

ROLE OF CEREBRAL GANGLIA IN REGULATION OF OXYGEN CONSUMPTION OF FRESHWATER BIVALVE MOLLUSC, *INDONAIA CAERULEUS* (PRASHAD, 1918) FROM GODAVARI RIVER DURING SUMMER

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ABSTRACT

The adult bivalve mollusc, *Indonaia caeruleus* of 50-53 mm shell length and 6.853-8.275g body weight were subjected to (a) control (normal) (b) removal of both the cerebral ganglia, (c) injection of their cerebral ganglionic extract to intact control as well as (d) injection of ice-cold distilled water and absolute alcohol (1:1) to normal control and (e) injection of this extract to ganglia removal bivalves for 10 days. The rate of oxygen consumption in bivalves from all four groups (including control) was measured on 2nd, 5th and 10th day. The study revealed that, the rate of oxygen consumption was significantly increased in cerebral ganglia removed, as well as cerebral ganglionic extract injected to ablated group on 2nd, 5th and 10th day compared to control. The rate also showed significant increase in injection of extract to normal control on 2nd day. The rate of oxygen consumption showed more increase in cerebral ganglia ablated group than extract injected one on 5th and 10th day. The results are discussed in the light of possible involvement of endogenous regulation in bivalve shell fishes.

INTRODUCTION

Many exogenous factors (temperature, salinity, pH, light, oxygen tension, turbidity etc.) affect the rate of oxygen consumption in bivalve molluscs (Bayne, 1976; Samant and Agrawal, 1978). Most of the vital activities in bivalves are regulated by neuroendocrine centers. The respiratory rate data of the animals reflect their general metabolic rate. The existence of neuroendocrine modulations of metabolic rate will be the adaptive significance for the freshwater bivalves, which have to live in ever fluctuating environments. Comparatively, very little work has been done on the neuro-endocrine regulation in bivalve shell fishes and also comparatively, very less attention has been given on the role of neuroendocrine centers in respiratory metabolism, particularly from freshwater bivalves. In the field of neuro-endocrinology, such neuroendocrine control on oxygen consumption has been reported for crustaceans (Nagabhushanam and Kulkarni, 1979). Hanumante *et al.* (1980). The role of cerebral and visceral ganglia in the respiratory metabolism has been reported by Mane *et al.* (1990) for estuarine clam, *Katelysia opima*, Shinde (2007) for freshwater bivalve, *Lamellidens corrianus*, Gaikwad (2008) and Wagh (2008) for *Lamellidens marginalis*. In bivalve molluscs, two types of neurosecretory-cycles like sudden change in the temperature, pH and salinity after cerebral neurosecretion and long cycle related to certain activities of reproduction and metabolism. Such neurosecretory cycles

from neurosecretory cells was reported by Nagabhushanam and Mane (1973) for estuarine clam, *Katelysia opima* and by Gaikwad (2008), Vedpathak and Wagh (2009) for freshwater bivalve, *Lamellidens marginalis*.

Review of literature shows that, very little information is known on the neuro-endocrine control in respiratory metabolism of freshwater bivalves. Since many features of aerobic metabolism can be studied directly by measurement of the rate of oxygen consumption by intact animals. Some reports are available on respiratory physiology of freshwater bivalves from India and abroad (Salanki and Lukacsovice, 1967; Bayne, 1976; Zs- Nagy, 1974; McMohan, 1979). Thus, considering the paucity of information on endogenous regulation in the respiratory metabolism the present study is undertaken on freshwater bivalves, *Indonaia caeruleus* from Godavari River at Paithan near Aurangabad.

MATERIALS AND METHODS

The adult freshwater bivalve molluscs, *Indonaia caeruleus* (50-53 mm in shell length) were collected from banks of Godavari River at Paithan, 45km away from Aurangabad, during summer season. After brought to the laboratory the shells of the bivalves were brushed and washed to remove the mud and fouling biomass. The bivalves were acclimatized for 24h in laboratory conditions and subsequent experimentation without food. After 24h acclimatization the

animals were arranged in five groups, each group containing 15 animals in 10 L of aerated reservoir water. The first group of animals were served as (a) normal control with intact ganglia and other four groups were experimental *i.e.* (b) removal of both the cerebral ganglia; (c) injection of cerebral ganglionic extract to control, and (e) injection of ganglionic extract to ablated bivalves. Total removal (ablation) of both the cerebral ganglia were done, with the help of fine sterilized forceps by inserting a rubber cork wedge of 3-4 mm thickness, between two valves of the shell, near anterior adductor muscles. The precaution was taken that the mantle should not get pinched in between the shell valves. For injection of cerebral ganglionic extract, it was prepared in ice-cold distilled water and ethyl alcohol 1:1 (10 ganglia in 1 mL solution), was centrifuged in refrigerated centrifuge and injected (0.2 mL extract per animal) *i.e.* equivalent to 2 ganglia per animal, into the muscular tissue-foot. For Sham operated control, the animals were injected by 0.2 mL mixture of ice cold distilled water and alcohol (1:1), (it was not run because it did not showed significant change). The experiment was run for 10 days. The physico-chemical characteristics of water used in experiments *i.e.* temperature, pH, hardness and dissolved oxygen contents were also measured. Temperature and pH were recorded daily, while hardness and dissolved oxygen contents of the water were determined on every two days throughout the experimental period.

The rate of oxygen consumption of individual animal from each group was determined by modified Winkler's method (Golterman *et al.*, 1978), in a specially prepared brown colored respiratory jar of 1.0 liter capacity. Four closed respiratory jars, each with an inlet and outlet, were kept in continuous circulation of water, in order to open the valves of animals. Once the animals were opened their valves, the flow of water was cutoff and sample of water from it, was drawn after 1hr for determination of oxygen consumption. The flesh of the individual animal was taken out carefully from the shell and socked on the blotting paper to remove the excess water. Blotted flesh was then weighted to obtain the wet-weight of the individual bivalve.

The oxygen consumed by each animal was then calculated and expressed as mg O₂/L/h/g wet-weight of the flesh. For confirmation of results all the values of four individual animals from each group were subjected to statistical analysis using 't' test (Dowdswell, 1957). Percentage differences were also calculated in experimental group.

RESULTS

The results of the experiments were shown in Fig.1. The physico-chemical characteristics of the water used in experiments during summer season were temperature 31.5°C – 33.0°C; pH 7.6 – 7.9; hardness in terms of bicarbonate 95-111 ppm and dissolved oxygen contents 5.496-6.823 mg/L/h.

As compared to control, the rate of oxygen consumption was significantly increased 0.3629 ± 0.0219 , 19.89%, $p < 0.01$ in cerebral ganglia ablated, 0.3438 ± 0.0218 , 13.44%, $p < 0.01$ in cerebral ganglionic extract injected to ganglia ablated animals on 2nd day. Similarly on 5th day, the rate of oxygen consumption also showed significant increase $0.2832 \pm$

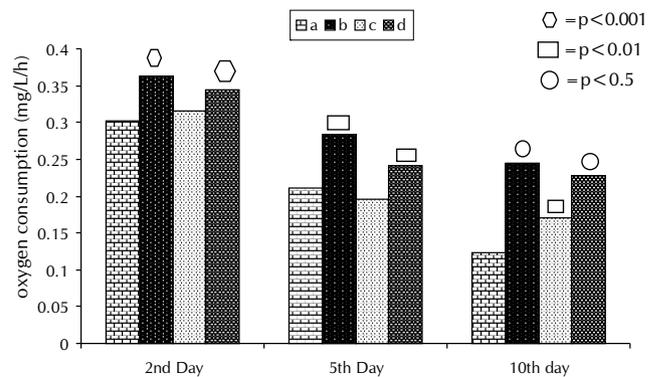


Figure 1: Changes in the rate of oxygen consumption of freshwater bivalve shell-fish *Indonaia caeruleus* from Godavari river during summer: as a function of removal of cerebral ganglia and injection of their extract

(a) = Normal control with intact ganglia (b) = Ablation of cerebral ganglia (c) = injection of cerebral ganglionic extract to control animals (d) = injection of cerebral ganglionic extract to ablated animals

0.0178, 34.027%; $p < 0.05$ in ganglia ablated group as well as 0.2409 ± 0.0187 , 14.0085%, $p < 0.05$ in ganglionic extract to ablated animals respectively. While on 10th day, the rate of oxygen consumption showed significant increase 0.2439 ± 0.0268 , 97.49%, $p < 0.001$; 0.1698 ± 0.0374 , 37.49%, $p < 0.05$ and 0.2276 ± 0.0236 , 84.29%, $p < 0.001$ in ablation of cerebral ganglia, injection of cerebral ganglionic extract to intact control and injection of extract to ablated animals respectively compared to control. But the rate of oxygen consumption does not showed significant change in injection of extract to control animals on 2nd and 5th day.

The rate of oxygen consumption in control group was 0.3027 ± 0.0148 , 0.2113 ± 0.029 and 0.1235 ± 0.0306 on 2nd, 5th and 10th day, respectively, while the rate of oxygen consumption in ganglionic extract injected to intact animals group was 0.3157 ± 0.0177 , 4.296% and 0.1963 ± 0.00986 , 7.099% on 2nd, 5th day respectively. The rate of oxygen consumption in extract injected to ablated animals was 0.3438 ± 0.0218 , 13.44% $p < 0.01$, 0.2409 ± 0.0187 , 14.0085% $p < 0.05$ and 0.2276 ± 0.0236 , 84.29% $p < 0.001$ on 2nd, 5th and 10th days respectively.

DISCUSSION

The present study on *Indonaia caeruleus* showed that, removal of both the cerebral ganglia in bivalves causes significant increase in the rate of oxygen consumption on 2nd, 5th and 10th day. The rate of oxygen consumption in ganglionic extract injected to ganglia removed animals also caused significant increase on any 2nd, 5th and 10th day compared to control. But the rate showed decrease in extract injected to cerebralectomised animals compared to cerebralectomised animals. The rate of oxygen consumption in extract injected to intact animal (control) also showed significant increase on 10th day, compared to control.

A significant increase in the rate of oxygen consumption in bivalves after cerebralectomy on 2nd, 5th and 10th day and decrease in the rate after injection of ganglionic extract to intact control on 5th day, suggest the possibility of feedback mechanism in regulation of oxygen consumption. The

existence of possible feedback mechanism could be because of further stimulation of rate of oxygen consumption after injection of cerebral ganglionic extract to the ganglia removed animals, which is receiving the cerebral ganglionic extract and hence restore or recover the rate of oxygen consumption.

From the results of this experiment, it can be suggested that cerebral ganglia must possess the hormonal factor which is responsible for regulation of oxygen consumption. Injection of cerebral ganglionic extract to the ganglia removed animals which did restore the rate of oxygen consumption. An increase in the rate of oxygen consumption following injection of ganglionic extract to the ablated animals which reached the normal intact control, this confirms that the regulating link is not through the nervous input but probably by neurosecretory. This contention can further be supplemented by the fact that even in intact (normal) control animals, as after injection of extract to animals from control significantly decreases the rate of oxygen consumption than ablated bivalves.

Hence, it is concluded that, cerebral ganglia must possess oxygen consumption controlling factor and which is neurosecretory. The integrity of these ganglia is essential in the normal functioning of physiological activities of the bivalve molluscs.

It has been shown that, in the earthworm, *Perionyx excavatus*, the rate of oxygen consumption be under the influence of neurosecretory release of one or more hormonal agents from central nervous system (Nagabhushanam and Hanumante, 1977). The brain and subpharyngeal ganglia of the earthworm have shown to be the site of oxygen inhibiting and elevating hormones respectively. The concept of hormonal control of oxygen consumption has been evidenced in number of poikilothermic organisms (Kale and Rao, 1973). In crab *Uca pugilator*, two independently acting hormones, regulates the rate of oxygen consumption- (1) eyestalk factor regulating oxygen consumption and (2) The removal of moulting inhibiting hormone which enhancing oxygen consumption (Silverthorn, 1975). In Penaid prawn, *Parapenaeopsis hardwickii*, eyestalk possess a hormone which decreasing the rate of oxygen consumption (Nagabhushanam and Kulkarni, 1979).

In gastropod mollusc, *Onchidium verruculatum* removal of whole central nervous system or pleuropedal ganglia significantly inhibited oxygen uptake (Hanumante et al., 1980). Replacement of pleurovisceral ganglia in pleurovisceralectomised gastropods recovers the rate of oxygen consumption upto the normal level. Similarly In freshwater gastropod, *Limnaea stagnalis*, lateral neurohormone stimulates oxidative phosphorylation (Geraerts, 1976).

In the present study, on freshwater bivalve, *Lamellidens marginalis*, it is possible that surgical bilateral ectomization of cerebral ganglia and injection of their extracts to bilateral cerebralectomised animals could have resulted in initiation of the release of large quantities of serotonin and catecholamines as stated by Lubet (1970) in *Mytilus edulis*. Another exciting possibly that these neuro- hormones after their entry may be enhancing the role of non - specific stressors (Gold and Ganong, 1977) or neuroendocrine transducers (Wurtman, 1972), there by indicating the endogenous

neurosecretory hormone or hormones involved in regulation of oxygen consumption. This idea gives strength to the fact that the biogenic amines act as neurotransmitter to induce the release of neurohormones from hypothalamic nuclei of vertebrates (Maclead and Lehmeier, 1977) and probably also from those of invertebrates e.g. crustaceans (Fingerman et al., 1974) and bivalve molluscs (Mane et al., 1990). These neurohumors are capable of inducing changes in the neurosecretory materials from cells in the cerebral and visceral ganglia of the bivalve shell fishes (Misal, 2011).

Since the presence of these neurohormones in the ganglia of bivalve molluscs have already been established, regulation of oxygen consumption may be tentatively suggested as one of the physiological roles for these neurohumors in the metabolic economy in case of freshwater bivalves shellfishes.

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