Biochimica et Biophysica Acta, 1019 (1990) 133–136
Elsevier

BBABIO 43255

Stimulation of phosphate transport in rat-liver mitochondria by thyroid hormones

Giuseppe Paradies and Francesca Maria Ruggiero

Department of Biochemistry and Molecular Biology and C.N.R. Unit for the Study of Mitochondria and Bioenergetics, University of Bari, Bari (Italy)

(Received 5 February 1990)

Key words: Phosphate transport; Hyperthyroidism; (Rat-liver mitochondria)

The effect of hyperthyroidism on the transport of phosphate in rat-liver mitochondria has been examined. Thyroid hormones administered in vivo increased carrier mediated (mersalyl-sensitive) phosphate transport. Kinetic analysis of the phosphate transport showed that the thyroid hormone affects the V_{max} of this process, while having no effect on the K_m values. The higher activity of the phosphate carrier was found not to be due to a change in the endogenous content of phosphate nor to a change in the transmembrane ΔpH value. Inhibitor titrations with mersalyl showed that mitochondria from both control and hyperthyroid rats required the same concentrations of inhibitor to produce total inhibition of phosphate transport, thus suggesting that the amount of functional translocase present is unaffected. The level of cardiolipin was significantly higher in mitochondrial membranes from hyperthyroid rats as compared to the control rats. The thyroid hormone induced change in the activity of the phosphate carrier appears to be due to a more favorable lipid microenvironment (cardiolipin content) surrounding the carrier molecule in the mitochondrial membrane.

Introduction

The thyroid hormones are known to play an important role in the regulation of hepatic metabolism. Mitochondria are considered a likely subcellular target of thyroid hormone action in view of their central role in energy metabolism. The transport of metabolites in mitochondria is an important step in the regulation of mitochondrial energy metabolism. Thyroid hormones have been shown to influence the activity of certain anion carrier proteins in mitochondria [1–6].

The synthesis of ATP during oxidative phosphorylation requires uptake of ADP and phosphate. The translocation of phosphate in mitochondria is mediated by a specific transporting system (for reviews see Refs. 7, 8). The phosphate carrier protein has been purified and its activity reconstituted in liposomes [9,10]. Lipids, in particular cardiolipin, have been shown to have a strong influence on the activity of the reconstituted phosphate carrier in liposomes [11,13]. Thyroid hormones are known to influence the lipid composition of the mitochondrial membrane [14–16].

In the present investigation the effect of hyperthyroidism on the activity of the phosphate carrier in rat liver mitochondria is studied. The results obtained indicate that the transport of phosphate is significantly enhanced in mitochondria isolated from hyperthyroid rats when compared with mitochondria isolated from control rats. The basis of this stimulation is also examined.

Materials and Methods

Chemicals. The radioactive [^{32}P]orthophosphate, ^3H_2O and [U-^{14}C]sucrose were obtained from Amersham International (U.K.). All other reagents were of reagent grade purity and were purchased from Sigma.

Animals. Male Wistar rats (180–230 g) were used throughout these studies. Animals were made hyperthyroid using 3,3′,5-triiodo-L-thyronine (30 μg/100 body wt.), injected intraperitoneally with a single daily injection for 5 consecutive days [17]. In the same way control animals received only the solvent for the same period. The drug dose and the treatment duration were chosen to obtain a variation of the haematic triiodothyronine level without significantly changing the body weight of the animals. Animals were killed 24 h after final administration.

Correspondence: G. Paradies, Dept. of Biochemistry and Molecular Biology and C.N.R., University of Bari, Bari, Italy.

Rat liver mitochondria were prepared by differential centrifugation of liver homogenates essentially as described in Ref. 18.

Mitochondrial phosphate transport. The transport of exogenous phosphate into mitochondria was measured at 0 °C by the 'inhibitor stop method' essentially as described in Ref. 19. Mitochondria corresponding to 2 mg of protein/ml were preincubated in a reaction medium that contained in a final volume of 1 ml: 100 mM sucrose, 50 mM KCl, 20 mM Tris-HCl, 1 mM $MgCl_2$, 0.5 mM EDTA, 1 mM *n*-butylmalonate (to inhibit dicarboxylate carirer which is also able to transport phosphate), 2 μg/ml rotenone. Final pH 7.4. After a period of equilibration of 2 min, phosphate transport was initiated by adding radioactive phosphate and stopped after time *t* by rapid addition of 0.2 mM mersalyl. The tubes were then rapidly centrifuged at 12000 × *g* for 2 min. The pellets were washed several times in 0.25 M sucrose and then the radioactivity was counted in a scintillation counter. Phosphate transport was considered as difference of noninhibited and mersalyl-treated samples (in the latter case mersalyl was added in the preincuation phase 2 min before radioactive phosphate).

Phosphate transport was also measured as phosphate–[^{32}P]phosphate exchange. In these experiments mitochondria from both control and hyperthyroid rats were first preloaded with unlabeled phosphate as follows. Aliquots of mitochondria (40–50 mg of protein) were incubated at 0 °C in 20 ml of the buffer containing 100 mM sucrose, 50 mM KCl, 20 mM Tris-HCl, 1 mM EDTA, 3 μg/ml rotenone and 2 mM unlabeled phosphate. Final pH 7.4. After a period of equilibration of 5 min, mitochondria were centrifuged at 9000 × *g* for 10 min. The mitochondrial pellet was resuspended in 0.25 M sucrose. The rate of phosphate–[^{32}P]phosphate exchange was then measured as described above.

Transmembreane Δ pH measurements. The transmembrane ΔpH values in mitochondria were determined essentially as described in Ref. 6.

HPLC analysis of phospholipids. Extraction and analysis of phospholipids were carried out as previously described [17].

Determination of phosphate and protein. The endogenous level of phosphate was determined chemically [20] in perchloric acid extracts. Protein concentration was measured by the usual biuret method, using serum albumin as standard.

Statistical analysis. Results are expressed as mean values ± S.E. Statistical significances were determined by Student's *t*-test.

Results and Discussion

Fig. 1 shows a representative of six separate experiments on the time-course of phosphate uptake by liver mitochondria from control and from hyperthyroid rats. At 0 °C and external phosphate concentration of 2 mM, phosphate uptake followed first-order kinetics for the first 10–15 s. When mersalyl was added to the incubation before starting the assay with radioactive phosphate, the amount of phosphate bound was the same in the two preparations of mitochondria. This indicates that thyroid hormone treatment had no effect on nonspecific phospahte binding. In contrast, at each of the subsequent times, phosphate uptake was significantly higher in mitochondria from hyperthyroid rats than in those from control rats. These results demonstrate that mitochondria isolated from hyperthyroid rats exhibit an enhanced activity of the phosphate carrier.

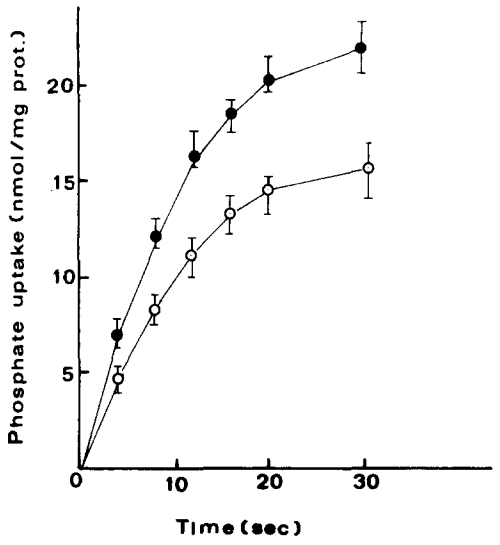

Fig. 1. Time-course of phosphate uptake by liver mitochondria from control and hyperthyroid rats. Phosphate uptake was measured as described in Materials and Methods. Mitochondria (2 mg of protein/ml) were preincubated in the standard reaction medium at pH 7.4 and 0 °C. After 2 min of preincubation 2 mM radioactive phosphate was added. The reaction was stopped at the times indicated by adding 0.2 mM mersalyl. The data represent the results of one of six experiments which gave similar results. ○, Mitochondria from control rats; ●, mitochondria from hyperthyroid rats.

The enhanced activity of the phosphate carrier in mitochondria from hyperthyroid rats could be due to an increased content of exchangeable endogenous phosphate. However, the amount of intramitochondrial phosphate was practically the same in the two populations of mitochondria (11.5 ± 1.4 and 12.2 ± 1.5 nmol per mg of protein in mitochondria from control and from hyperthyroid rats, respectively).

The transmembrane ΔpH appears to be the major driving force for the net uptake of phosphate in mitochondria [7,8]. Thus, the observed increase in the activity of the phosphate carrier in mitochondria from hyperthyroid rats could be due to an increase in the transmembrane ΔpH value. However, no substantial changes in the transmembrane ΔpH values were observed in mitochondria from either control or hyper-

thyroid rats, the values being $+0.94 \pm 0.09$ and $+0.96 \pm 0.1$ (means $\pm$ S.E. for five different experiments), respectively.

The dependence on substrate concentration of the rate of phosphate uptake by mitochondria from normal and hyperthyroid rats was studied at 0 °C by changing the concentration of externally added [^{32}P]phosphate. The results from a typical experiment (see Fig. 2) show that the concentration dependence of phosphate uptake by both these two preparations of mitochondria reveals hyperbolic saturation characteristics. However, while the affinity of phosphate for its carrier remained practically the same, the maximal velocity of phosphate uptake significantly increased in mitochondria from hyperthyroid rats. The values for the kinetic parameters of the phosphate transport, obtained from six different experiments, were the following: K_m 1.75 ± 0.18 and 1.68 ± 0.19 mM and V_{max} 172 ± 18 and 255 ± 26 nmol per min per mg of protein in mitochondria from control and from hyperthyroid rats, respectively. Very similar results were obtained when mitochondria from both control and hyperthyroid rats were first preloaded with 2 mM unlabeled phosphate and then the activity of the phosphate carrier was measured as rates of phosphate–[^{32}P]phosphate exchange (results not reported). It should be noted that the observed values for the kinetic parameters of the phosphate carrier in mitochondria from control rats, are in good agreement with previous data reported in the literature [21,22].

The enhanced activity of the phosphate carrier in mitochondria from hyperthyroid rats could also be due

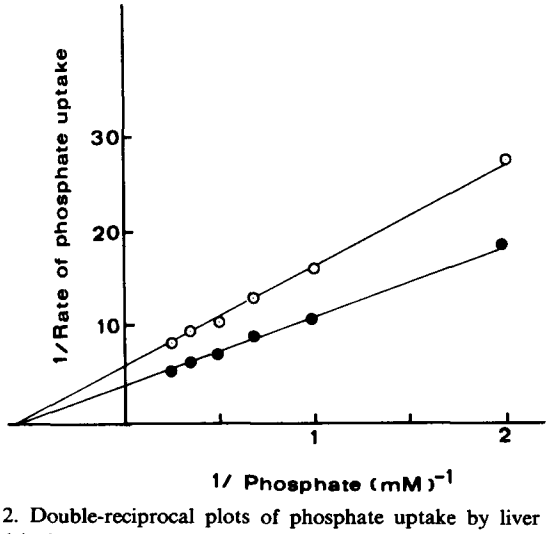

Fig. 2. Double-reciprocal plots of phosphate uptake by liver mitochondria from control and hyperthyroid rats. The rate of phosphate uptake was measured as described in Materials and Methods and in the legend of Fig. 1. Mitochondria (2 mg of protein/ml) were added to the standard reaction medium. After 2 min of preincubation, radioactive phosphate was added at concentrations indicated and 6 s later, 0.2 mM mersalyl was added to stop the transport reaction. The rate of phosphate uptake is expressed as μmol/min per mg protein. ○, Mitochondria from normal rats; ●, mitochondria from hyperthyroid rats.

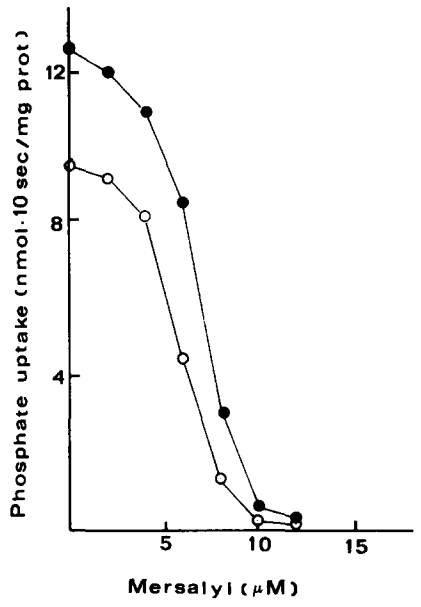

Fig. 3. Mersalyl inhibition of phosphate transport in mitochondria from control and from hyperthyroid rats. Experimental conditions as in Fig. 1. Mitochondrial protein was 2 mg/ml. Mersalyl was added, at concentrations indicated, during the preincubation phase. The data represent the results of one of five experiments which gave similar results. ○, Mitochondria from control rats; ●, mitochondria from hyperthyroid rats.

to an increase in the amount of functional translocase enzyme. To assess this, inhibitor titrations of phosphate transport with mersalyl were carried out in both mitochondria from control and from hyperthyroid rats. Mersalyl is a very powerful inhibitor of the phosphate transport in mitochondria [7]. Thus, the minimum amount of the inhibitor added which produces complete inhibition of phosphate transport, should give an indirect measure of the amount of phosphate translocase present in the mitochondria. Maximum inhibition of the phosphate transport was achieved in both cases by approximately the same amount of mersalyl (Fig. 3). This suggests that the hyperthyroid state did not affect the amount of functional translocase.

Thyroid hormones have been shown to alter the lipid composition of the mitochondrial membranes [14–16]. On the other hand, the activity of certain anion carrier proteins appears to be influenced by the lipid composition of the mitochondrial membrane [2–4]. The phospholipid composition of the mitochondrial membranes from control and from hyperthyroid rats is reported in Fig. 4. No substantial changes in the mitochondrial phospholipid composition of either type of animal occurred, with the exception of negatively charged phospholipid cardiolipin, the level of which increased by more than 50% in the mitochondrial membrane from hyperthyroid rats.

It has been reported that cardiolipin is specifically required for the reconstitution of the isolated phosphate carrier activity in artificial membranes such as lipo-

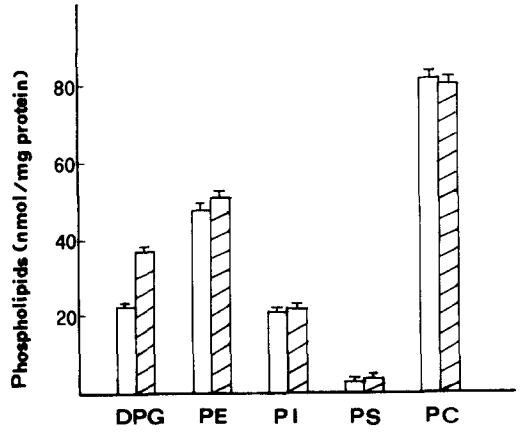

Fig. 4. Phospholipid distribution in mitochondrial membranes from normal and from hyperthyroid rats. For phospholipid extraction and analysis, see Materials and Methods. Abbreviations: DPG, Cardiolipin; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PC, phosphatidylcholine. Each value represent the mean ± S.E. obtained from six different experiments. Unhatched bars, mitochondria from normal rats; hatched bars, mitochondria from hyperthyroid rats.

somes [11,12]. Furthermore, the transport of phosphate in mitochondria is inhibited by doxorubicin [23] an antitumoral agent which is known to form specific complexes with cardiolipin [24]. Thus, it appears that cardiolipin provides the environment necessary for the activity of the phosphate carrier in the mitochondrial membrane. If this in vitro requirement for cardiolipin reflects the in vivo situation, then it can be proposed that the enhanced activity of the phosphate carrier in hepatic mitochondria from hyperthyroid rats, can be ascribed to a more favorable lipid microenvironment (specifically to an increase in the cardiolipin content) surrounding the phosphate carrier molecule in the mitochondrial membrane. In addition to this specific effect, the thyroid-hormone induced change in the activity of the phosphate carrier could also be due to a more general change in the mitochondrial membrane lipid composition and hence membrane fluidity.

The transport of phosphate in mitochondria may be involved in regulating the supply of phosphate to the mitochondrial matrix for the reactions of oxidative phosphorylation. Furthermore, the transport of phosphate is closely linked to that of ADP and Ca^{2+} and it is an obligatory requirement for the net transport of malate, citrate and oxoglutarate [25–28]. Thus, the enhanced activity of the phosphate carrier in mitochondria from hyperthyroid rats may account, in addition to other factors, for the enhanced mitochondrial oxidative capacity, typical of the hyperthyroid state.

Acknowledgments

This work has been accomplished with funds from Ministero Pubblica Istruzione (60%) and Consiglio Nazionale delle Ricerche. The authors would like to thank Drs. P. Dinoi, F. Cafagna and S. Pierno for excellent technical assistance and Mr. L. Gargano for the expert management of the experimental animals.

References

1 Babior, B.M., Cregan, S., Ingbar, S.H. and Kipnes, R.S. (1973) Proc. Natl. Acad. Sci. USA 70, 98–102.
2 Hoch, F.L. (1977) Arch. Biochem. Biophys. 178, 535–545.
3 Chen, Y.D. and Hoch, F.L. (1977) Arch. Biochem. Biophys. 181, 470–483.
4 Mak, L.T., Shrago, E. and Elson, C.E. (1983) Arch. Biochem. Biophys. 266, 317–323.
5 Paradies, G. and Ruggiero, F.M. (1988) Biochim. Biophys. Acta 935, 79–86.
6 Paradies, G. and Ruggiero, F.M. (1989) Arch. Biochem. Biophys. 269, 595–602.
7 Fonyo, A. (1979) Pharmac. Ther. 7, 627–645.
8 LaNoue, K.F. and Schoolwerth, A.C. (1979) Annu. Rev. Biochem. 48, 871–922.
9 Wohlrab, H., Collins, A. and Costello, D. (1984) Biochemistry 23, 1057–1064.
10 Wohlrab, H. (1986) Biochim. Biophys. Acta 853, 115–134.
11 Kadenbach, B., Mende, P., Kolbe, H.V.J., Stipani, I. and Palmieri, F. (1982) FEBS Lett. 139, 109–112.
12 Mende, P., Kolbe, V.J., Kadenbach, B., Stipani, I. and Palmieri, F. (1982) Eur. J. Biochem. 128, 91–95.
13 Krämer, R. and Palmieri, F. (1989) Biochim. Biophys. Acta 974, 1–23.
14 Hulbert, A.J. (1978) J. Theor. Biol. 73, 81–100.
15 Clejan, S., Collip, P.J. and Maddaiah, V.T. (1980) Arch. Biochem. Biophys. 203, 744–752.
16 Hoch, F.L., Subramanian, C., Dhopeshwarkar, G.A. and Mead, J.F. (1981) Lipids 16, 328–335.
17 Ruggiero, F.M., Gnoni, G.V. and Quagliariello, E. (1984) Lipids 22, 148–151.
18 Paradies, G. and Papa, S. (1977) Biochim. Biophys. Acta 462, 333–346.
19 Palmieri, F. and Klingemberg, M. (1979) Methods Enzymol. 56, 279–301.
20 Wahler, B.E. and Wollenberg, A. (1958) Biochem. Z. 329, 508–520.
21 Coty, W.A. and Pedersen, O. (1974) J. Biol. Chem. 249, 2593–2598.
22 Ligeti, E., Brandolin, G., Dupont, Y. and Vignais, P.V. (1984) Biochemistry 24, 4423–4428.
23 Cheneval, D., Muller, M. and Carafoli, E. (1983) FEBS Lett. 159, 123–126.
24 Goormaghtigh, E., Chatelain, P., Caspers, J. and Ruysschert, J.M. (1980) Biochim. Biophys. Acta 597, 1–14.
25 McGivan, J.D. and Klingemberg, M. (1971) Eur. J. Biochem. 20, 392–399.
26 Lehninger, A.L. (1974) Proc. Natl. Acad. Sci. USA 71, 1520–1524.
27 Coty, W.A. and Pedersen, P.L. (1975) Mol. Cell. Biochem. 9, 109–124.
28 Reed, K.C. and Bygrave, F.L. (1975) Eur. J. Biochem. 55, 497–504.