PHYSIOLOGY OF LOW TEMPERATURE ACCLIMATION : CHANGES IN THE ACTIVITY LEVELS OF ADENOSINE-TRI-PHOSPHATASE IN THE DIFFERENT REGIONS OF THE NERVOUS SYSTEM OF SELECTED POIKILOTHERMS

THE activity level of adenosine-tri-phosphatase exhibited a significant increase on cold ($12 \pm 1°$ C) acclimation in the cerebral and thoracic ganglionic pools of the Crab, *Paratelphusa hydrodromus*. The increase was more significant in the Thoracic Ganglionic Pools.

A significant increase in the activity level of ATPase was seen on cold acclimation in the fore, mid and hind brain regions of the Frog, *Rana cyanophlictis*. The elevation in the activity level of ATPase was the highest in the fore brain and least in the hind brain. The significance of the changes observed is discussed.

Introduction

The role of adenosine-tri-phosphatase in energy metabolism and active transport is well established[1]. Adenosine-tri-phosphatase is the chief enzymatic mechanism asscciated with active monovalent cation transport[2] that characterizes the action potential. For instance, changes occurring in the activity of ATPase was found to influence the different parameters of neuronal activity[3] in the giant nerve fibers of the ventral nerve cord of earthworm, *Lumbricus terrestris*. An augmentation in the level of ATPase on cold acclimation exhibiting partial compensation, was found in the nerve cord of earthworms, and in different tissues of the goldfish, *Carassius auratus*[4], However, information about changes in the activity levels of ATPase on cold acclimation in the different regions of the nervous system of Crabs and Frogs is lacking. Hence the present study was proposed in view of the importance of ATPase in the neuronal activity which in turn is involved in the adaptive changes occurring during thermal acclimation.

Materials and Methods

Crabs, *Paratelphusa hydrodromus* and frogs *Rana cyanophlictis* were purchased from a local dealer and maintained in the laboratory, in glass aquaria at ($26 \pm 2°$ C). The experimental animals (Crabs and Frogs kept in separate bread boxes in tap water) were acclimated in low temperature ($12 \pm 1°$ C) for 15 days to get them completely acclimated to that temperature. Animals maintained at the laboratory temperature ($26 \pm 2°$ C) constituted controls. Crabs were fed with earthworms, thrice a week and frogs were force-fed once in three days with the thigh muscle of the frog. Water from the containers was changed daily.

The crabs were frozen in the freezing jacket of the refrigerator. The carapace was cut open and the cerebral and thoracic ganglia were removed with sterlized instruments and immediately kept in cavity glass containing Ringer, kept in ice blocks. The fore, mid and hind brain regions were separated with sterilized instruments.

The tissues were weighed immediately in a single pan electric balance in Ringer at 0° C. 1% (W/V) homogenates of the above mentioned tissues were prepared in distilled water in an all glass homogenizer and centrifuged at 3000 rpm for 15 minutes to remove the cell debris. The supernatants were used for the assay of ATPase by the method of Lowry *et al.*[5]. Standards and blanks were carried through from the beginning and the readings were taken against the blank at 870 nm in a Du-2-Beckman's spectrophotometer.

Results and Discussion

The data after statistical analysis is presented in Tables I and II.

TABLE I

Activity levels of adenosine-tri-phosphatase in the cerebral and thoracic ganglionic pools of control ($26 \pm 2°$ *C*) *and acclimated* ($12 \pm 1°$ *C*) *crabs,* Paratelphusa hydrodromus

		Control	Test	Per cent increase
Cerebral	**a*	3·945 ±1·7	6·000 ±1·76	52·0
Thoracic	a_1	3·390 ±0·58	5·833 ±0·54	71·5

* Activity expressed as micromoles of inorganic phosphate (pi) liberated/mgm wet tissue/hour. Values are mean ± SD of 6 observations; for each observation ganglia from 15 crabs were pooled.

Levels of significance: a—$P > 0·01$; a_1—$P > 0·001$.

TABLE II

Activity levels of adenosine-tri-phosphatase in the fore mid and hind brain regions of control ($26 \pm 2°$ *C*) *and acclimated* ($12 \pm 1°$ *C*) *Frogs,* Rana cyanophlictis

		Control	Test	Per cent increase
Fore brain	**a*	4·466 ±0·77	9·000 ±1·183	102·0
Mid brain	a_1	3·940 ±1·025	6·000 ±0·806	55·00
Hind brain	a_2	4·160 ±0·755	4·800 ±0·608	15·0

* Activity expressed as micromoles of inorganic phosphate (Pi) liberated/mgm/hour. Values are mean ± SD of 5 observations.

Levels of significance: a—$P < 0·001$; a_1—$P < 0·05$; a_2—not significant.

The activity level of ATPase increased significantly on cold acclimation in both the cerebral and thoracic ganglionic pools (+52%; +71·5% respectively). Thoracic ganglia exhibited higher response. Similarly the activity levels of ATPase increased in the fore, mid and hind brain regions of the frog on cold acclimation +102%; +55%; +15% respectively). However, forebrain exhibited the highest response and hind brain the least (Table II).

The increase observed in the activity level of ATPase in the present study can be correlated to the higher level of metabolic activity of the organism, characteristic of the cold acclimated state[6].

A decrease in the resting potential with a lowering of ambient temperature has been reported for giant nerve fibers of earthworm and *Aplysia*[7,8]. Lagerspetz[2] suggested that such changes are based on the changes in ionic conductance and on the cation pump. In the light of above studies the present increase in ATPase in the nervous tissue of a crab and a poikilothermic vertebrate frog can be suggested to be indicative of higher neuronal activity in them to achieve thermal compensation.

However, the differences in magnitude of response in the different regions of the nervous system of the animals studied point out to the different functional organisations of these regions. A correlation in the dstribution of ATPase and AChE was observed in the different regions in the brain of frog (unpublished data) in the course of the present study. Nistratova[9] postulated such a relationship between AChE and ATPase while working with the nervous system of bivalves. In the light of such a study, the present co-relation between the two enzymes in the brain of frog points to a functional similarity between the two enzyme systems in achieving compensation during acclimation to low temperature.

The authors are thankful to Dr. A. R. Kasturi Bai Head of the Department of Zoology, Bangalore University, Bangalore, for offering facilities to conduct the study and to C.S.I.R. for financial assistance.

Department of Zoology, Bangalore University, Bangalore 560 001 (South India),
August 4, 1978.

U. P. NALINI.
NAYEEMUNNISA.

1. George, J. Siegel and Wayne Albers, R., In *Hand Book of Neurochemistry*, edited by Abel Lajtha, Plenum Press, New York, p. 13.
2. Lagerspetz, K. Y. H., Kohonen, J. and Tirri, R., *Compl. Biochem. Physiol.*, 1973, 44 B, 823.
3. — and Talo, A., *J. Exp. Biol.*, 1967, 47, 471.
4. Smith, M. W. and Ellory, J. C., *Compl Biohem. Physiol.*, 1971, 39 A, 209.
5. Lowry, O. H. and Passonneau, J. V., *J. Biol. Chem.*, 1964, 239, 31.
6. Rao, K. P., In *Molecular Mechanisms of Temperature Adaptation*, edited by C. L. Prosser, AAS Publication, 1967, p. 227.
7. Dierolf, B. H. and McDonald, H. S., *Z. Vergl. Physiol.*, 1969, 62, 284.
8. Carpenter, D. O., *J. Gen. Physiol.*, 1967, 50, 1469.
9. Nistratova, S. N., *Neurobiology of Invertebrates*, edited by J Salanki, Plenum Press, N.Y., 1968, p. 315

PRESENCE OF NON-PATHOGENIC BACTERIA IN THE GUT OF RICE YELLOW STEM BORER, *TRYPORYZA INCERTULAS* WLK. (LEPIDOPTERA : PYRALIDAE)

LEYDIG[7] made the first observation on the association of microorganisms with the insect gut. Subsequently a number of reports[1–10] appeared in literature about the association and possible role of the microorganisms in the insect gut. Trager[9] reported that symbiotic yeasts and bacteria are most prevalent in insects with relatively restricted or peculiar diets. The yellow stem borer of rice, *Tryporyza incertulas* is highly specific in its diet and feeds only on rice (*Oryza sativa*).

Healthy larva of *T. incertulas* were collected from the field and conditioned in the laboratory at 30–32° C on cut stem pieces of a high yielding rice cultivar, *Jaya for three days. The larvae were surface sterilised* with 0·1% $HgCl_2$. The entire gut was removed intact on sterilised wax plates under aseptic condition. The major components of the alimentary canal (*viz.*, stomodaeum, mesenteron and proctodaeum) and the cuticle were separated out and transferred separately to nutrient agar plates and incubated for 48 hrs at 30° C. In a second set of experiments, all the major components of the gut and the cuticle were oriented on the nutrient agar plate at a distance of 3 cm. Two sets of controls were maintained in every case: (i) plates *having unsterilised components, (ii) plates having* only nutrient agar for ascertaining the possibility of contamination, if any, due to surface microorganisms under unsterilised conditions. Healthy *T. incertulas* larvae were also inoculated with freshly grown bacteria which were reisolated after 48 hours, following the same procedure. Reisolation was made from both sterilised and unsterilised and treated and untreated larvae.

The study revealed that *T. incertulas* larvae harbour two bacteria, *viz.*, *Klebsiella pneumoniae* (Schroter) Trevisan and *Enterobacter aerogenes* (Kruse) Hormeche and Edwards in all the major components, *viz.*, stomodaeum, mesenteron and proctodaeum. These bacteria, could not be recovered from cuticle and in the controls.

Association of *K. pneumoniae* and *E. aerogenes* with the alimentary canal of *T. incertulas* is being