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In vitro Effects of Thyroid and Gonadal Hormones on the Activity of Mitochondrial Oxidative Enzymes in a Teleost (*Anabas testudineus* Bloch) and an Apodan Amphibian (*Gegenophis carnosus* Beddome)

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The specific activity of two important oxidative enzymes — viz. cytochrome oxidase and α-glycerophosphate dehydrogenase (α-GPDH) — in isolated hepatic mitochondria of a telecost, *Anabas testudineus* and an apodan amphibian, *Gegenophis carnosus* was investigated in response to the *in vitro* administration of thyroid and gonadal hormones. Addition of different concentrations of L-thyroxine (T_4), triiodo-L-thyronine (T_3), testosterone (T) or estradiol 17 β (E_2) *in vitro* to liver mitochondria prepared from normal male/female fish and apodans significantly stimulated the activity of cytochrome oxidase and α-glycerophosphate dehydrogenase (α-GPDH). The activity of cytochrome oxidase and α-GPDH in the mitochondria isolated from thiouracil-pretreated fish and apodans was also stimulated by thyroid hormones. It is suggested that thyroid and gonadal hormones have a direct influence on mitochondrial metabolism of these two non-mammalian vertebrate species.

Key Words : Thyroxine, Triiodothyronine, Testosterone, Estradiol-17β, Mitochondrial metabolism, Oxidative enzymes, Fish, Amphibian

Introduction

Although the endocrine control of metabolism in lower vertebrates has been the subject of numerous studies (Higgs et al. 1982, Plisetskaya et al. 1983, Plisetskaya 1985, Peter & Oommen 1989, Matty 1985, Jacob & Oommen 1992, Sutharam et al. 1991, Baby & Oommen 1993, Kaippallil & Oommen 1994), the precise role of thyroid and gonadal hormones in the mitochondrial oxidative metabolism in relation to different enzyme systems remains to be investigated. Recent in vivo studies have shown that thyroid and gonadal hormones influence the activity of various oxidative enzymes in *A. testudineus*

(Peter & Oommen 1987, 1988, 1989a, b) and in *G. carnosus* (Sutharam & Oommen 1989, Sutharam et al. 1990, 1991).

In spite of the phyletic significance of apodan amphibians, reports on the endocrine function in this species are still sparse (Sutharam et al. 1990). Stimulation of mitochondrial oxidative metabolism *in vivo* by thyroid and gonadal hormones administration has been reported in *G. carnosus* (Sutharam & Oommen 1989, Sutharam et al. 1990, 1991). A number of investigations in fishes and amphibians unequivocally revealed the important role of sex steroids in hepatic oxidative metabolism (Peter & Oommen 1988, 1989 b, c, Sutharam et al. 1991, Kaippallil & Oommen 1994).

In addition to the binding sites for thyroid hormones on nucleus, high affinity binding sites are also identified in mitochondria, cytosol, microsomes and plasma membrane (Tata 1980, Schwartz et al. 1994) Likewise, androgen binding sites in the cytosol and nuclei have been located in the rat liver (Sato et al. 1980). Shivakumar and Jayaraman (1986) have reported the presence of a thyroxine binding protein in the gill mitochondria of *Sarotherodon mossambicus*. Likewise a sex-steroid binding protein in the spotted sea trout (Ladliey & Thomas 1994) and an androgen receptor in the ovaries of Coho salmon (Fitzpatrick et al. 1994) have been identified. A direct action of triiodothyronine on mitochondria has been reported in mammals too (Sterling 1979, Goglia et al. 1989). However, convincing demonstration of a direct effect of T_3 on mitochondrial functions is lacking (Hafner 1987). A large number of enzymes such as α-GPDH and cytochrome oxidase, the synthesis of which is affected by thyroid hormones, are associated with mitochondria (Thomas & Keenan 1986).

In order to study the comparative aspects of *in vitro* action of hormones in lower vertebrates, we examined the direct effect of thyroid and gonadal hormones in two representatives of lower vertebrates viz. a fish (*A. testudineus*) and an apodan amphibian (*G. carnosus*). The isolated hepatic mitochondrion, capable of showing oxidative capacity by means of enzyme activity, has been used in the present study as an *in vitro* model.

Materials and Methods

Healthy adult fish weighing 40±5g, fed with fish feed every alternate day were used in the present study. *G. carnosus* is a primitive apodan amphibian found in the marshy area of Western Ghats in Kerala. Adult *G. carnosus* comprising both sexes (approximate body weight 7-10g) were collected from the Ghat region of Trivandrum and housed in large tanks containing wet sand and mud. The animals were acclimated to laboratory conditions at 28-30°C on 12 hr light : dark schedule. The animals were fed on live earthworms everyday through out acclimation and experimentation periods. The study was conducted during February-March when the animals used were in the non-breeding phase.

The experimental animals were sacrificed by decapitation and liver was excised. Liver mitochondria were isolated at 4°C by differential fractionation (Johnson & Lardy 1967), washed twice and suspended in 0.25 M sucrose medium. The purity of mitochondrial preparation was tested by assaying P/O ratio and a value of 1.6 ± 0.2 was obtained, when succinate was used as substrate.

In order to study the dose-dependent effects of thyroid and gonadal hormones *in vitro*, different doses of L-thyroxine (T_4), triiodo-L-thyronine (T_3), testosterone (T) and estradiol - 17 β (E_2) were added to the reaction mixture containing isolated mitochondria. The doses of thyroid hormones used in the present study were selected on the basis of *in vitro* experiments conducted by Peter and Oommen (1989c).

The specific activity of cytocrome oxidase (Umbreit et al. 1972) and α-GPDH (Reugamer et al. 1964) was assayed manometrically in a Gilson differential respirometer at 30°C. After 10 min of equilibration the reaction was started by tipping the substrate along with the respective hormones from the side arm. The enzyme's specific activities are expressed in nanogram atom oxygen consumed/min/mg mitochondrial

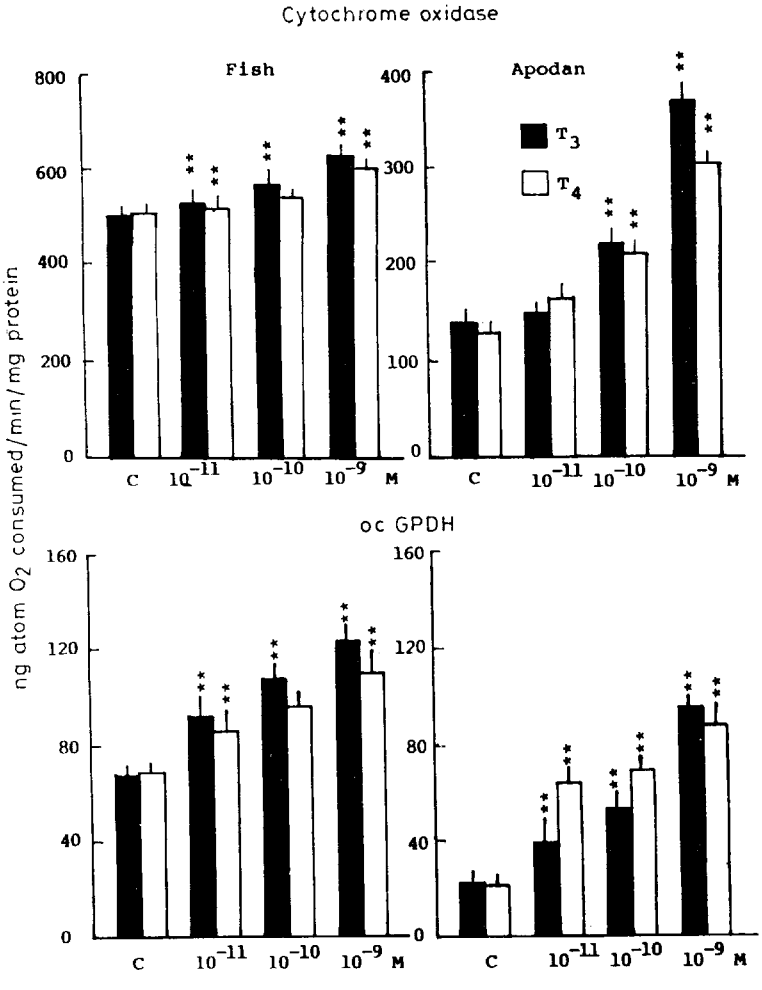

Figure 1. Effect of varied concentrations of T_3 and T_4 *in vitro* on the activity of cytochrome oxidase and α-GPDH in liver mitochondria isolated from normal fish and apodans. C: control; ** $p < 0.01$)

protein. Proper control experiments were also run with respective hormone vehicles. Mitochondrial protein was estimated by Biuret reaction (Gornall et al. 1949).

Statistical analysis of data was done by one way classification of analysis of variance (Snedecor & Cochran 1967) supplemented by multiple range test (Kramer 1956). All the hormones and chemicals were procured from Sigma Chemical Co., USA, except estradiol-17β which was obtained from Ciba-Geigy, Bombay.

Results

The mitochondria isolated from *A. Testudineus* and *G. carnosus* showed significant increase in the activity of cytochrome oxidase at concentrations of 10^{-10} and 10^{-9} M T_3 and 10^{-9} M T_4 (figure 1). Addition of 10^{-11}, 10^{-10} and 10^{-9} M concentrations of T_3 and T_4 significantly stimulated α-GPDH activity in fish and apodan mitochondria in a dose-dependent manner (figure 1). Cytocrome oxidase and α-GPDH activities in the mitochondria

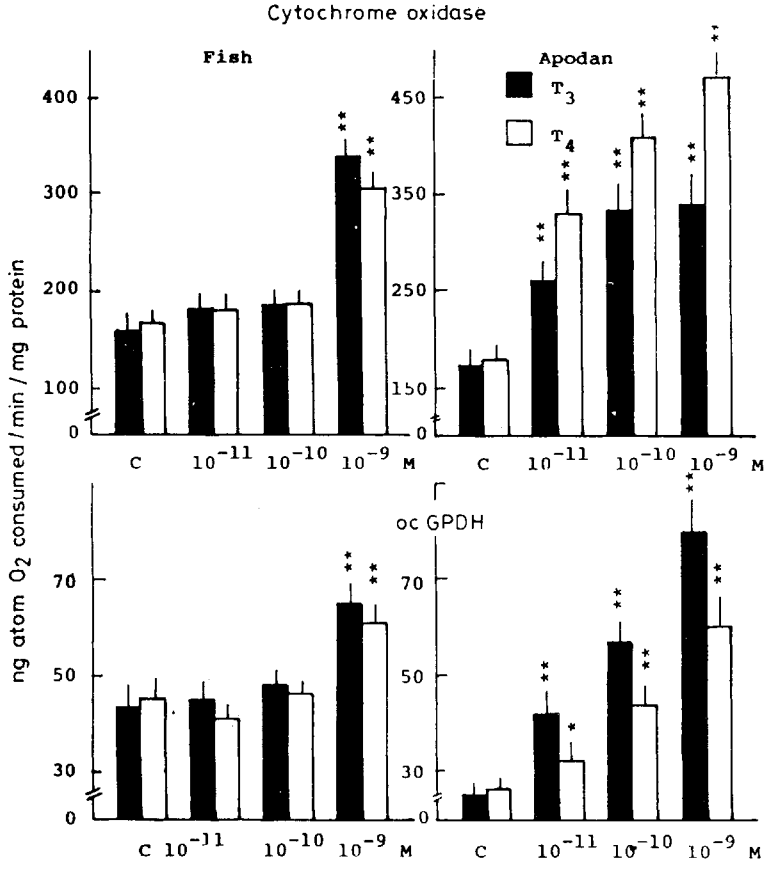

Figure 2. Effect of varied concentrations of T_3 and T_4 *in vitro* on the activity of cytochrome oxidase and α-GPDH in liver mitochondria isolated from thiouracil treated fish and apodans.C: control *P < 0.05 **P <0.01

prepared from thiouracil (antithyroidal drug)-treated fish were stimulated significantly by T_3 and T_4 only at 10^{-9} M level and not at any other concentration (figure 2). However a significant increase in α-GPDH activity was observed in mitochondria isolated from thiouracil-treated apodans by the addition of various concentrations of T_3 and T_4.

Addition of 10^{-10} and 10^{-9} M T and 0.5×10^{-10} M E_2 concentrations in normal male/female fish mitochondria stimulated the activity of cytochrome oxidase significantly (figure 3). The activity of cytochrome oxidase in mitochondria isolated from apodans showed significant increase at all concentrations of T and E_2 used. α-GPDH activity showed significant stimulation to various concentrations of T and E_2 in male/female fish and apodan mitochondria except at 10^{-9} M T in fish mitochondria (figure 3).

Discussion

The present *in vitro* studies in *A. testudineus* and *G. Carnosus* clearly reveal that thyroid and gonadal hormones have a direct stimulatory influence on mitochondrial

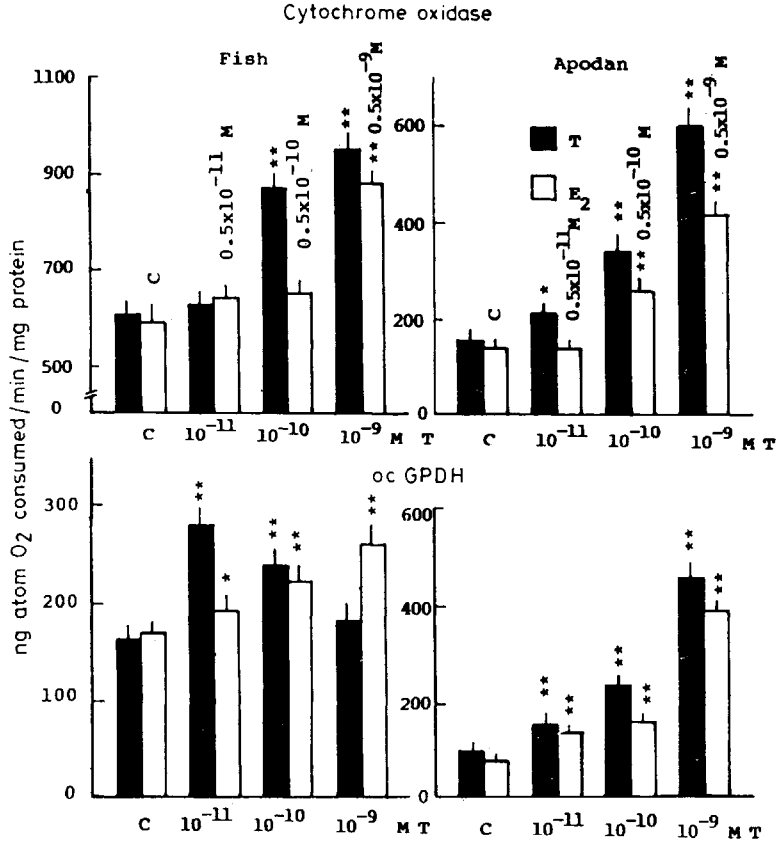

Figure 3. Effect of different concentration of T and E_2 *in vitro* on the activity of cytochrome oxidase and α-GPDH in liver mitochondria isolated from normal male/female fish and apodans. C: control *P < 0.05 **P < 0.01

oxidative metabolism of these representatives of lower vertebrates.

Stimulation in the activity of cytochrome oxidase and α-GPDH after thyroid hormones additions indicates increased mitochondrial respiration and oxidative metabolism. Thyroid hormones *in vivo* are reported to stimulate cytochrome oxidase activity in the liver of *A. testudineus* (Peter & Oommen 1987, 1988 b, 1989 a), *Mugil auratus* (Leray et al. 1970) and in the gill mitochondria of *Sarotherodon mossambicus* (Shivakumar & Jayaraman 1984) and in *G. carnosus* (Sutharam & Oommen, 1989, Sutharam et al. 1990). Hepatic mitochondrial cytochrome oxidase activity is reported to increase in the tadpole of *Rana catesbeiana* during metamorphosis by T_4 or T_3 administration (Goto et al. 1982). *In vitro* studies in brain homogenates of the teleost, *Channa punctatus* reveal that presence of T_4 (3.12 μM) in the incubating medium stimulates SDH activity (Begum et al. 1984).

The increased activity of α-GPDH after T_3 and T_4 additions indicates that thyroid hormones stimulate mitochondrial oxygen consumption as there exists an excellent correlation between α-GPDH activity and oxygen consumption (Oppenheimer 1975). A direct cellular action of T_3 on α-GPDH activity in rat hepatocyte culture medium has also been reported (Ismail-Beigi et al. 1979, Jane & McMurry 1983). A dose-dependent stimulatory activity of α-GPDH has been reported by *in vivo* thyroid hormone adminsitration in *A. testudineus* (Peter & Oommen 1987, 1989a) and in *G. carnosus* (Sutharam & Oommen 1989). It is known that hormone doses higher than the circulating levels are necessary for eliciting the thyroid hormone-mediated responses in fishes (Plisetskaya et al. 1983). Moreover, it is now known that the media in which the mitochondira are incubated often contain little protein and the effective concentration of thyroid hormones may be higher than *in vivo* doses (Gorbman et al. 1983).

The stimulation of cytochrome oxidases and α-GPDH activities in mitochondria isolated from thiouracil-treated fish is achieved only by high concentrations of thyroid hormones. Further more, the data are consistent with the earlier report in *A. testudineus* and *G. carnosus* that thiouracil decreases and administration of thyroid hormones *in vivo* to thiouracil-treated fish and apodan increases the activity of cytochrome oxidase and α-GPDH (Peter & Oommen 1988b, Sutharam et al. 1990). The present results also reveal that thyroid hormones *in vitro* have a direct and rapid stimulatory action on hepatic mitochondrial energy metabolism, thus supporting Sterling's (1979) view that the inner mitochondrial membrane is a target for the primary and immediate action of these hormones. This could also be at true in *A. testudineus* and *G. carnosus*.

Addition of T or E_2 *in vitro* stimulates cytochrome oxidase and α-GPDH activities, suggesting that like thyroid hormones, sex steroids also have a direct effect on liver mitochondria and its oxygen consumption. An increase in hepatic cytochrome oxidase activity after *in vivo* administration of testosterone and estradiol-17β has been observed in *A. testudineus* and in *G. carnosus* (Peter & Oommen 1988a, 1989b, Sutharam 1990, Sutharam et al. 1990, 1991). It appears that E_2 administration *in vitro* stimulates α-GPDH activity in *A. testudineus* despite its lack of

effect on this enzyme when administered *in vivo* (Peter & Oommen 1989b).

It is assumed that the observed stimulation in the activity of oxidative enzymes in response to thyroid and gonadal hormones addition could also be due to the activation of enzymes as a result of structural changes, since thyroxine is well known for its uncoupling action in oxidative phosphorylation. There are reports on the stimulatory action of thyroid hormone on mitochondrial proton leak and ATP turnover in rat hepatocytes without affecting the overall kinetics of substrate oxidation (Harper & Brand 1994). However, the present study demonstrates that thyroid and gonadal hormones *in vitro* have a direct effect on liver mitochondria and its oxidative enzyme activities. It is generally accepted that the biochemical action of a hormone *in vivo* is better appreciated when confirmed *in vitro*. Thus the present study confirms the *in vivo* effects of these hormones in non-mammalian species reported from our laboratory. However, comparison of the *in vitro* with the *in vivo* condition is to be made with caution.

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