greenberg (1965) and Painter and Greenberg (1979) have shown that the responses of isolated ventricles to 5HT, FMRFamide and acetylcholine often vary within a given species. Thus our results are not surprising given the complexity of cardic pharmacology of isolated ventricles.

Isolated ventricles of *Mytilus edulis*, *Geukensia demissa*, and *Crassostrea gigas* are relatively insensitive to tetrodotoxin (TTX) (Irisawa *et al.*, 1967; Wilkens, 1972). Moreover, isolated nerve fibers of *Mytilus* and *Geukensia* are also relatively insensitive to both TTX and saxitoxin (STX) (Twarog and Yamaguchi, 1972). Disruption of cardiac activity in *Mytilus*, *Geukensia* and *Ostrea* in response to GT429 is probably not related to a direct action of STX on the heart, although STX is but one of 12 toxins produced by *Protogonyaulax tamarensis* (Boyer *et al.*, 1985). To our knowledge, these toxins have not been tested on either isolated ventricles, or neurons. The burst pattern of cardiac activity that we observed in *Mytilus* has also been observed by Helm and Trueman (1967) in response to aerial exposure, by Bayne (1971) in response to declining oxygen tension and by Grace and Gainey (1987) in response to copper. We are unable to conclude whether the patterns of cardiac activity in response to exposure to GT429 are a result of the action of toxin(s) on the heart, the nervous system, or are simply a general response to physiological stress.

Widdows (1973) found that heart rates of starved *Mytilus* increased after the addition of food, but the rates did not increase until 6 days after the treatment. The increased heart rates that we observed in several *Mytilus*, along with the transient increases in *Geukensia*, are unlikely to be the result of utilization of *Protogonyaulax* as a food source since cardiac activity in our experiment increased within several hours to 1 day after exposure. Moreover, exposure of *Mytilus* to a mixture of algae resulted in no change in heart rates for 4 days after exposure, thus cardiac excitation is likely related to the complexity of the pharmacology of bivalve hearts as discussed previously, and not to the presence of food.

Shumway and Cucci (1987) and Shumway *et al.* (1985) found that *Mytilus edulis* from Maine, which had prior exposure to *Protogonyaulax*, were relatively unaffected by laboratory exposure to GT429. They also found that *Mytilus edulis* from Rhode Island and Spain, which had no prior exposure to *Protogonyaulax*, had a variety of responses upon laboratory exposure to GT429: increased oxygen consumption, increased valve closure, increased siphon closure, decreased clearance rates, and increased mortality. In spite of these differences in *Mytilus* populations, we found no obvious differences in the distribution of responses of cardiac activity of *Mytilus* from Long Island in comparison with *Mytilus* from Maine.

In summary, although our sample of bivalve species is miniscule in comparison to the number of species extant, we tentatively conclude that exposure of pteriomorph bivalves results in a mixed set of responses ranging from inhibition to excitation, while heterodonts are unaffected.

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