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The role of equivalent commercial hormones and cerebral ganglionic extract injections in regulation of oxygen consumption of fresh water bivalve mollusc, *Indonaia caeruleus* (Prasha, 1918) during monsoon season

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Abstract
Considering the importance of neuroendocrine control on the metabolic activities of freshwater bivalves, we report here the effect of injections of cerebral ganglionic extract and equivalent commercial hormones (Progesterone & Estradiol) on respiratory metabolism of freshwater bivalve mollusc *Indonaia caeruleus* (Prashad, 1918) from Godavari River. During monsoon season, the adult bivalve mollusc, *Indonaia caeruleus* (50-55 mm shell length) were subjected to a) control (normal) (b) injection of a cerebral ganglionic extract of same species to intact individuals (c) injection of equivalent commercial hormone progesterone to normal control and (d) injection of estradiol to normal control for 8 days. The rates of oxygen consumption in bivalves from all four groups (including control) were measured on 2nd, 5th, and 8th day. The study revealed that, the rate of oxygen consumption was significantly decreased in cerebral ganglionic extract injected group on the 2nd day. The rate of oxygen consumption also showed significant increase in estradiol injected group and ganglionic extract injected group on 5th day. The rate of oxygen consumption showed significant decrease on 8th day in progesterone injected as well as an insignificant decrease in ganglionic extract and estradiol injected groups.

Keywords: Cerebral ganglionic extract, progesterone, estradiol, oxygen consumption, freshwater bivalve

1. Introduction
In general, most of the vital activities in bivalve are regulated by neuro-endocrine centers. The respiratory rate data of the animals reflects their general metabolic rate and is proportional to its instantaneous ATP demand. The synthesized ATP is utilized in various processes, including ion pump activity, muscular activity, neural activity, growth, and gametogenesis and catabolite excretion [1]. The existence of neuro-endocrine modulation of the metabolic rate will be the adaptive significance for the freshwater bivalves. Comparatively, very little work was done on the endocrine regulation in bivalve molluscs and also comparatively; very less attention has been given to the role of neuroendocrine centers in respiratory metabolism. In the field of neuro-endocrinology, such neuro-endocrine control on oxygen consumption has been reported for crustaceans [20].
Hanumante *et al*. [6]. Has been shown that neurohormones from pleurovisceral ganglia regulate the rate of oxygen consumption in gastropod molluscs. The role of cerebral and visceral ganglia in respiratory metabolism has been reported for estuarine clam, *Katelysia opima* [11], for freshwater bivalve mollusc, *Lamellidens marginalis* [25], *Indonaia caeruleus* [26] and Effect of cerebral ectomy on the rate of respiration in *Lamellidens corrianus* [21].
In bivalve molluscs, two types of neuro-secretory cycles occurs in cerebral ganglia[10], the short term cycle (*i.e.* sudden change in temperature, pH and salinity) and long term cycle related to the certain activity of reproduction and metabolism. Such neuro-secretory cycles of neuro-secretory cells were reported by [18], for estuarine clam, *Katelysia opima*. Though considerable data is accumulating on several aspects of endogenous and exogenous regulation of reproduction and energy metabolism in bivalve molluscs, the data appears to be restricted exclusively and specially for marine dioecious species. Very little work on the involvement of neuro-secretion in reproduction and energy metabolism reported in the case of freshwater species [9]. Review of literature shows very little information is known on neuro-endocrine control of oxygen consumption in freshwater bivalves.

Since many features of aerobic metabolism can be studied directed by measurement of the rate of oxygen consumption by intact animals. Several reports are available on respiratory physiology of freshwater bivalves from India and abroad [22, 1, 29, 14].

Thus, considering the paucity of information on endogenous regulation in the respiratory metabolism, because the respiration is considered as one of the important aspects for understanding the physiological adaptation of a species. In bivalve shellfishes from the Inland waters, the present study is taken on freshwater bivalve, *Indonaia caeruleus* from Godavari River.

2. Materials and Methods

The adult freshwater bivalves, *Indonaia caeruleus* (50-55mm in shell length) were collected from Godavari River near Aurangabad, during monsoon season (June –September 2014). Sampling was done between 8 am to 11 am by using simple hand picking method with wearing hand gloves. As soon as sample brought to the laboratory the bivalve rinse with water thrice and then brushed to remove the mud, fouling fungal and algal biomass. Then they were acclimatized for 24 hr. in laboratory conditions. No food was given to the animals during laboratory acclimatization and subsequent experimentation. Considering the role of cerebral ganglia on the rate of oxygen uptake in freshwater bivalve, we designated experimental plan of 10 days i.e. the injection of cerebral ganglionic extracts and their equivalent commercial hormones (progesterone and estradiol) to intact freshwater bivalves during monsoon season, the results are compared to respective controls of 2nd, 5th and 8th days. After 24hr. acclimatization the animals were arranged in four groups i.e. in individual aquarium, each group containing 20 animals in 10 liter of aerated water. The first group of animals was served as normal control and other three groups were experimental with (i) injection of cerebral ganglionic extract to intact control; (ii) injection of equivalent progesterone to normal intact control and (iii) injection of the equivalent commercial hormone estradiol to normal control bivalves. For injection of cerebral ganglionic extract, extract was prepared in 1:1 ice cold distilled water and ethanol (*i.e.*20 ganglia in 2mL ice cold distilled water and ethanol), it was centrifuged and injected (0.2 mL extract/animal *i.e.* equivalent to 2 ganglia/animal), into the foot (muscular region). The experiment was run for 10 days. The physicochemical characteristics of water used in experiments i. e. temperature, pH, hardness and dissolved oxygen contents of the water were determined on every two days throughout the experimental period. The temperature determined with the help of thermometer, pH by ELICO pH meter, Hardness determined by EDTA method and dissolved oxygen of reservoir water determined by modified Winkler's technique [5].

The rate of oxygen consumption of individual animal from each group was determined by modified Winkler's technique [5], in a specially prepared brown colored respiratory jar of 1 liter volume. Five closed respiratory jars, each with an inlet and outlet. Every time five marked animals on their shells from each group were kept individually in the continuous circulation of water inside the jar by attaching inlet to the water reservoir with the help of plastic pipe, in order to open their shell valves. Once the animals were opened their valves, the flow of water was cutoff and animals were kept for 1 hour. Then sample of water from it was drawn after 1 hour. For determination of oxygen consumption, the bivalves from each group dissected carefully and 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 weighed to obtain the wet-weight of the individual bivalve, which required for calculating the rate of oxygen consumption of each individual animal.

The oxygen consumed by each animal was then calculated and expressed as mg O2/l/h/gm wet-weight of the flesh. The mean values of five individual animals from each group were used for statistical analysis. For confirmation of results all the values were subjected to statistical analysis using student't' test [2]. Percentage differences were also calculated in the experimental group compared to their respective control.

3. Results

The results of the experiments were shown in (Fig. 1 and table 1). The physico-chemical characteristics of the water used in experiments during monsoon season were – Temperature (24.0C- 29.0C); pH (8.2- 8.35); hardness in terms of bicarbonate (125 - 142 ppm) and dissolved oxygen content (6.40 – 7.0 mg/L/h).

The rate of oxygen consumption was significantly decreased (0.2103± 0.0181, 66.52%, $P<0.01$) from ganglionic extract injected group, on 2nd day compared to control. Similarly the rate of oxygen consumption showed significant increase (0.2325 ± 0.0121, 74.38%, $P<0.001$) in estradiol injected group, on 5th day. On 8th day, the rate of oxygen consumption also showed significant decrease (0.1218 ± 0.0174, 41.83%, $P<0.05$) in progesterone injected group and also insignificantly decreased (0.1617 ± 0.0326, 22.78%) in ganglionic extract injected group as well as (0.1673 ± 0.0628, 20.10%) in estradiol injected group. The rate of oxygen consumption in control group was (0.2811 ±0.0173), (0.1339 ± 0.0102) and (0.2094± 0.0113) on 2nd, 5th and 8th day respectively. While the rate of oxygen consumption in ganglionic extract injected to intact animals group was (0.2103 ± 0.0181, 66.52%), (0.2099 ± 0.0329, 56.76%) and (0.1617 ± 0.0326, 22.78%) on 2nd, 5th, and 8th day respectively. The rate of oxygen consumption in progesterone injected and estradiol injected were (0.1218 ± 0.0174, 41.83%) and (0.1673 ± 0.0628, 20.10%) on 8th day respectively.

Table 1: Effect of hormone injection Progesterone, Ganglionic extract, Sham operation, and Estradiol on the rate of respiration of fresh water bivalve *Indonaia caeruleus* during Monsoon season. •••=$p<0.001$; ••=<0.01; •=<0.05.

Oxygen Consumption					
Days	**Control (mg O2/l/h/gm)**	**Injection of progesterone (mg O2/l/h/gm)**	**Injection of Ganglionic Extract (mg O2/l/h/gm)**	**Sham operation (mg O2/l/h/gm)**	**Injection of Estradiol (mg O2/l/h/gm)**
2nd Day	0.2811 ±0.0173	0.2528 ±0.0432 (59.75%) •	0.2103 ±0.0181 (66.52%) ••	0.2431 ±0.0226 (61.30%) •	0.2359 ±0.0145 (62.44%) •
5th Day	0.1339 ±0.0102	0.1668 ±0.0292	0.2099 ±0.0329	0.2216 ±0.0143	0.2325 ±0.0121

		(24.57%)	(56.76%) •	(65.50%) ••	(74.38%) •••
8th Day	0.2094 ±0.0113	0.1218 ±0.0174 (41.83%) ••	0.1617 ±0.0326 (22.78%)	0.1643 ±0.034 (21.54%)	0.1673 ±0.0628 (20.10%)

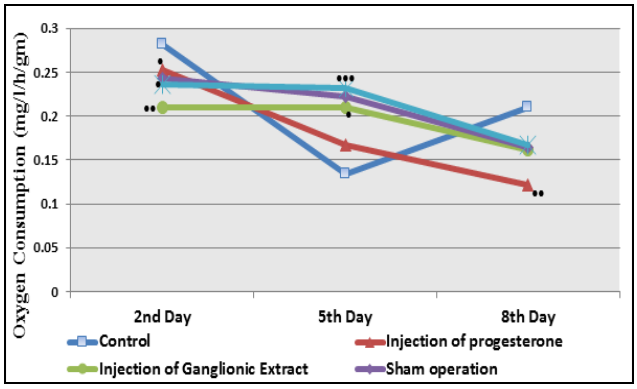

Fig 1: Effect of hormone injection Progesterone, Ganglionic extract, Sham operation, Estradiol on the rate of respiration of fresh water bivalve *Indonaia caeruleus* during Monsoon season. •••=p<0.001; ••=<0.01; •=<0.05.

4. Discussion

The present study on freshwater bivalve, *Indonaia caeruleus*, revealed that, injection of cerebral ganglionic extracts to intact bivalves causes a significant and insignificant decrease in the rate of oxygen consumption on 2nd and 8th day respectively. The rate of the oxygen consumption in estradiol injected animals also caused significant decrease on any 2nd and insignificant decrease on the 8th day compared to respective control. But the rate showed an increase in all groups on 5th day compared to the 2nd day.

A significant decrease in the rate of oxygen consumption after injection of ganglionic extract, progesterone and estradiol compared to control on 2nd day and again recovery of metabolic regulation with significant increase 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 intact animals, which is receiving the cerebral ganglionic extract and restore the rate of oxygen consumption.

From the data, it can be suggested that cerebral ganglia must possesses 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 [25, 26, and 12]. A decrease in rate of oxygen consumption following injection of ganglionic extract to the intact animals which reached the normal intact control, confirms that the regulating link is not through the nervous input but possibly by neuro-secretory. This contention can further be supplemented by the fact that even in intact control animals, as injection of extract of cerebral ganglia significantly decreases the rate of oxygen consumption.

Hence, it is concluded that, cerebral ganglia must possesses oxygen consumption controlling factor and which is neuro-secretory. This integrity of these ganglia is essential in normal functioning of physiological activities of the bivalve molluscs. Similarly equivalent commercial hormones (progesterone and estradiol) also essentially playing a principle role in the normal functioning of respiratory activity.

In the earth worm, *Perionyx excavates*, the rate of oxygen consumption has been suggested to be under the influence of neurosecretory release of one or more hormonal agents from central nervous system [19]. The brain and subpharyngeal ganglia of earthworm have shown to be the oxygen inhibiting and elevating hormones respectively. The concept of hormonal control of oxygen consumption has been evidenced in number of poikilothermic organisms [7]. In crab *Uca pugilator* two independent active hormones, controls the rate of oxygen consumption (1) Eyestalk factor regulating oxygen consumption and (2) The removal of moult inhibiting hormone which enhancing oxygen consumption [24]. In Penaid prawn, *Parapenaeopsis hardwickii*, eyestalk possesses a hormone which decreasing the rate of oxygen consumption [20]. In gastropod, *Onchidium verruculatum* removal of whole central nervous system or pleuropedal ganglia significantly inhibit oxygen uptake [6] Replacement of pleurovisceral ganglia in pleurovisceralectomised gastropod restores the rate of oxygen consumption to the normal level.

5. Conclusion

In the present study, on freshwater bivalve, *Indonaia caeruleus*, it is possible that injection of their cerebral ganglionic extracts to intact animals could have resulted in the initiation of the release of large quantities of serotonin and catecholamine as stated by [10] in *Mytilus edulis*. Another exiting possibly that these neuro hormones after their entry into "milieu interieur" may be enhancing the role of non–specific stressors [4] or neuroendocrine transducer [28], there by indicating the endogenous neurosecretory hormones involved in regulation of oxygen consumption. This idea gives strength to the fact that the biogenic amines act as a neurotransmitter to elicit the release of neurohormones from hypothalamic nuclei of vertebrate [13] and probably also from those of invertebrates e.g. crustaceans [3] and bivalve molluscs [11]. These neurohumors are capable of inducing changes in the neurosecretory cells in the cerebral and visceral ganglia of the bivalve mollusc [8]. Administration of sex steroids in bivalves may also stimulate gonadal differentiation by accelerating the metabolic rate to provide more materials and energy [27]. Evidence exists for possible actions of sex steroids in the regulation of the metabolism of glycogen, protein and lipids in bivalves. For example, estradiol may stimulate glycogenolysis and lipogenesis by regulating the activities of some important enzymes such as glucose- 6-phosphate dehydrogenase and malate dehydrogenase in molluscs [15, 16, and 17].

Since the endogenous presence of these neurohormones in the ganglia of bivalve molluscs as well as equivalent commercial hormones like progesterone and estradiol have already been established, regulation of oxygen consumption may be tentatively suggest as one of the physiological roles for these neuro-humors in the metabolic economy of the bivalves.

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