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Influence of Neuroendocrinological and Pharmacological Stimulations on the Oxygen Consumption of a Freshwater Leech, *Poecilobdella viridis* (Blanchard)*

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Significant decrease in oxygen-consumption was observed 24 hrs after debraining. Injection of brain-homogenate (50 µl=2 brains/leech) caused a significant hike in the oxygen-consumption of normal as well as debrained (24 hrs) leeches. A significant elevation in the oxygen consumption was noticed in normal leeches after reserpine (RSP : 50µl= 50µg/leech) administration, and the decrease in oxygen-consumption was more dramatic and significant in debrained leeches. Chlorpromazine CPZ : 50 µl=25 µg/leech)induced a significant decrease in the oxygen-consumption of both normal and debrained leeches. The results indicate that the brain of leech secretes an oxygen-consumption accelerating factor and the secretory activity of brain is influenced by the tranquillizers in *P. viridis*.

Key Words: Oxygen consumption, Debraining, Tranquillizers, CPZ, RSP

Introduction

The implications of hormonal regulation over oxygen-consumption were reported for non-annelid invertebrates, such as crustaceans (Silverthorn 1975 a, b, Nagabhushanam & Kulkarni 1978) and molluscs (Hanumante et al. 1980). However, no such findings for annelids in general, and Hirudineans and Polychaetes, in particular, are available; Except that in the case of Oligochaetes, *Lampito mauritii* (Rao 1966) and *Perionyx excavatus* (Kale & Rao 1973, Nagabhushanam & Hanumante 1977), a relationship between the oxygen-consumption and neurohumoral agents was established.

As is well known, numerous neuropharmacological agents and other chemicals provoke changes in the physiological processes of animals by affecting directly or indirectly the

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endocrine centres of vertebrates (Sarkar et al. 1977) and invertebrates (Masner et al. 1976, Hanumante et al. 1978, 1979 a, b, c, 1980). Reserpine was reported to deplete 5-hydroxytryptamine (5-HT) content (Giachetti & Shore 1978) and catecholamines (Muscholl & Vogt 1958) in the brain of vertebrates, whereas, Fingerman et al. (1974) demonstrated that reserping lowers serotonin (5-HT) levels in the eyestalks and brain of invertebrate, *Uca pugilator*. Chlorpromazine, another tranquillizer, although showed effects similar to reserpine, yet it differed in its mode of action (Hess 1954). According to him, reserpine affects by releasing 5-HT, while chlorpromazine acts by blocking the release of noradrenaline. The natural existence of adrenaline, acetylcholine and 5-HT has been demonstrated by many workers in leeches (Ehingar et al. 1968, Marsden & Kerkut 1969). Biogenic amines are known to participate in the release of neurohormones in insects (Samarnayaka 1976), crustaceans (Fingerman & Fingerman 1977) and Oligochaete annelids (Nagabhushanam & Hanumante 1977).

The present study was undertaken to show the neuroendocrine involvement in the regulation of oxygen-consumption and the possible involvement of biogenic amines in the release of neurohormones in *P. viridis*.

Meterial and Methods

The leeches, *P. viridis*, were collected locally from the freshwater ponds and maintained in wet mud under normal laboratory conditions for 24 hr before use. The leeches having approximately equal weight (7–7.5 g) were selected and grouped into 10 categories of 10 each as follows :

Group 1 : Normal control

Group 2 : Normal leeches receiving distilled water (50 μl/leech)

Group 3 : Normal leeches injected with brain homogenate (2 brains= 50 μl/leech)

Group 4 : Normal leeches receiving chlorpromazine (CPZ: 25 μg =50μg/ leech)

Group 5 : Normal leeches administered with reserpine (RSP : 50 μg = 50μl/leech)

Group 6 : Debrained for 24 hrs, and

24 hrs debrained leeches of groups 7, 8, 9 and 10 received the same quantity of distilled water and same concentrations of brain extract and CPZ and RSP as mentioned above. Methods for debraining, preparations of the brain extracts and drug concentrations and their injections were the same as described earlier (Kulkarni 1976).

The oxygen-consumption rates were measured by the Standard Winkler's technique at the start of the experiment in group 1, after 24 hrs of debraining in group 6 and after 2 hrs following the respective treatment in the remaining groups of leeches. The rates of oxygen-consumption were expressed as μl/hr/g/l.

Results

The results presented in figure 1 clearly indicate that there was a significant ($P<0.05$) decrease (—14.80%) in the rate of oxygen-consumption by *P. viridis* after 24 hrs of debraining (group 6). Brain-homogenate injection elevated the rate of oxygen-consumption significantly ($P<0.05$) in both normal (group 3; +8.98%) as well as 24 hrs debrained (group 8; +5.91%) leeches as compared to the normal leeches of group 1. The rate of oxygen-consumption decreased significantly ($P<0.05$) in normal (group 4; —13.99%) as well as debrained leeches (group 9; —58.58%) injected with CPZ. Oxygen-consumption rate increased. significantly ($P<0.01$) in RSP-treated normal leeches (group 5;+30.75%). But surprisingly, the RSP treatment induced

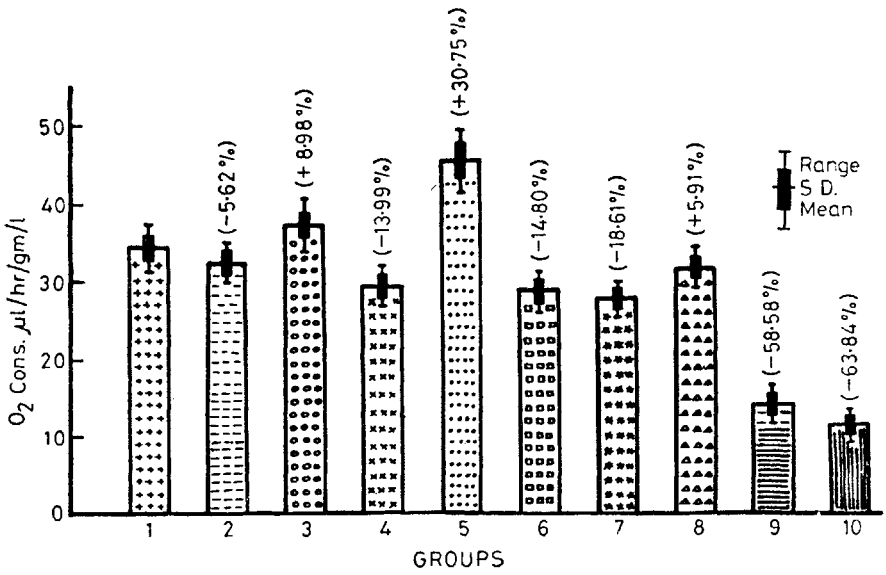

Figure 1 Effect of different treatments on the rate of oxygen consumption of a freshwater leech, *P. virids* (Groups are explained in text)

severe fall (group10; —63.84%) in the rate of oxygen-consumption in 24-hr-debrained leeches.

Discussion

The present data obviously show that in *P. viridis* the brain regulates the oxygen-consumption. A decrease in the rate of oxygen-consumption might be due to removal of some regulating nervous link during operation. But an elevation in the oxygen-consumption following the injection of brain-homogenate into 24 hrs debrained leeches and not in control (injected with distilled water) confirms that the regulating link is not the nervous input but probably neurosecretory. This conclusion is additionally strengthened by the fact that even in normal leeches, the brain-homogenate injections stimulated significant hike in oxygen-consumption. Therefore, it is expected that the neuroendocrine armour of the brain of *P. viridis* is the site for the synthesis of "oxygen-consumption-accelerating factor". The pres ence of a "respiration-regulating factor" in the brain (suprapharyngeal ganglion) and the subpharyngeal ganglion have already been demonstrated in an Oligochaete, *Perionyx excavatus* (Nagabhushanam & Hanumante 1977). The existence of neurohormonal regulation of metabolic rate will be of adaptive significance for *P. viridis* which has to thrive in labile tropical hydrobiotic conditions.

The occurrence of serotonergic and catecholaminergic neurons as well as the biogenic amines in the nervous system of different invertebrates, has been reported. Biogenic amines act as neurotransmitter to elicit the release of neurohormones in vertebrate hypothalamic nuclei (Macleod & Lehmeyer 1974) and probably also from those of invertebrates (Fingerman et al. 1974, Heldman & Alkone 1978, Kulkarni et al. 1980). The increased oxygen-consumption in normal *P. viridis*, after RSP-treatment may be due to its pharmacological effect. RSP, owing to its

neuroleptic effect can bring about the release of monoamines which in turn presumably influence the secretion of hormones that increase the oxygen-consumption. Previously, it was reported that 5-hydroxytryptamine and adrenaline, when injected exogenously in normal *P. viridis*, elevated its oxygen-consumption rate significantly (Hanumante et al. 1978) and brought about spectacular changes in the brain neurosecretory structure (Kulkarni et al. 1980). Thus, the effect of RSP is quite specific in releasing the monoamines, but the severe decline in the oxygen-consumption in 24-hr-debrained leeches, after RSP injection is difficult to explain. It could be expected due to the brain-removal, no target tissue remained to act upon for the released monoamines under the influence of RSP and to cause to release the oxygen-consumption-levating factor. It is also possible that RSP could act nonspecifically, i. e. toxic effect, in brainless leeches. It has been already demonstrated that RSP produces toxic effects by its non-pharmacological action (Samarnayaka 1976). However, it is difficult to explain as to how RSP actually caused lowering of the oxygen-consumption of debrained leeches. The significant decrease in the rate of oxygen-consumption of normal *P. viridis*, produced by CPZ, indicates that this tranquillizer has its specific effects and inhibits the release of biogenic amines from the nervous system, which may stimulate brain to secrete oxygen-consumption-elevating factor. At the same time, perhaps, it may exert non-specific, i. e. toxic effect, in both normal as well as de-brained leeches. Since, the same dose (25μg/leech) of CPZ was administered to normal and debrained leeches, it is surprising as to how CPZ would be so toxic (51.58% decrease in oxygen-consumption) to the debrained leeches. Earlier, it was reported that in leeches, the mode of oxygen-uptake is by pumping of the coelomic fluid round the body. Injections of monoamines increases the rate of the pumping which in turn increases the respiratory rate (Mann 1962). It is expected that CPZ might be having toxic effect upon the vascular system, reducing its efficiency of pumping the coelomic fluid, in both normal as well as in debrained-leeches. The more prominent effect which was seen in operated leeches may be due to the lack of an endocrine centre, which can protect the pumping system by its secretion, as expected in normal one, but the mechanism of action to prevent or to minimize the toxicity of CPZ is not known.

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