Proc. Indian Acad. Sci. (Anim. Sci.), Vol. 95, No. 6, December 1986, pp. 723–727.

Blood glucose in a freshwater leech, *Poecilobdella viridis* (Blanchard): Neuroendocrine regulation

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MS received 29 March 1984; revised 4 September 1986

Abstract. Hormonal involvement in the control of blood glucose level of *Poecilobdella viridis* was tested by performing debraining, supra- and sub-oesophageal ganglionectomy, followed by injections of the extract of neuroendocrine tissues in operated leeches. Anatomically, the brain ring, through which the oesophagus passes, of this leech is composed of two separate ganglia, the supra-oesophageal and sub-oesophageal, connected to each other by circum-oesophageal connectives. In normal leeches blood glucose level was found to be 10·2 μg%. Removal of complete brain and only the supra-oesophageal portion of the brain produced after 24 h a significant ($P < 0{\cdot}01$) decrease in the blood glucose concentration. Sub-oesophageal ganglionectomy produced no effect. Injections of brain and supra-oesophageal ganglion extracts into 24 h debrained and supra-oesophageal and sub-oesophageal ganglionectomized leeches increased the blood glucose level significantly, but the injections of sub-oesophageal ganglion homogenates had no effect. These data indicate that supra-oesophageal ganglionic portion of the brain of *Poecilobdella viridis* may be the production site of hyperglycemic hormone.

Keywords. Blood glucose; neuroendocrine regulation; supra-oesophageal ganglion; hyperglycemic hormone.

1. Introduction

Sugars in some of annelids have been analysed and generally measured as reducing sugars like glucose and certain disaccharides, such as maltose (Oglesby 1978). Clark (1964) found 72–264 mg/100 ml of total carbohydrates in the coelomic fluid of the polychaete, *Nephtys hombergi* and reported that glucose was the major and maltose was the minor component. Hanumante (1975) also reported that 14·5 mg% of blood glucose present in an earthworm, *Perionyx excavatus*. However, the reports on blood glucose levels in hirudinean annelids are very sparse, except a few on the free sugar levels of body fluids in *Glossiphonia complanata* and *Helobdella stagnalis* (Florkin and Scheer 1969).

In *Poecilobdella viridis*, various physiological phenomena, like respiration, osmoregulation, nitrogen excretion and digestion (Bhaskarrao 1983) and gametogenesis (Nagabhushanam *et al* 1976; Kulkarni and Nagabhushanam 1980) were already found to be under the influence of brain hormone. The present study is undertaken to determine the blood glucose level and the role of brain hormone, if any, in the regulation of blood glucose level of the freshwater leech, *P. viridis*.

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2. Materials and methods

The leeches, *P. viridis* used for experiments were adult and healthy, having approximately equal size (15–16 cm) and weight (8–10 g), collected from the freshwater ponds around Aurangabad city. In the laboratory leeches were maintained in glass troughs containing wet mud and kept under normal day/night illumination at 25 ± 0·5°C. A 3 days pre-experimental period was allowed to get leeches acclimatized to the prevailing laboratory conditions. To determine the blood glucose levels and to ascertain the role of brain as a whole and of its components like supra- and sub-oesophageal ganglia hormones individually in regulating the blood glucose level, leeches were divided into two groups with following protocol.

Group-I: It was further divided into 7 sub-groups, each of 5 leeches.
(i) Normal control (brain intact).
(ii) 24 h sham operated controls (brain was exposed, slightly agitated but left intact).
(iii) 24 h debrained.
(iv) 24 h debrained leeches received 2 injections of distilled water after every 6 h (50 µl/leech).
(v) 24 h debrained leeches received 2 injections of whole brain extract after every 6 h (50 µl = 2 brains/leech).
(vi) 24 h debrained leeches received 2 injections of supra-oesophageal ganglion (SPOG) homogenate after every 6 h (50 µl = 2 SPOG/leech).
(vii) 24 h debrained leeches received 2 injections of sub-oesophageal ganglion (SOG) extract after every 6 h (50 µl = 2 SOG/leech).
In addition to the first two sub-groups of Group-I, the Group-II was further divided into 4 more sub-groups and each sub-group contained 5 leeches.

Group-II
(i) 24 h supra- and sub-oesophageal ganglionectomized leeches.
(ii) 24 h supra- and sub-oesophageal ganglionectomized leeches received 2 injections of distilled water after every 6 h (50 µl/leech).
(iii) 24 h supra- and sub-oesophageal ganglionectomized leeches injected with 2 injections of supra-oesophageal ganglion homogenate (50 µl = 2 SPOG/leech) after every 6 h.
(iv) 24 h supra- and sub-oesophageal ganglionectomized leeches administered with 2 injections of sub-oesophageal ganglion extract (50 µl = 2 SOG/leech) after every 6 h.

In leeches it is already reported that blood circulation takes place through 4 main longitudinal coelomic sinuses: a dorsal sinus above the gut, a ventral sinus covering the ventral nerve cord and anterior and posterior ganglionic masses, and two lateral sinuses (Bhatia 1977). In order to determine the blood glucose levels of *P. viridis* from both control and experimental groups leeches were dissected after the stipulated time period by taking an incision latero-dorsally all along the body length and exposed the sinuses. 0·25 ml of blood was taken out directly from these sinuses with the help of a hypodermic syringe, having 27 gauze needle and 0·1 ml of blood was then transferred to separate test tubes. The methods of deproteinization and estimation of blood glucose using photoelectric colorimeter was the same as described by Oser (1976). Results of 3 replications were averaged and blood glucose levels were represented as µg%.

3. Results

Data from figure 1 clearly show that an average blood glucose level in normal leeches was found to be 10·2 μg%. 24 h debrained leeches showed a significant ($P < 0{\cdot}01$) decrease in their blood glucose concentration as compared to the sham operated controls. 24 h debrained leeches when administered with the whole brain homogenate produced a quite remarkable increase in blood glucose level as compared to the values of 24 h debrained leeches injected with distilled water. Supra-oesophageal ganglion homogenate alone when administered into the brainless leeches, a significant ($P < 0{\cdot}01$) increase in the blood glucose level was observed as compared to the brainless leeches injected with distilled water. The extracts of sub-oesophageal ganglion when injected into the brainless leeches could not produce any effect.

24 h supra-oesophageal ganglionectomized leeches showed a significant ($P < 0{\cdot}01$) decrease in the blood glucose levels of *P. viridis*. Administration of supra-oesophageal ganglion homogenate into the supra-oesophageal ganglionectomized leeches increased the blood glucose levels significantly ($P < 0{\cdot}05$). Whereas, in the supra-oesophageal ganglionectomized leeches the injections of sub-oesophageal ganglion homogenate produced no effect. Sub-oesophageal ganglionectomy did not produce any significant change in the blood glucose levels even after 24 h of operation. The injection of the supra-oesophageal ganglion homogenate elevated the blood glucose level significantly ($P < 0{\cdot}05$) in the sub-oesophageal ganglionectomized leeches, whereas the injections of sub-oesophageal ganglion did not show any effect.

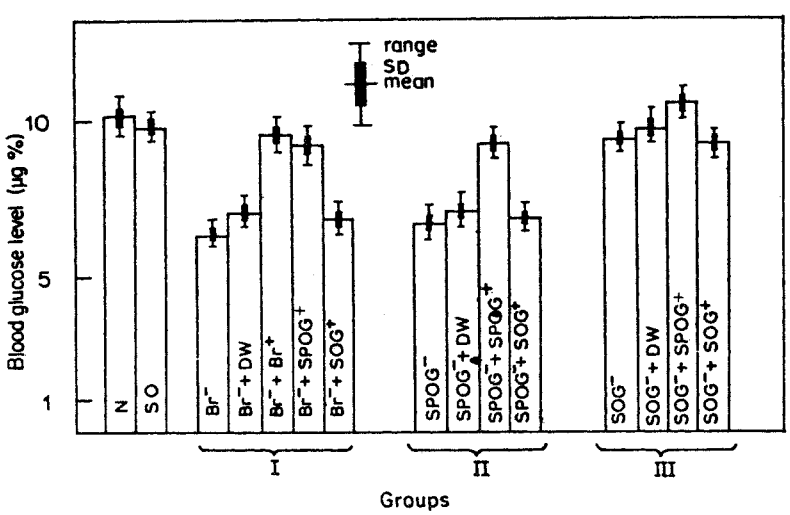

Figure 1. Blood glucose levels in normal and experimental *P. viridis*.
(N), Normal (brain intact); (DW), distilled water; (SO), Sham operated; (Br^-), brain removed; ($SPOG^-$), Supra-oesophageal ganglionectomized (Sub-oesophageal ganglion intact); (SOG^-) Sub-oesophageal ganglionectomized (Supra-oesophageal ganglion intact); (Br^+) Brain homogenate injected; ($SPOG^+$) Supra-oesophageal ganglion homogenate-injected; (SOG^+) Sub-oesophageal ganglion extract injected.

4. Discussion

The blood glucose level in the normal *P. viridis* was found to be 10·2 μg%. Earlier Clark (1964) reported 72–264 mg% of total carbohydrates in the coelomic fluid of a polychaete, *N. hombergi* containing glucose as a major constituent. Hanumante (1975) reported 14·5 mg% of blood glucose concentration in an earthworm, *P. excavatus*. It is obvious that the blood glucose concentration of *P. viridis* is quite low as compared to the concentration of total carbohydrates in *Nephtys* (Clark 1964) and blood glucose in *Perionyx* (Hanumante 1975).

The data of preliminary experiments showed that the injection of the extract of whole brain into 24 h debrained leeches produced a significant increase in blood glucose levels. Since brain is composed of supra-oesophageal and sub-oesophageal ganglia (Bhaskarrao 1983) further experiments were designed to detect which ganglion is involved in the regulation of blood glucose level in *P. viridis*. It was observed that the homogenate of supra-oesophageal ganglia when injected into leeches having their brain, supra- and sub-oesophageal ganglia been removed, increased the blood glucose levels. Injection of sub-oesophageal ganglion extracts had no effects. These results indicate that only the supra-oesophageal ganglionic portion of the brain of *P. viridis* is responsible for increase in the blood glucose level and inferred that supra-oesophageal ganglion is the production site for hyperglycemic hormone in this leech. Similar incidences of neuroendocrine involvement in the control of blood glucose levels have been previously reported for an oligochaete, *Lumbricus terrestris* (Lawrence *et al* 1972) wherein supra-oesophageal ganglion was responsible for the hyperglycemic factor. Interestingly, a contrary finding to Lawrence *et al* (1972) was reported by Hanumante (1975) in another oligochaete worm, *Perionyx excavatus* who observed that the sub-oesophageal ganglion was responsible to increase blood glucose level in that earthworm. No information has come to our notice so far regarding the mechanism and site of hormonal action and the exact responses of the target tissues of leeches to neurohormone(s) which influences the blood glucose levels.

Acknowledgements

We are thankful to Prof. R Nagabhushanam, for providing laboratory facilities and to the University Grants Commission, New Delhi for financial assistance through a project on 'Neuroendocrine Mechanisms in Hirudinean Annelids'.

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