Kinetics of Thyroid Hormone Induced Changes in Liver and Skeletal Muscle Enzymes of *Clarias batrachus*

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Kinetics of triiodothyronine (T_3) induced changes were studied in cytoplasmic malate dehydrogenase (cMDH), mitochondrial malate dehydrogenase (mMDH) and lactate dehydrogenase (LDH) of the liver and skeletal muscle of a catfish, *Clarias batrachus*. The rates of gradual inductions in the activities of all the three metabolic enzymes were faster in skeletal muscle than those of the liver. These time-dependent and tissue-specific inductions may be due to the possible differences in the rates of different enzymic syntheses. The maximum inductions in the activities of cMDH, mMDH and LDH were recorded around 19 hr after T_3 treatment. Thereafter, the activities of all the enzymes gradually declined to their half levels within the next 12 hr which reflected the physiological half-life of these metabolic enzymes in the freshwater catfish.

Introduction

Trickle of reports are available on the hormone-induced enzymatic changes in the metabolism of vertebrates. Basically the similar mode of thyroid hormone action persists throughout the vertebrate phylogeny, nevertheless, there are major differences about its effects on various enzymes in aquatic animals. In fishes it is not yet certain whether T_4 and/or T_3 or one or more of their metabolic derivative(s) is an active form(s) at the cellular level (Donaldson *et al.*, 1979). Some experiments in fishes have been related to the calorigenic and anabolic effects of thyroid hormones (Higgs *et al.* , 1982). It has been documented that the treatment of thyroid hormones increased the activity of cytochrome oxidase and malate dehydrogenase but decreased the activity of glucose-6-phosphate dehydrogenase in the liver of *Mugil auratus* (Le Ray *et al.* , 1970). Exposure of thyroxine to *Ophiocephalus punctatus* increased the activity of acid and alkaline phosphatases (Ray and Medda, 1976). Effects of thyroid hormones on the activities of some selected enzymes have been reported in freshwater teleosts (Peter and Oommen, 1989; Tripathi and Shukla, 1989). Infact the information on this line is too meager to draw any significant conclusion. As far my knowledge is concerned there is not even a single report on the kinetics of thyroid hormone induced enzymatic changes in the metabolism of fishes.

In retrospection of the above view, it was considered of an interest to perform a time course study on T_3- induced changes in some cytoplasmic and mitochondrial enzymes of the freshwater catfish, *Clarias batrachus*.

Results and Discussion

The activity of cytoplasmic malate dehydrogenase (cMDH) of the liver and skeletal muscle of the fish exposed to thiouracil for 28 days increased

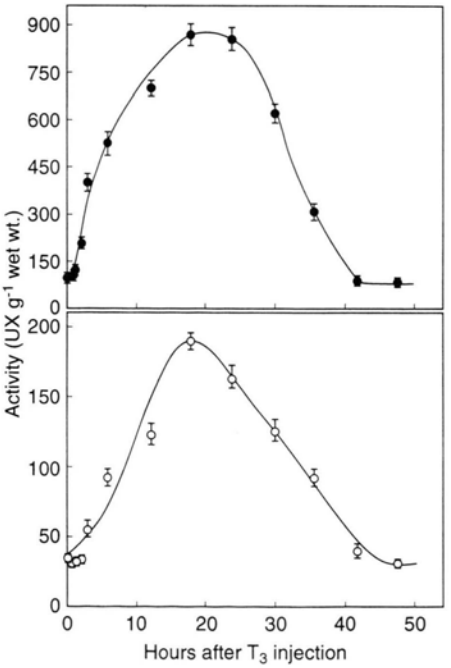

Fig. 1. Activity of cMDH at different time intervals from the liver (●—●) and skeletal muscle (○—○) of the catfish.

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gradually after a single injection of T_3 (Fig. 1). A significant ($P < 0.05$) increase (27%) in the activity of liver cMDH was found at 1 hr of T_3 treatment. Subsequently, the activity increased rapidly and became about 9 fold higher at 18 hr as compared to the vlaue at 0 hr. It followed a gradual decline and approached the control level within 42 hr of T_3 administration. A significant ($P < 0.005$) increase (67%) in the activity of skeletal muscle cMDH was observed only at 3 hr of T_3 treatment to thiouracil exposed individuals. It reached maximum (5.7 fold) at 18 hr and then started declining and approached the control level at 48 hr of hormonal administration (Fig. 1). Like cMDH, the activity of mitochondrial malate dehydrogenase (mMDH) of the liver and skeletal muscle was induced in response to T_3 treatment (Fig. 2). T_3 induced (3 fold) significantly ($P < 0.001$) the activity of mMDH of liver firstly at 3 hr and peaked (7.9 fold) around 18–20 hr which remained constant upto 24 hr. There occurred a gradual decrease in its activity reaching the control level at 48 hr of hormonal treatment. Similarly, T_3 significantly ($P < 0.001$) induced the activity of skeletal muscle mMDH by 100% at 3 hr and the peak activity (6.6 fold) was found around 18–20 hr (Fig. 2). It started declining and reached the control level at 48 hr of T_3 injection.

Administration of T_3 to thiouracil exposed fish gradually increased the activity of lactate dehydrogenase (LDH) of the liver and skeletal muscle (Fig. 3). The earliest increase (103%) in liver LDH was significantly noticed at 3 hr of T_3 injection. It subsequently followed a gradual trend of induction which peaked around 18–20 hr and remained maintained upto 24 hr. Thereafter, the activity declined gradually and touched the control level at 42 hr of T_3 administration. Likewise the activity of skeletal muscle LDH increased (88%) significantly ($P < 0.001$) at 6 hr and the maximum induction (4.5 fold) was achieved at 18–20 hr of hormonal administration. This rise in the enzymic activity was maintained upto 24 hr and then followed a gradual decline approaching the control level within 42 hr of T_3 treatment.

The patterns of gradual increases as well as declines in all the enzymic activities (cMDH, mMDH and LDH) showed somewhat faster rates of inductions of different enzymes in skeletal muscle than

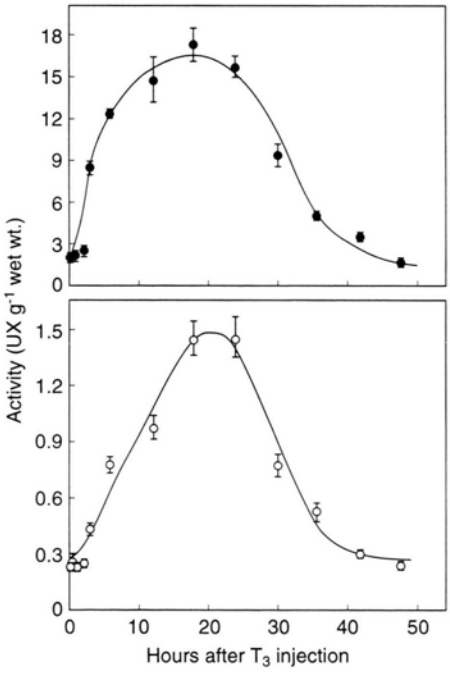

Fig. 2. Activity of mMDH at different time intervals from liver (●—●) und skeletal muscle (○—○) of the catfish.

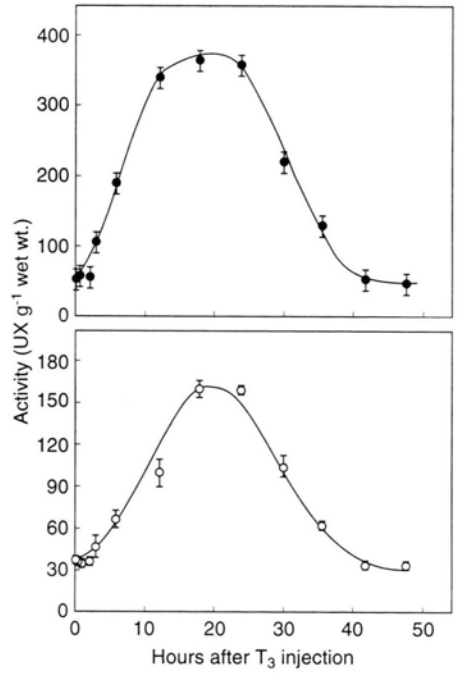

Fig. 3. Activity of LDH at different time intervals from liver (●—●) und skeletal muscle (○—○) of the catfish.

those of the liver as evidenced from Figs. 1, 2 and 3. These time – dependent and tissue – specific differences in enzymic inductions may be due to the possible differences in the rates of syntheses of different enzymes. This may be supported by the reports on thyroid hormone induced *de novo* syntheses of enzymes in vertebrates (Tarentino *et al.*, 1966; Bulos *et al.*, 1972; Schultz *et al.*, 1988; Tripathi and Shukla, 1989).

The maximum inductions in the activities of cMDH, mMDH and LDH varied from 7–9 fold in liver. However, there were 5–7 fold maximum inductions in the enzymic activities of skeletal muscle. This suggests the differences in the efficiencies of T_3 induced DNA- depended RNA syntheses in different tissues of a fish. After attaining the maximum induction the activities of liver and skeletal muscle cMDH, mMDH and LDH started declining gradually (Figs 1–3) and indicated about 12 hr of physiological half-life of these metabolic enzymes in the freshwater catfish.

Experimental Part

Clarias batrachus were collected from local ponds during winter season (November to February) and they were acclimatized to laboratory condition for 2 weeks prior to experimentation. Fish were fed on minced goat liver on alternate day and maintained for 28 days in water containing 0.4% thiouracil. They were injected only a single dose (20 μg per 100 g body mass) of T_3 intraperitoneally and then a group of 4 individuals each were sacrificed at 0 (control), 0.5, 1, 2, 3, 6, 12, 18, 24, 30, 36, 42 and 48 hr for enzymatic assays.

Specimens were sacrificed and the liver and caudal skeletal muscle were removed. A 10% homogenate was prepared in 0.25 mM ice-cold sodium phosphate- buffered sucrose (pH 7.4) using teflon pestle. The homogenate was centrifuged at 700 g and the obtained supernatant was centrifuged at 12,100 g for 20 min to get the mitochondrial pellet. The resulting supernatant was recentrifuged at $30{,}000 \times g$ for 30 min and thus the obtained supernatant was taken as cytoplasmic fraction for the assay of cMDH and LDH. The extraction of mitochondrial enzyme was done according to the procedure of Casadó *et al.* (1980). The principles adopted for the assay of MDH isozymes and LDH were that of Ochoa (1955) and Kornberg (1955), respectively.

The optimum concentrations of substrates, coenzymes and enzymes were used in the assays and the activities were measured in a recording spectrophotometer as referred earlier (Tripathi and Shukla, 1989). One unit of an enzyme activity was defined as the amount of enzyme catalyzing the oxidation of one μ mole of NADH per minute under the above specified conditions. This activity was expressed as units x g^{-1} wet wt. (mass) of the tissue. The students t-test was employed to ascertain the level of significance. Values obtained at different time intervals were compared with the control data (values at 0 hr).

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