

Oxygen Consumption of an Oligochaete, *Metaphire houlleti* as a Function of Neuroendocrine Manipulations

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The suprapharyngeal ganglionectomy for 24 hr induced an increase in the oxygen consumption of worms. A significant ($P < 0.05$) decline in oxygen consumption rates of normal and 24 hr suprapharyngeal ganglionectomized worms was observed after injecting suprapharyngeal ganglion homogenate. The subpharyngeal ganglionectomy for 24 hr produced a significant ($P < 0.05$) decrease in the rate of oxygen consumption as compared to the sham-operated controls. Injection of subpharyngeal ganglion homogenate, but not that of saline, provoked significant increase in the oxygen consumption rates of normal as well as 24 hr suprapharyngeal ganglionectomized worms. The results suggest that the process of oxygen consumption in *Metaphire houlleti* is under the influence of two antagonistic hormones, secreted in two parts of the brain.

Key Words: Oxygen consumption, Brain, Supra and subpharyngeal ganglia, Hormonal involvement

Introduction

There have been a few reports on the hormonal control of oxygen consumption of leeches, *Poecilobdella viridis* (Nagabhusanam & Kulkarni 1978, Kulkarni et al. 1982) and *Hirudo birmanica* (Anand 1984). Kale and Rao (1973) and Nagabhusanam and Hanumante (1977) have stated that oxygen consumption in *Lampito mauritii* and *Perionyx excavatus* respectively might be under the influence of neurohumoral agents. But for these couple of reports, no precise information is available regarding the role of brain hormone, in the process of oxygen consumption of tropical oligochaetes. The present work is an attempt in this direction, using the worm, *Metaphire houlleti*.

Material and Methods

The worms, *Metaphire houlleti*, after collection were immediately transferred to the laboratory and held on simulated natural habitat for a period of 3 days to enable them to get acclimated to the prevailing laboratory conditions.

A group of 10 adult, fully clitellate worms of approx. same size (3-4 cm) and weight (2-3 g) were introduced into the respiratory chamber of 1 L capacity

(size 10" × 4"). Winkler's technique as described by Welsh et al. (1968) was employed for measuring oxygen content of the initial and final samples. For all observations final samples were siphoned out after 1 hr. Average oxygen consumed was calculated in relation to unit wet weight of the worm based on 3 replications.

Extirpation of the suprapharyngeal and subpharyngeal ganglia was performed after anaesthetizing the worms in 5% ethanol (V/V) for about 2 min. A mid-dorsal incision was taken between 3rd and 6th segment and the exposed suprapharyngeal ganglion was removed by severing the circumpharyngeal connectives. Subpharyngeal ganglionectomy was effected in the same fashion but through a midventral incision after severing the ganglion from the ventral nerve cord. After recovery from anaesthesia, the worms were wrapped in moist filter paper and returned to the bedding soil. Minced suprapharyngeal and subpharyngeal ganglia were kept immersed in earthworm physiological saline, prepared according to Oka et al. (1984), and or frozen if not used immediately. The sham operated control leeches received the same treatment as the ones from which the ganglia were extirpated, except for the actual

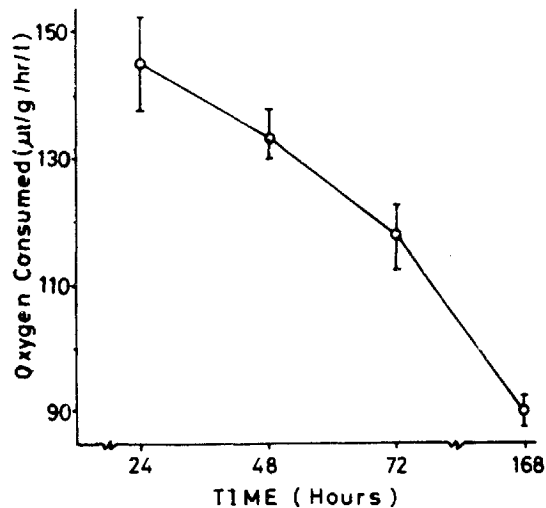


Figure 1 Oxygen consumption of *Metaphire houlletii* at different time intervals after supratharyngeal ganglionectomy. Vertical bars around each mean represent the range of variability

severing of the ganglia. Both the ganglia were homogenized separately and their extracts prepared. The supernatant after centrifugation at 2000 rpm was injected. The desired concentration was obtained by mincing the specific number of ganglia in the appropriate amount of physiological saline. Each worm received in all 100 µl of the homogenate.

Wherever needed the significance of the data was tested by applying students 't' test (Ostle 1966).

Results

The rate of oxygen consumption of worms maintained at two different habitats and designated as normal and standard controls are depicted in table 1. The average oxygen uptake was 80.4 and 77.1 µl/g/hr/l for normal and traumatized worms respectively.

24 hr after the supratharyngeal ganglionectomy there was a drastic elevation in oxygen consumption (145 µl/g/hr/l) which showed a gradual decline over the next seven days but did not come within the range of normal worms (figure 1). The rate of oxygen consumption decreased unabated in worms having anaesthetization and anaesthetization plus incision, after 6 through 24 hr (table 2).

The supratharyngeal ganglionectomy induced a constant increase in the rate of oxygen consumption, but subpharyngeal ganglionectomy produced a significant ($P < 0.05$) decrease in the rate of oxygen consumption after 6 through 24 hr of treatment (table 2).

Injection of the supratharyngeal ganglion homogenate significantly ($P < 0.05$) slashed the oxygen

Table 1 Oxygen consumption of normal *Metaphire houlletii* in different habitats and of traumatized worms

Habitat or treatment	Mean O ₂ consumption µl/g/hr/l ± SD
Paper toweling	80.4 ± 2.1
Moist soil	79.8 ± 2.2
Incision only (tested 24 hr later)	77.1 ± 1.9

Table 2 Oxygen consumption of *Metaphire houlletii* over a period of 24 hr following different treatments

Hours	Oxygen consumption (µl/g/hr/l) ± SD			
	Anaestheti- after treatment	Anaestheti- zation plus incision	Anaestheti- zation plus supratharyn- geal gang- lionectomy	Anaestheti- zation plus subpharyn- geal gang- lionectomy
6	95.3 ± 1.2	105.2 ± 2.1	125.2 ± 1.9	114.1 ± 2.3
12	92.7 ± 1.3	98.0 ± 1.9	134.9 ± 1.7	88.2 ± 1.7
24	81.7 ± 1.5	80.6 ± 1.4	152.0 ± 2.1	51.4 ± 1.8

consumption by 52.8% in normal and by 16.8% in 24 hr supratharyngeal ganglionectomized worms over those injected with saline (table 3). 24 hr after subpharyngeal ganglionectomy (table 4) the oxygen consumption of *Metaphire* was decreased by 38.55%. The injection of subpharyngeal ganglion homogenate produced a significant ($P < 0.05$) rise in the oxygen consumption of both normal and 24 hr subpharyngeal ganglionectomized worms.

Discussion

Rao and Saroja (1963) have reported that addition of the body fluids from cold acclimated worms increased the oxygen consumption of normal as well as warm acclimated tissues of *Lampito mauritii*. Enhanced metabolic rates and other compensatory changes occurring during acclimation to low temperature are triggered by the neurosecretory release of one or more humoral agents (Rao & Saroja 1963, Rao 1966). This idea has been extended further by Kale and Rao (1973) who observed a significant rise in the oxygen consumption of *Perionyx* worms acclimated to low temperature and of normal earthworms following the injection of the central nervous system extract of worms acclimated to cold. The present study shows that the supra- and subpharyngeal ganglia are probably the centres localizing the chemical messengers involved in the regulation of respiratory metabolism of *Metaphire*.

Two possible explanations were put forth for the rise in oxygen consumption following supratharyngeal ganglionectomy. One was the stress (anaesthesia plus incision) to which the worms were subjected during the operation and secondly the induction of regeneration

Table 3 Oxygen consumption of normal (control) and 24 hr suprpharyngeal ganglionectomized *Metaphire houlletii* after the injection of saline or suprpharyngeal ganglion extract

Worm category	Mean oxygen consumption ($\mu\text{l/g/hr/l}$) \pm SD after the injection of	
	Saline (100 μl /worm)	Suprpharyngeal ganglion extract (100 μl /worm)*
Normal (Control)	84.4 \pm 1.2	43.1 \pm 1.1
24hr suprpharyngeal ganglionectomized	161.2 \pm 2.7	134.4 \pm 2.4

*The concentration of extract was 2 suprpharyngeal ganglia per worm

spurt as a consequence of the removal of suprpharyngeal ganglion. Merely a glance through tables 1 and 2 will quickly brush aside the first cause. Immediate increase in oxygen consumption after traumatization is followed by a subsequent gradual decrease and 24 hr after the trauma the rate of oxygen consumption returned within the limits of the normal values.

In the present study over a period of several days following the suprpharyngeal ganglion removal the rate of oxygen consumption of *Metaphire houlletii* did not come on par with the normal values though a general decline was observed. This may be due to the restraining influence exerted by the substances originating in the other part of the central nervous system. Significant decline in oxygen uptake induced by the injections of suprpharyngeal ganglion homogenate into normal as well as 24 hr suprpharyngeal ganglionectomized worms as compared to the saline injected controls (table 3) provides an additional evidence for the presence of an oxygen consumption inhibiting or depressing hormone secreted in the suprpharyngeal ganglion of *Metaphire houlletii*.

Table 4 Oxygen consumption of normal (control) and 24 hr subpharyngeal ganglionectomized *Metaphire houlletii* administered with saline or subpharyngeal ganglia homogenate

Worm category	Mean oxygen consumption ($\mu\text{l/g/hr/l}$) \pm SD after the injection of	
	Saline (100 μl /worm)	Subpharyngeal ganglion homogenate (100 μl /worm)*
Normal (Control)	85.5 \pm 1.8	106.3 \pm 2.1
24 hr subpharyngeal ganglionectomized	57.3 \pm 1.1	78.4 \pm 1.6

*The concentration of homogenate was 2 subpharyngeal ganglia per worm

24 hr after suprpharyngeal ganglionectomy there was a 38.55% decline in the oxygen uptake and injections of the homogenate of subpharyngeal ganglia elicited a significant increase in normal as well as 24 hr subpharyngeal ganglionectomized worms over that of the controls. This suggests that subpharyngeal ganglion too contributes in the form of some chemical factors possibly neurosecretory one, which accelerate the rate of oxygen consumption. This statement stands substantiated by the emphatic evidences from the work of Rao and Saroja (1963), Kale and Rao (1973), Nagabhushanam and Hanumante (1977) on a few other tropical oligochaetes. Similar incidences of the occurrence of two antagonistic hormones controlling the rate of oxygen consumption are also reported in leeches, *Poecilobdella viridis* (Nagabhushanam & Kulkarni 1978) and *Hirudo birmanica* (Anand 1984)

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