BRAIN LACTATE DEHYDROGENASE AS A FUNCTION OF METHYL PARATHION EXPOSURE IN THE DEVELOPING TADPOLES OF FROG, *RANA CYANOPHLICTIS*

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ABSTRACT

The molecular heterogeneity of brain lactate dehydrogenase (LDH) was studied by polyacrylamide gel electrophoresis in the control and methyl parathion exposed developing tadpoles of frog, *Rana cyanophlictis*. A profound suppression of slowest moving isozymes and induction of the fastest moving molecular form was detected. The alterations observed in the isozymic spectrum are considered to be due to the stress caused by methyl parathion.

INTRODUCTION

METHYL PARATHION, an organophosphorus pesticide extensively used in agriculture[1] is known to be responsible for a number of physiological and biochemical disturbances[2, 3]. The onset of the crippling disease called 'Handigod syndrome' reported in Karnataka, India has been supposed to be due to the consumption of crabs and fish exposed to endrin and parathion by local population[4].

The critical stage of development is known to have specific isozymic spectrum of enzyme lactate dehydrogenase[5]. A decrease in the levels of selected oxidative enzymes like SDH and LDH has been reported in the metabolically active tissues of fish (muscle, gill and liver) during methyl parathion exposure[6]. Impact of methyl parathion on LDH isozymes of a teleost *Tilapia mossambica* has also been reported[7]. However, no information is available on the effects of methyl parathion on the molecular heterogeneity of brain LDH during critical stages of brain development in a vertebrate. Hence, in view of the need to understand the disruptions occurring in the molecular mechanisms on methyl parathion exposure in a developing vertebrate brain, the present study was undertaken. The paper presents information on the changes occurring in the isozymic profile of brain LDH on exposure to methyl parathion in the developing tadpoles of frog, *Rana cyanophlictis*.

MATERIALS AND METHODS

About three-week-old tadpoles of frog, *Rana cyanophlictis* in the weight range of 1.5–3 g were obtained from local ponds and acclimated to laboratory conditions for a week, prior to experimentation. They were maintained in aquaria at 28 ± 2°C and fed on Hydrilla; 2.5 PPM of the methyl parathion (O,O-dimethyl-O-nitrophenyl-thiophosphate) (EC 50%, Bayer Ltd., India) in tap water was prepared and the animals were exposed to this sublethal concentration after due standardization by probit analysis[8].

After exposure to methyl parathion for 24 hr, the control and methyl parathion exposed tadpoles were sacrificed and the brain tissue was excised at 0°C. LDH isozyme distribution was determined in clear supernatants from brain samples homogenized in deionized distilled water (100% w/v) and centrifuged at 7000 rpm for 1 hr at 4°C. The isozymes were separated by polyacrylamide gel electrophoresis[9]. The electrophoresis was run for 3–4 hr at room temperature using 0.05 M tris HCl buffer, pH 8.9 at a voltage of 100V and 3 m.A[13]

After the run, the gels were removed from the glass tubes and stained for the resolution of isozymes of LDH[10]. The incubation mixture consisted of 1 M sodium lactate, 0.1 M NaCl, 5 mM $MgCl_2$ 0.5 M phosphate buffer pH 7.4, 2.5 mg nitrobluetetrazolium, 1 mg phenazine methosulphate and 10 mg NAD in total volume of 9.5 ml. The gels were incubated in darkness, in the staining solution at 37°C until purple bands appeared. The stained gels were removed, washed with water and placed in 7.5% acetic acid. The gels were scanned under a transmission densitometer (Biochemistry Instrument, India) and the readings were presented graphically.

RESULTS AND DISCUSSION

It is clear from the isozymic spectra of control and methyl parathion exposed animals (figure 1) that methyl parathion substantially affected the qualitative nature of the brain LDH isozymes in the developing

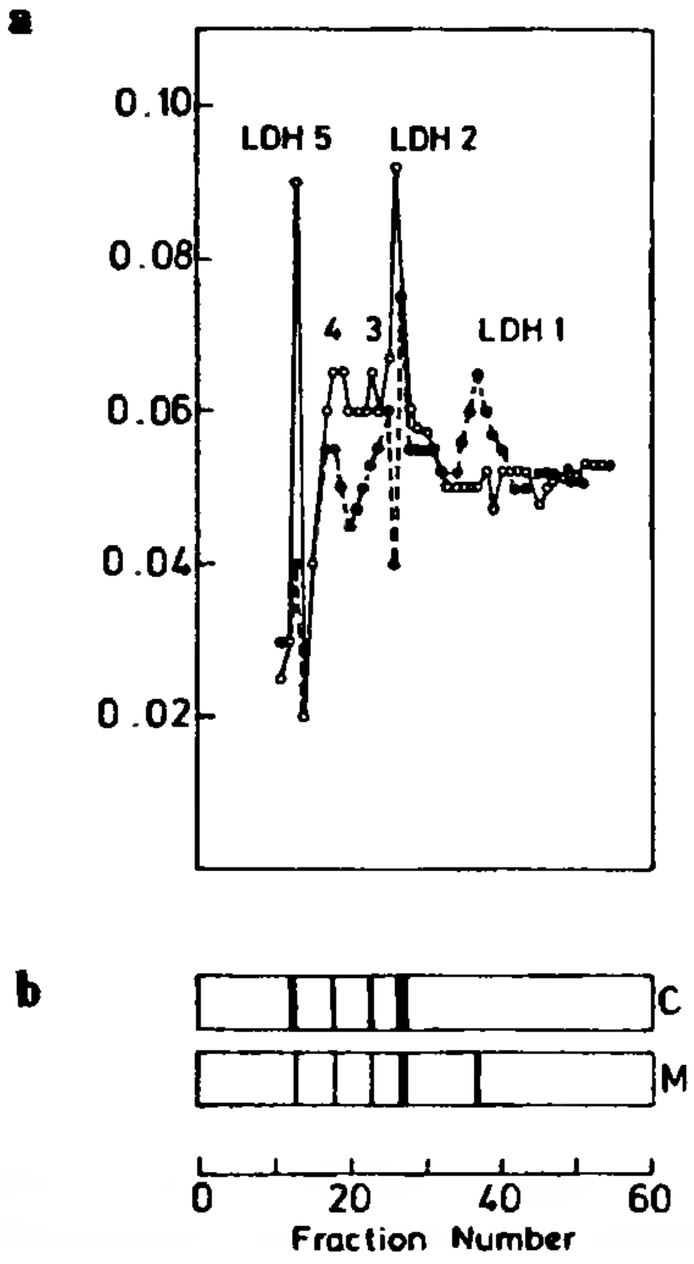

Figure 1. Polyacrylamide gel electrophoresis for the molecular heterogeneity (isozymes) of Lactate dehydrogenase in 0.05 M tris HCl buffer pH 8.9. Profile of lactate dehydrogenase isozymes in the brain of control and methyl parathion exposed tadpoles of frog, *Rana cyanophlictis*. Solid lines represent the electrophonetic pattern of LDH isozymes of control tadpoles. Broken lines represent the electrophoretic pattern of LDH isozymes of tadpoles exposed to methyl parathion. **a.** Semidiagrammatic representation of the gel. **b.** Photograph of gels C-Control, M-Methyl parathion exposed.

tadpoles of frog, *R. cyanophlictis*. For instance, LDH_1 which was absent in the controls was induced on MPE (figure 1). LDH_4 showed 16.6% reduction and LDH_3 was ineffectively suppressed (8.3%). The slowest moving LDH_5 was profoundly suppressed following which LDH_2 exhibited 24% suppression in its expression due to MPE (figure 1). Thus the isozymic profile of LDH was substantially affected due to MPE.

It is known that any change in the external environment of the organisms is capable of consistently modulating the activities of regulatory enzymes like LDH, which appear to be well adapted for channelling the metabolism of pyruvate in direction consistent with the demands imposed by the stress condition[11]. It is therefore plausible that the specific changes observed in the isozymic spectrum of brain LDH in the present study on methyl parathion exposure reflect the demands imposed by the neurotoxicity resulting from methyl parathion exposure. Supporting this the disruption of isozymic profile of brain AChE on MPE has been observed[12] (unpublished observations).

30 September 1985

1. Sreenivasa, A. and Soundar Raj, R., *Pesticides*, (ed.) S. K. Majumdar, Academy of Pest Control Sciences, Manipal Power Press, India, 1968, 316.
2. Koundinya, R. P. and Ramamurthy, R., *Curr. Sci.*, 1979, **48**, 877.
3. Koundinya, R. P. and Ramamurthy, R., *Indian J. Exp. Biol.*, 1978, **16**, 809.
4. Radhakrishna Rao, *Indian Express* (*Magazine Section*), Jan. 22, 1984, 4.
5. Blanco, A. and Zinkham, W. H., *Science*, 1963, **139**, 601.
6. Siva Prasad Rao, K. and Ramana Rao, K. V., *Curr. Sci.*, 1979, **48**, 526.
7. Siva Prasad Rao, K., Kabeer Ahamed, I. and Ramana Rao, K. V., *Indian J. Fish.*, 1982, **29** (1 & 2), 185.
8. Finney, D. J., In: *Probit analysis*, 3rd edition, Cambridge Univ. Press, London, 1971, 28.
9. Davis, B. J., *Ann. N. Y. Acad. Sci.*, 1964, **121**, 404.

10. Vander Helm, Dietz, A. A. and Lubrane, T., *Anal. Biochem.*, 1967, **20**, 246.
11. George Somero, *Comp. Biochem. Physiol.*, 1973, **B44**, 205.
12. Naiyara Yasmeen, 1985, (unpublished observations).
13. Tsao, M. V., *Arch. Biochem. Biophys.*, 1960, **90**, 234.

NEWS

ASPRIN RISK

Researchers have revealed that there is a link between Reye's Syndrome, a rare disease that affects children, and the use of asprin during the flu or chicken pox. Reye's Syndrome typically occurs four to seven days after a child has the flu or chicken pox. Rather than recovering, the child begins to vomit and becomes delirious. In severe cases, the child lapses into a coma and 10 percent die. A pilot study by Boston's Public Health Services concludes that if a child develops the disease there is a 93 percent chance that the child received salicylates, an asprin ingredient during a previous illness. Such children are 16 times more likely to get Reye's than those who do not.

BREAKTHROUGH IN LIVER CANCER CURE

Researchers at the Johns Hopkins University in Baltimore (USA) say they have found the first effective treatment for advanced liver cancer, which until now has almost always been fatal.

The treatment involved the use of antibodies with drugs and radiation treatment to increase chances of curing the disease.

The treatment using radioactive isotopes attached to antibodies – proteins that bind to cancer cells to deliver radiation directly to the tumour has shrunk tumours by at least 30 percent in almost half of the patients tested.

NEW TEST FOR EARLY DIABETES DETECTION

Researchers say that screening tests that pinpoint tell-tale antibodies in the blood could help identify those who risk getting diabetes.

One newly developed test reveals the presence of antibodies that attack islet cells, specialised cells within the pancreas that produce insulin; when these cells are destroyed, the body can no longer make its own insulin, an essential hormone, and the result is Juvenile diabetes which is a devastating disease with many side effects including blindness and kidney failure.

The latest research shows that people with islet – cell antibodies risk getting the disease. And doctors can estimate how soon it will strike measuring their insulin production.

Juvenile diabetes runs in families, but doctors do not fully understand the underlying defect that causes it. However, they suspect that it results from an abnormality of helper 'T' cells, a variety of white blood cells that control antibody production.

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