Indexed, Abstracted and Cited: Index Copernicus International (Poland), ISRA Journal Impact Factor, International Impact Factor Services (IIFS), Directory of Research Journals Indexing (DRJI), International Institute of Organized Research and Scientific Indexing Services, Cosmos Science Foundation (South-East Asia), International Innovative Journal Impact Factor, Einstein Institute for Scientific Information {EISI}, Directory of Open Access Scholarly Resources, Science Indexing Library (UAE), Swedish Scientific Publication (Sweden), citefactor.org journals indexing, Directory Indexing of International

World Journal of Biology and Medical Sciences

Published by Society for Advancement of Science®

ISSN 2349-0063 (Online/Electronic)

Volume 5, Issue-3, 9-16, July - September, 2018

Journal Impact Factor: 4.197

WJBMS 05/03/003/2018

www.sasjournals.com

A Double Blind Peer Reviewed Journal / Refereed Journal

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REVIEW ARTICLE

Received: 03/06/2018 Revised: 08/07/2018 Accepted: 09/07/2018

Role of Cerebral Ganglionic Extract and Equivalent Commercial Hormone injection in regulation of Oxygen Consumption of freshwater bivalve mollusc *Indonaia caeruleus* (Prashad, 1918) during winter 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 winter 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 2^{nd}, 5^{th}, and 8^{th} day. The study revealed that, the rate of oxygen consumption was significantly increased in all injected group on the 2^{nd} day. The rate of oxygen consumption also showed significant decrease in progesterone injected group and ganglionic extract injected group on 5^{th} day. The rate of oxygen consumption showed insignificant increase on 8^{th} day in progesterone, ganglionic extract and estradiol injected groups.

Keywords: - Cerebral ganglionic extract, Progesterone, Estradiol, Oxygen consumption and Freshwater bivalve.

INTRODUCTION

The rate of oxygen consumption is an integral metabolic index, which allows us to determine physiological state of the organism under various ecological conditions. Oxygen consumption by edible mussels as well as other mollusks depends on a number of internal and external factors. Internal (developmental stage, the corresponding body weight, or sexual cycle stage) and external factors (geographic latitude, season, temperature oscillations, feeding conditions, water saturation with oxygen, and tidal conditions) affect the rate of oxygen consumption (Thompson and Bayne, 1972; Bayne *et al*., 1973; Braiko and Dereshkevich, 1978; Famme, 1980; Hamburger *et al*., 1983; Sukhotin, 1988). Naturally, oxygen consumption rate depends on growth conditions of mollusks.

In general, many exogenous environmental variables (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 neuro-endocrine centers. The respiratory rate data of the animals reflect their general metabolic rate. The existence of neuro-endocrine modulations of metabolic rate will be the adaptive significance for the freshwater bivalves, which have to live in ever fluctuating environments.

In the field of neuroendocrinology, neuroendocrine regulation of oxygen consumption has been reported for crustaceans (Nagabhushnam and Kulkarni, 1979). Hanumante *et al.* (1980) has been shown that, neurohormones from pleurovisceral ganglia regulate the rate of oxygen consumption in gastropod mollusks. 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* from Godavari River at Kaigaon. Jadhav (2011) studied on *Lamellidens marginalis* from Paithan some reports are available on respiratory physiology of freshwater bivalves mollusc from India and abroad (Salanki and Lukascsovice, 1967; Bayne, 1976; Zs- Nagy, 1974).

In freshwater bivalve, *Lamellidens corrianus* (Jadhav et al., 2012), it is possible that surgical bilateral decerebration and injection of their extracts to bilateral cerebralectomised animals as well as injection of hormone to intact animals could have resulted in initiation of the release of large quantities of serotonin and catecholamine as stated by Lubet (1970) in 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/ hormones involved in regulation of oxygen consumption. This idea gives strength to the fact that the biogenic amines act as neurotransmitters to induce the release of neurohormones from hypothalamic nuclei of vertebrate (Maclead and Lehmeyer, 1977) and probably also from those of invertebrate e.g. crustaceans (Fingerman *et. al.,* 1974) and bivalve mollusk (Mane *et al.,* 1990) these neurohormones are capable of inducing changes in the neurosecretory materials from cells in the cerebral and visceral ganglia of the bivalve shell fishes (Kapoor, 1986).

MATERIALS AND METHODS

The adult freshwater bivalves, *Indonaia caeruleus* (50-55mm in shell length) were collected by hand picking method from Godavari River near Aurangabad, during winter season (December-January) 2014. After brought to the laboratory the shells of the bivalves were brushed and washed with water to remove the mud and fauling fungal and algal biomass and 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 2^{nd}, 5^{th} and 8^{th} 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. Injections were prepared before every experimentation i.e. commercial hormone injection progesterone and estradiol 0.1 mg/ml respectively and 0.1 ml quantity have been injected; 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 O_2/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.

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 winter season were – Temperature (18.0C- 24.0C); pH (8.13- 8.42); hardness in terms of bicarbonate (112- 130 ppm) and dissolved oxygen content (6.10 – 7.45 mg/l/h).

Table 1. Effect of hormone injection Progesterone, injection Ganglionic extract, injection Sham operation, hormone injection Estradiol on rate of respiration of fresh water bivalve, *Indonaia caeruleus* during winter season (Bracket values represent percentage differences compared to control). •••=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	0.1218 ±0.0266	0.1825 ±0.0323 (49.84%) •	0.2281 ±0.0243 (87.27%) •••	0.1683 ±0.0393 (38.18%)	0.4323 ±0.0255 (254.93%) •••
5th Day	0.1880 ±0.0189	0.0981 ±0.0258 (47.82%) ••	0.1129 ±0.0157 (39.75%) ••	0.1660 ±0.0286 (11.70%)	0.1543 ±0.0349 (17.93%)
8th Day	0.1205 ±0.0193	0.1584 ±0.0102 (31.45%)	0.1323 ±0.0264 (9.79%)	0.1229 ±0.0337 (1.99%)	0.1407 ±0.0637 (16.76%)

During winter season, the rate of oxygen consumption in control group was (0.1218 ± 0.0266) on 2nd, (0.1880 ± 0.0189) on 5th and (0.1205 ± 0.0193) on 8th day. The rate of oxygen consumption was increased on 5th and decreased on 10th day, compared to 2nd day. The rate of oxygen consumption was significantly increased (0.1825 ± 0.0323, 49.84 %, P < 0.05) on 2nd and significant decreased (0.0981 ± 0.0258, 47.82 %, P < 0.01) on 5th day, in hormone progesterone injected animals, while the rate was non significantly increased (0.1584 ± 0.0102, 31.45 %) on 8th day, compared to respective controls. The rate of oxygen consumption also significantly increased (0.2281 ± 0.0243, 87.27 %, P < 0.001) on 2nd and significantly decrease (0.1129 ± 0.0157, 39.75 %, P < 0.01) on 5th day, while it was non significantly increased (0.1323 ± 0.0264, 9.79 %) on 8th day in ganglionic extract injected animals, compared to respective control. The rate of oxygen consumption significantly increased (0.4323 ± 0.0255, 254.93 %, P < 0.001) on 2nd day and non significantly decreased (0.1543 ± 0.0349, 17.93 %) on 5th day, while non significantly increased (0.1407 ± 0.0637, 16.76 %) on 8th day in hormone estradiol injected animals compared to respective controls.

DISCUSSION

The present study on freshwater bivalve, *Indonaia caeruleus* revealed that, injection of progesterone, estradiol and cerebral ganglionic extracts to intact bivalves causes a significant increase in the rate of oxygen consumption on 2nd day and it is significantly decreased on 5th day from progesterone and ganglionic extract injected group respectively. The rate of oxygen consumption on 8th day increased insignificantly from all experimental groups as compared to normal intact control group.

A significant increase 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 and insignificant decrease 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; while similar pattern of restoration for oxygen consumption have been observed from hormone progesterone and estradiol injected group.

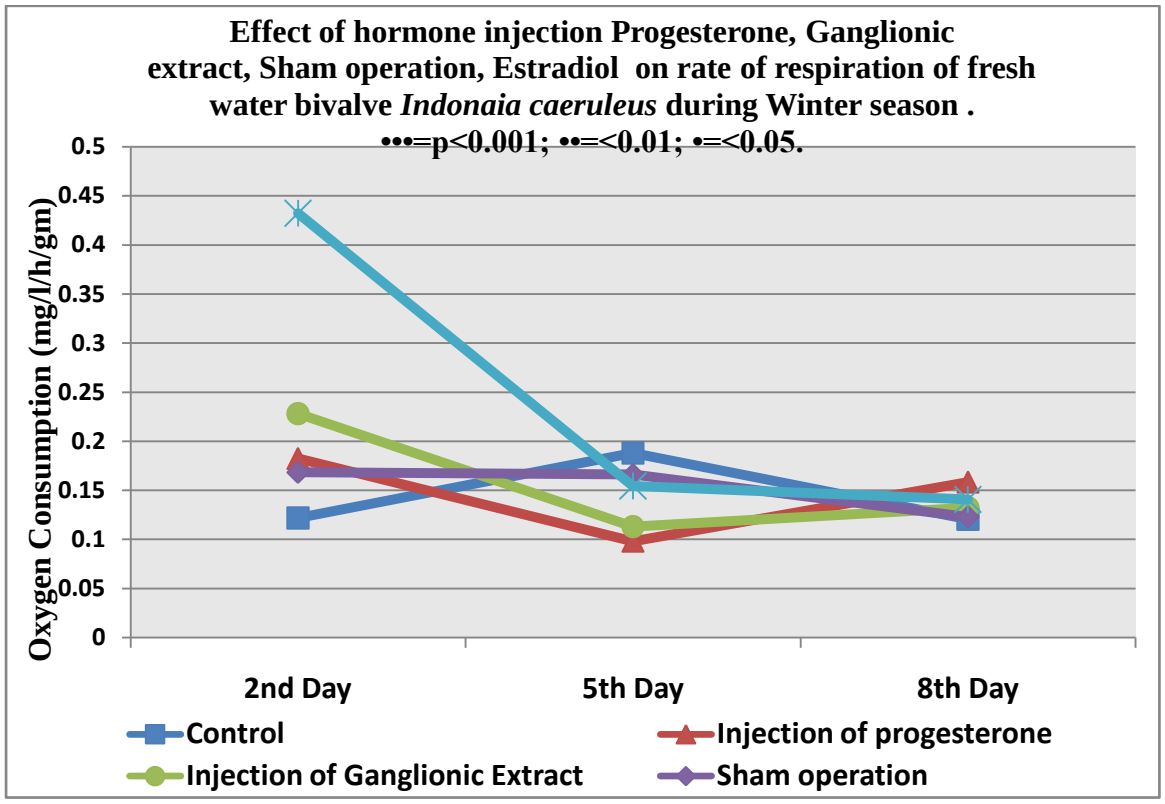

Figure 1.

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 (Vedpathak and Wagh, 2009; Vedpathak et al., 2011; Jadhav et al., 2012). A decrease in rate of oxygen consumption following injection of ganglionic extract and commercial hormones 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, after initial increase in respiratory metabolism.

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 (Nagabhushanam R and Hanumante MM, 1977). 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 (Kale RD and Rao KP, 1973). 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 (Silverthorn, 1975). In Penaid prawn, *Parapenaeopsis hardwickii*, eyestalk possesses a hormone which decreasing the rate of oxygen consumption (Nagabhushanam and Kulkarni, 1979). In gastropod, *Onchidium verruculatum* removal of whole central nervous system or pleuropedal ganglia significantly inhibit oxygen uptake (Hanumante et al., 1980). Replacement of pleurovisceral ganglia in pleurovisceralectomised gastropod restores the rate of oxygen consumption to the normal level.

CONCLUSION

In the present study, on freshwater bivalve, *Indonaia caeruleus*, it is possible that injection of their cerebral ganglionic extracts and equivalent commercial hormones (progesterone and estradiol) to intact animals could have resulted in the initiation of the release of large quantities of serotonin and catecholamine as stated by Lubet (1970) in *Mytilus edulis*. Another exiting possibly that these neuro hormones after their entry into “milieuinterieur” may be enhancing the role of non–specific stressors (Gold and Ganong, 1977) or neuroendocrine transducer (Wurtman, 1972), 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 (Maclead and Lehmeyer, 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 cells in the cerebral and visceral ganglia of the bivalve mollusc (Kapoor, 1986). Administration of sex steroids in bivalves may also stimulate gonadal differentiation by accelerating the metabolic rate to provide more materials and energy (Croll and Wang, 2007). 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 lipidogenesis by regulating the activities of some important enzymes such as glucose- 6-phosphate dehydrogenase and malate dehydrogenase in molluscs (Mori, 1969; Mori et al., 1972a,b).

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.

ACKNOWLEDGEMENTS

Authors are thankful to UGC New Delhi, India for awarding Rajiv Gandhi National Research Fellowship and also thankful to Department of Zoology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS), India for providing the laboratory facilities.

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