HORMONAL REGULATION OF THE IONIC BALANCE IN THE HAEMOLYMPH OF THE LARVAE OF THE DRAGONFLY *ORTHETRUM CHRYSIS* (SELYS) (ANISOPTERA: LIBELLULIDAE)

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Immersion of larvae in various external salinities indicates a sound ability to maintain the haemolymph ionic balance. Removal of the pars intercerebralis region of the brain and ligature at the neck region do not provide any indication of involvement of the hormones of the brain and other endocrine organs of the cephalic region. Destruction of the thoracic ganglia apparently creates an imbalance in the concentrations of sodium and potassium in the haemolymph, suggesting the thoracid ganglia as a source of a hormonal factor controlling ionic balance. The regulation of internal ionic balance under hormonal influence of thoracic ganglia has become evident from the experiments performed during the present study.

INTRODUCTION

Although adult dragonflies are terrestrial, larvae are well adapted to the aquatic mode of life. Along with the radical modifications in body organisation (SNODGRASS, 1954; CORBET, 1962) a well-balanced osmoregulatory mechanism under freshwater conditions appears to be the major physiological adaptation in the dragonfly larvae (KOCH, 1934; SCHOFFENIELS, 1951; SUTCLIFFE, 1962; MOENS, 1973, 1975; KOMNICK, 1977).

A good deal of work has been carried out on the larvae of *Aeshna cyanea*, pointing to an efficient ability to regulate internal water balance (MOENS, 1973), ionic balance (MOENS, 1975), an active role of rectal chloride epithelia in hyper--osmoregulation (KOMNICK, 1978), an antidiuretic influence of the cerebral neurosecretory cells on water balance (CHARLET, 1974) and involvement of neurosecretory A cells of the thoracic ganglia in the regulation of osmotic and

ionic balance (TEMBHARE, 1979).

The present work was undertaken as an extension to earlier studies of the mechanism of hormonal regulation of ionic balance in the last-instar larvae of the dragonfly, *Orthetrum chrysis.*

MATERIAL AND METHODS

Penultimate larvae of *Orthetrum chrysis* were collected from local freshwater ponds and acclimatized in the same medium separately (to prevent cannibalism) in the laboratory for a week. They were fed daily on mosquito larvae *ad lib.* and reared under controlled conditions of temperature and photoperiod.

Experimental design

About 160 larvae were divided into four equal groups and transferred separately to (i) distilled water (DW), (ii) saline 1 (S1) (NaCl-0.68 g, KCl-0.02 g, DW-100 ml), (iii) saline 2 (S2) (NaCl--0.95 g, KCl-0.042 g, DW-100 ml) and (iv) dechlorinated freshwater (FW). The larvae of the last group served as controls for the other groups. The external aqueous media were removed every 12 hours to maintain the original concentration of external salinities. The larvae approaching ecdysis were excluded from the experimental studies.

At the time of commencement of experiments, 5-10 larvae were taken out from each medium at intervals of 6, 12, 24, 48, 72 and 96 hours. The haemolymph from individual larvae was pooled immediately for the estimation of Na^+ and K^+ concentrations.

For the next experiment, 150 acclimatized larvae of another lot were divided into three groups. Half of the larvae from these groups were operated for (i) cauterization of the pars intercerebralis region of the brain, (ii) neck ligation and (iii) cauterization of the lateral region of the thoracic ganglia, respectively. In the remaining larvae of groups (i) and (iii) the respective areas were exposed without disturbing the ganglia, while those of group (ii) were simply maintained as controls. Not less than five experimental and an equal number of control larvae from each group were immersed in DW, FW and Saline. After three days, haemolymph was collected from each larva for estimation of Na^+ and K^+ concentrations.

For the last experiment, thoracic ganglia from 50 to 60 larvae were cauterized and after 3 hours into half of them the aqueous extract of these ganglia was injected, equivalent to a single complex of pro-, meso- and metathoracic ganglia per larva. The remaining larvae were injected with the equal quantity of distilled water, as controls. The larvae were exposed to FW. The concentration of Na^+ and K^+ was determined from the larvae at the intervals of 3, 6 and 12 hours. Those sacrificed at the commencement of the experiment served as the initial controls (IC).

Na^+ K^+ estimation

The haemolymph was centrifuged immediately and the estimations of the sodium and potassium concentrations were made on Eel's Flame Photometer at appropriate dilutions.

Aqueous extracts of the thoracic ganglia

Ice-cold aqueous extracts of thoracic ganglia from larvae of the same stage were prepared according to the method adapted previously (TEMBHARE & THAKARE, 1976a).

RESULTS

EFFECT OF EXTERNAL MEDIA ON THE HAEMOLYMPH IONIC CONCENTRATION

The concentrations of sodium and potassium in DW, FW, S1 and S2 media have been shown in Figure 1.
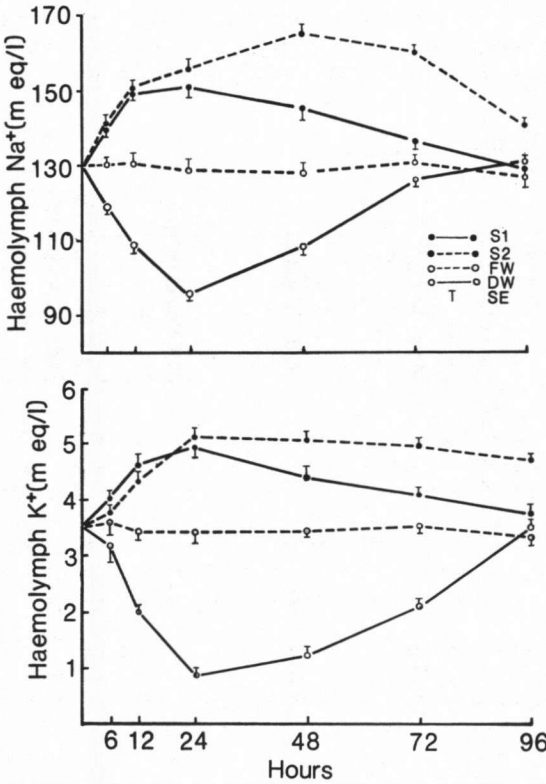

Fig. 1. Effect of external media on the haemolymph Na$^+$and K$^+$ concentrations in the larvae of *Orthetrum chrysis*. Haemolymph samples were taken 6, 12, 24, 48, 72 and 96 hrs after the larvae were transferred to DW (distilled water), FW (freshwater), S1 (saline 1) and S2 (saline 2) media. Each point represents the mean of five animals and the vertical bars represent standard error (SE).

In the larvae transferred to DW, the concentration of Na$^+$ and K$^+$ declines constantly from the time of immersion during a period of 24 hours, showing enhanced hyponatremia as well as hypokalemia, respectively. Thereafter, the levels of haemolymph Na$^+$ and K$^+$ start rising gradually. The concentrations of Na$^+$ and K$^+$ do not show any significant difference from those of controls at 72 and 96 · hours, respectively, suggesting their return to normal levels by these intervals.

The S1 and S2 media induce enhanced hypernatremia, which falls rapidly after 24 hours and returns to normal by 72 hours. In the S2 condition, however, hypernatremia increases for 48 hours and declines after 72 hours. Hyperkalemia also develops along with hypernatremia but only under S1 conditions does it drop after 24 hours and no significant variation can be seen by 96 hours from that of the controls. The high hyperkalemia does not recover during a period of four days in the larvae subjected to S2 medium.

EFFECTS OF SURGICAL OPERATIONS

Results of surgical operations on the levels of Na^+ and K^+ in haemolymph of the larvae under different salinities are plotted in Figure 2. The presence of prominent medial neurosecretory cells (MNC) in the pars intercerebralis (PI) region of the brain has been well demonstrated in the larvae of *Orthetrum chrysis* (TEMBHARE & THAKARE, 1976b).

Removal of the PI region of the brain does not disturb the Na^+-K^+ concentration of haemolymph in the larvae exposed to various salinities for three days. The neck ligation also causes no significant change in the haemolymph Na^+-K^+ contents in the larvae during the entire experiment. The cauterization of the lateral region of the proto-, meso- and metathoracic ganglia containing the well recognized neurosecretory cells (TEMBHARE & THAKARE, 1977) has induced normal changes in the haemolymph Na^+-K^+ levels in comparison to that of sham-operated controls. The levels of both electrolytes decrease strongly in larvae kept in DW and FW media showing enhanced hyponatremia as well as hypokalemia. On the other hand, concentrations of both cations become elevated in those immersed in S1 and S2 media showing marked hypernatremia and hyperkalemia.
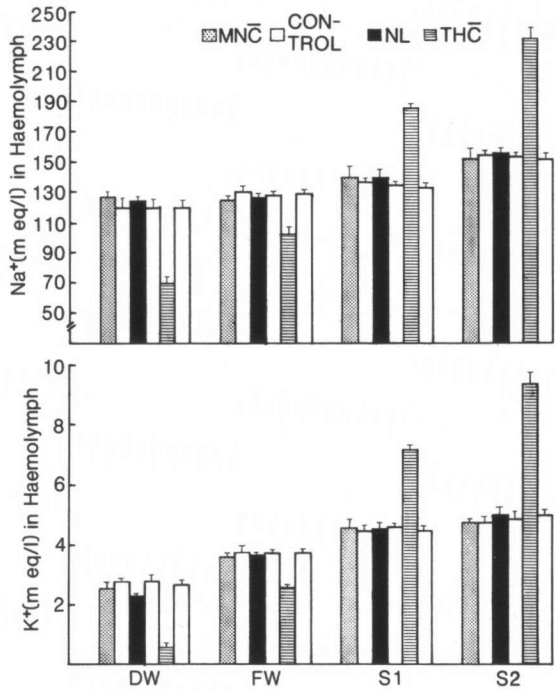

Fig. 2. Effects of some surgical operations on the haemolymph Na^+ and K^+ concentrations of the larvae immersed in DW (distilled water), FW (freshwater), S1 (saline 1) and S2 (saline 2). Haemolymph samples were taken 3 days after operations. MNC̄: cauterization of medial neurosecretory cells of the brain; — NL: neck ligation; — THC̄: cauterization of the cells of thoracic ganglia.

EFFECTS OF AQUEOUS EXTRACTS OF THORACIC GANGLIA IN THE OPERATED LARVAE

Under FW conditions, after cauterization of the thoracic ganglia the Na^+-K^+ concentration drops rapidly as is evident in larvae sacrificed 3, 6 and 12 hours after the commencement of the experiment. In the larvae cauterized after 3 hours, and injected with the extract of thoracic ganglia, the levels of Na^+ and K^+ increase gradually and compense hyponatremia as well as hypokalemia, without showing any significant difference from those of controls within a period of 12 hours, suggesting the initiation of ionic homeostasis in the haemolymph (Tab. I).

Table I

Effects of the aqueous extract of thoracic ganglia on the haemolymph Na^+, K^+ concentration (m eq/lit) in the thoracic ganglia-cauterized larvae of *Orthetrum chrysis* under freshwater condition.

Experiment duration	No. of insects	Na^+			K^+		
		Intact	Operated	Operated + extract	Intact	Operated	Operated + extract
Initial control	5 x 2	130 ± 3.10	130 ± 4.70	—	3.80 ± 0.08	3.84 ± 0.14	—
3 h	5 x 3	132 ± 2.50	120 ± 4.10***	—	3.82 ± 0.12	3.40 ± 0.10**	—
6 h	5 x 3	129 ± 4.10	108 ± 4.50**	122 ± 4.20*	3.81 ± 0.10	3.15 ± 0.10*	3.56 ± 0.16**
12 h	5 x 3	128 ± 0.80	91 ± 2.80*	129 ± 3.80ns	3.79 ± 0.28	2.50 ± 0.10*	3.76 ± 0.22ns

h hour; — * P < 0.001; — ** P < 0.01; — *** P < 0.05; — ns not significant.

DISCUSSION

The larvae of *Orthetrum chrysis* are FW inhabiting insects found in seasonal ponds and tanks of the local region, and are often facing sudden variations in the concentration of solutes in the external media. The data available in the present study show clearly that they are efficiently capable of osmoregulation following acclimation to external salinities ranging trom distilled water to highly concentrated saline. Similar to other freshwater insects studied so far (SHAW & STOBBART, 1963), the larvae of *O. chrysis* maintain haemolymph osmolarity always above the external salinity and inclined to that of the FW conditions. SHAW & STOBBART (1963) further emphasized that the osmoregulation in FW insects depends on the regulation of the ionic composition of the haemolymph. This is equally true for dragonfly larvae (MOENS, 1973). Haemolymph Na^+ and K^+ concentration changes closely parallel in larvae exposed to various external media, indicating a major role of these two ions in maintaining the osmolarity of haemolymph as has also been reported in *Aeshna*. Essentially, solutes which are excreted must be replenished by tissues from the surrounding medium through rectal gills.

Like the larvae of the chironomids *Chironomus plumosus* and *Procladius nubifer,* the damselfly *Enallagma clausum* (LAUER, 1969); the hemipterans *Cenocorixa bifida* and *C. expleta* (SCUDDER et al. 1972); the dragonfly *Aeshna cyanea* (TEMBHARE, 1979), the larvae of *Orthetrum chrysis* can perform both hypo- and hyper-osmotic regulation.

It is now well established that neurohormones, irrespective of their source, play a vital role in water and electrolyte homeostasis in the insects (NOVAK, 1975; GERSCH. 1975).

The hormonal regulation of ionic balance appears to be mostly confined to the neurohormone(s) produced by A cells. In some insects, however, the cells of the pars intercerebralis of the brain (BERRIDGE, 1966; VIETINGHOFF, 1966, 1967; MILLS, 1967; WALL, 1967; PILCHER, 1970), while in others those of the thoracic ganglia are assumed to perform this function (MADDRELL, 1963, 1964; STOBBART, 1971; GEE, 1975). In larvae of *Aeshna cyanea* the active involvement of the A cells of the thoracic ganglia is evident from experimental as well as electron-microscopic studies (TEMBHARE, 1979). The present study also provides evidence in favour of the neurohormone(s) of thoracic ganglia controlling the ionic balance of haemolymph in the larvae of *O. chrysis.*

There are some reports suggesting diuretic action of the neurohormone(s) on the Malpighian tubules stimulating rapid secretion of fluid from the haemolymph and in turn causing steep diuresis (HIGHNAM et al., 1969; CAZAL & GIRARDIE, 1968; MORDUE, 1969, 1972; GIRARDIE, 1970; PILCHER, 1970; GELDIAY & EDWARDS, 1976). However, in some insects the hormonal stimulation of rectal reabsorption has been clearly demonstrated (WALL & RALPH, 1964; VIETINGHOFF, 1967; WALL, 1967; CAZAL & GIRARDIE, 1968) to evoke an antidiuretic mode of action especially in terrestrial insects in which the rectum plays a key role in osmoregulation mostly by absorbing rectal fluid and discharging it into the haemocoel. This seems true for adult dragonflies also, where neurohormone of pars intercerebralis of the brain A cells plays an antidiuretic role (THAKARE et al., 1977). In FW insects, on the other hand, conservation of water is not so important as they are habituated for drinking the surrounding water (SHAW, 1955; JARIAL et al., 1969; FRICK & SAUER, 1974; MOENS, 1973).

MOENS (1973) noticed that the retroperistaltic movements of the pyloric chamber force the fluid from the Malpighian tubules into the midgut, from which it is again reabsorbed into the haemolymph. The same process has also been reported in freshwater larvae of the mosquito *Aedes aegypti* (STOBBART, 1977).

Besides the Malpighian tubules and the midgut, the rectum is playing an active role in osmoregulation in dragonfly larvae (KOMNICK, 1977). KOMNICK (1978) found that the rectal chloride epithelia adsorb ions from the external solution ingested during rectal ventilation via the anus, but not from urine or from fluid that may have been ingested orally.

It seems that the neurohormone(s) of thoracic ganglia in the larvae of *O. chrysis* can regulate efficiently the ionic balance by stimulating the Malpighian tubules to accelerate the secretion of salts from the haemolymph or by stimulating the rectal chloride epithelia to adsorb ions from the external media. In this way, according to the environmental situation, the internal ionic balance of the haemolymph is being maintained by performing hypo- or hyper-osmoregulation.

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