

TOXLET 02942

3,3',5-Triiodothyronine-induced hepatic respiration: Effects of desferrioxamine and allopurinol in the isolated perfused rat liver

Virginia Fernández and Luis A. Videla

Departamento de Bioquímica, Facultad de Medicina, Universidad de Chile, Santiago (Chile)

(Received 17 November 1992)

(Accepted 3 February 1993)

Key words: Thyroid hormone; Liver respiration; Antioxidant; Desferrioxamine; Allopurinol; Oxidative stress

SUMMARY

The effect of daily doses of 0.1 mg 3,3',5-triiodothyronine (T_3)/kg for three consecutive days on hepatic O_2 uptake was studied in the isolated perfused rat liver, in the absence and presence of the antioxidants desferrioxamine (DFO) and allopurinol (A). T_3 treatment elicited a thermogenic condition in the animals, together with a 25% increase of total O_2 consumption by the liver, which is inhibited by 0.5 mM DFO or 1 mM A. The antioxidant-sensitive respiration is enhanced by 62–64% by T_3 over control values and represents 16–25% of the net increase in O_2 uptake elicited by the hormone treatment. The respiratory components suppressed by the antioxidants are suggested to represent O_2 equivalents related to T_3 -induced oxidative stress, and correlate with elevated rates of fractional lactate dehydrogenase efflux from the perfused livers.

INTRODUCTION

The liver tissue is one of the target organs for thyroid hormone action leading to an enhanced respiratory rate [1]. This calorigenic response of the liver has been shown to be associated with increased electron fluxes through microsomal [2], mitochondrial [3] and peroxisomal [3] electron transport systems, with the respective increases in superoxide radical (O_2^-) and/or hydrogen peroxide (H_2O_2) production. The toxicological relevance of these findings is strengthened by the significant enhancement in he-

patic lipid peroxidation indexes [2,4–6], parameters related to the development of cell injury at the membrane level by oxidative stress [7]. In order to assess the influence of thyroid hormone on the O₂ equivalents used in the generation of O₂-derived reactive metabolites in the intact organ, liver O₂ uptake was measured in the absence and presence of the antioxidants desferrioxamine (DFO) and allopurinol (A) in the isolated perfused rat liver, both in control rats and in animals treated with 3,3',5-triiodothyronine (T₃).

MATERIALS AND METHODS

Male Sprague-Dawley rats (Instituto de Salud Pública, Santiago) weighing 185–230 g were fed ad libitum and received daily i.p. injections of either T₃ (0.1 mg/kg for three consecutive days) or equivalent volumes of T₃ diluent (0.1 M NaOH) (controls). After treatment, serum T₃ levels were measured by the GammaCoat™ [¹²⁵I]T₃ Radioimmunoassay Kit (Baxter Healthcare Corp., Cambridge, MA) and the rectal temperature was measured with a thermocouple Cole-Parmer 8112-20 (Cole-Parmer Instrument Co., Chicago, IL), in animals exhibiting comparable body weights (controls, 215 ± 4 (*n* = 8) g; T₃-treated rats, 203 ± 11 (*n* = 8)) and liver weight/body weight ratios (controls, 3.46 ± 0.19 (*n* = 8) g liver/100 g body weight; T₃-treated rats, 3.62 ± 0.30 (*n* = 8)).

Livers obtained from rats under Nembutal (50 mg/kg, i.p.) anaesthesia were perfused with Krebs bicarbonate buffer [8,9] containing 10 mM glucose, equilibrated with an O₂/CO₂ mixture (19:1 v/v) to give pH 7.4, at constant flow rates (3.21 ± 0.10 (*n* = 16) ml/g liver/min). Perfusions were carried out at 36–37°C without recirculation of the perfusate. Livers were allowed to recover from surgery for 15 min, and oxygen consumption was continuously measured polarographically [10] in the effluent perfusate collected via a cannula placed in the vena cava and allowed to flow past a Clark-type oxygen electrode [8]. After obtaining the basal rate of hepatic O₂ uptake in both experimental groups, 0.5 mM DFO or 1 mM A were infused for 15 min as shown in Figure 1, and perfusions were continued for an extra 10 min to assess the reversibility of the changes induced by the antioxidants. Lactate dehydrogenase (LDH) activity [11] in the perfusate was measured every 10 min as integrity parameter in relation to that found in the tissue, and average values were expressed as fractional LDH efflux [8,9]. Values shown are means ± SE for the number of separate experiments indicated in parentheses. Statistical comparisons were performed by one-way analysis of variance, using the random model. All chemicals used were obtained from Sigma Chemical Co. (St. Louis, MO), except for desferrioxamine (Ciba-Geigy, Chile).

RESULTS

Treatment of fed rats with 0.1 mg T₃/kg for three consecutive days resulted in a significant increase in serum T₃ levels compared to controls (Table I). This was observed concomitantly with elevated rectal temperatures in the animals (Table I), sug-

gesting a T_3 -induced thermogenic condition. T_3 treatment elicited a significant increment in the total rate of O_2 uptake by the liver (25%; $P < 0.05$) (Fig. 1; Table I), assessed in perfusion experiments, together with an enhancement in the fractional rate of LDH efflux from the liver (controls, 0.023 ± 0.008 ($n = 8$) h^{-1} ; T_3 -treated rats, 0.166 ± 0.012 ($n = 8$); $P < 0.05$). Results presented in Figure 1 show that the addition of 0.5 mM DFO to the perfused liver from a control rat reduced the O_2 uptake by 190 nmol/g liver/min, with a mean value of 197 ± 14 nmol/g liver/min (Table I), which represents 10% of total respiration. Addition of DFO to perfused livers from T_3 -treated rats diminished O_2 consumption by 320 nmol/g liver/min (Table I), representing a 62% increase in the respiratory component inhibitable by the antioxidant. Similar results were obtained after the infusion of 1 mM A to the perfused livers from control rats and T_3 -treated animals (Table I). Calculation of the net increase in hepatic respiration elicited by T_3 treatment, in the absence and presence of DFO or A, indicates that the enhancement in the respiratory component suppressed by the antioxidants corresponds to 25% (DFO) and 16% (A) of the net increment in liver O_2 uptake elicited by the hormone treatment (Table I).

DISCUSSION

Thyroid calorigenesis in the rat resulted in a significant 25% increment in the rate of hepatic O_2 uptake, which was partially inhibited by the infusion of either DFO or A to the perfused liver. These agents exhibit an antioxidant behaviour due to their

TABLE I

SERUM T_3 LEVELS, RECTAL TEMPERATURE OF THE ANIMALS, AND EFFECTS OF DESFERRIOXAMINE (DFO) AND ALLOPURINOL (A) ON THE RATE OF OXYGEN CONSUMPTION BY PERFUSED LIVERS FROM CONTROL RATS AND T_3 -TREATED ANIMALS

Parameters	Control rats (C)	T_3 -treated rats (T_3)	$\Delta(T_3-C)$	% ^a
Serum T_3 (ng/dl)	53 ± 2 (8)	320 ± 52 (8) ^b		
Rectal temperature ($^{\circ}C$)	37.3 ± 0.1 (8)	38.7 ± 0.1 (8) ^b		
Liver oxygen uptake (nmol/g liver/min)				
No additions (NA)	2030 ± 63 (4)	2520 ± 102 (4) ^b	490 ± 36 (4)	
DFO (0.5 mM)	1833 ± 76 (4)	2200 ± 100 (4) ^b		
$\Delta(NA - DFO)$	197 ± 14 (4)	320 ± 28 (4) ^b	123 ± 14 (4)	25
No additions (NA)	2080 ± 25 (4)	2607 ± 38 (4) ^b	527 ± 22 (4)	
A (1 mM)	1950 ± 30 (4)	2394 ± 27 (4) ^b		
$\Delta(NA - A)$	130 ± 12 (4)	213 ± 11 (4) ^b	83 ± 9 (4)	16

Fed rats were given 0.1 mg T_3 /kg i.p., for three consecutive days, or equivalent amounts of T_3 diluent (0.1 M NaOH) (controls).

^a Percentage of the net increment in O_2 uptake elicited by T_3 treatment which is suppressed by the antioxidants. Values represent means \pm SE for the number of animals indicated in parentheses.

^b $P < 0.05$, compared to control values.

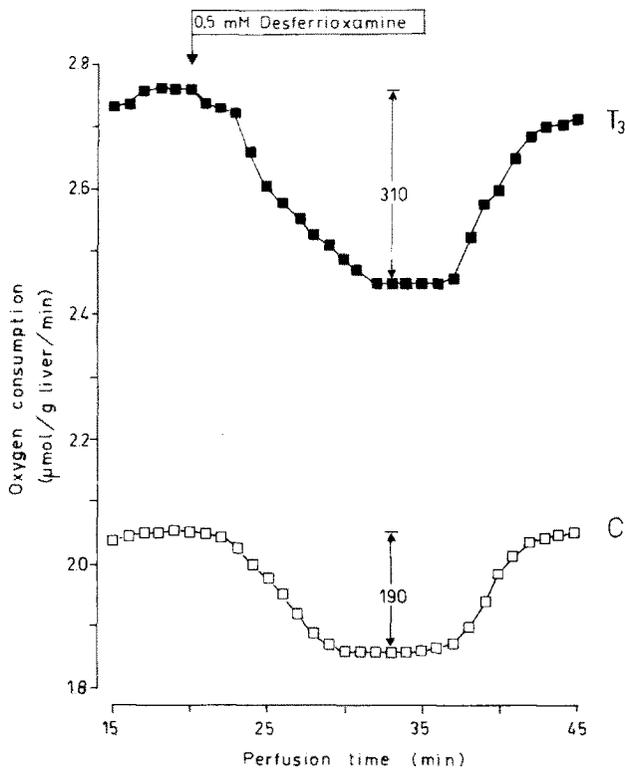


Fig. 1. Representative study of the effect of desferrioxamine (DFO) (0.5 mM) on the rate of oxygen consumption by perfused livers from a control (C) (□) and a T₃-treated (daily doses of 0.1 mg/kg for three consecutive days) (T₃) (■) rat. The values shown in the traces correspond to the respiratory component suppressed by DFO for both experimental animals, in nmol O₂/g liver/min.

effective free-radical scavenging activity in biological systems [12,13], in addition to the powerful iron chelating capacity exhibited by DFO [14] and the inhibition of xanthine oxidase exerted by A [15]. Furthermore, both agents do not seem to interfere with mitochondrial energetics, as shown by the lack of effects of DFO on mitochondrial oxidative phosphorylation [12] and on the hepatic levels of adenine nucleotides by A [16]. Thus, the respiratory component suppressed by the studied antioxidants might represent O₂ equivalents utilized either in reactive O₂ metabolites generation, at the different stages of the lipid peroxidative process, or both, and represents a net 16–25% of the total increment in O₂ consumption of the liver elicited by T₃ treatment. This antioxidant-sensitive respiration observed in the intact liver under the influence of T₃ is in agreement with the enhanced rates of O₂⁻ and H₂O₂ production reported in subcellular fractions [2,3], and occurred in parallel with elevated fractional rates of LDH efflux from the perfused livers (correlation between antioxidant-sensitive respiration and LDH efflux, $r = 0.85$; $P < 0.05$). The data presented suggest a derange-

ment of the permeability properties of the plasma membrane of the hepatocyte, which coincides with the development of an oxidative stress condition [1] imposed by thyroid calorogenesis. This prooxidant condition could constitute a major hepatotoxic mechanism underlying abnormalities of thyroid gland function, which have been shown to induce liver damage in hyperthyroid patients without congestive heart failure, described prior to the advent of antithyroid therapy (see Ref. 17). Recent reports indicate the appearance of mild histological changes in the liver of patients with Graves' disease, together with elevated serum alkaline phosphatase and γ -glutamyl-transferase activities [17] and increased sulphobromophthalein retention [18].

ACKNOWLEDGEMENTS

This work was supported by grants from FONDECYT (1109/91) and DTI-Universidad de Chile (B-3241). The excellent technical assistance of C. Almeyda and M. Suárez is acknowledged.

REFERENCES

- 1 Fernández, V. and Videla, L.A. (1989) Thyroid hormone, active oxygen, and lipid peroxidation. In: J. Miquel, A.T. Quintanilha and H. Weber (Eds.), *Handbook of Free Radicals and Antioxidants in Biomedicine*, CRC Press Inc., Boca Raton, Vol. I, pp. 105-115.
- 2 Fernández, V., Barrientos, X., Kipreos, K., Valenzuela, A. and Videla, L.A. (1985) Superoxide radical formation, NADPH oxidase activity, and cytochrome P-450 content of rat liver microsomal fractions in an experimental hyperthyroid state: relation to lipid peroxidation. *Endocrinology* 117, 496-501.
- 3 Fernández, V. (1991) Hepatic respiration in experimental hyperthyroidism. *Arch. Biol. Med. Exp.* 24, R-109 (Abstr.).
- 4 Fernández, V., Llesuy, S., Solari, L., Kipreos, K., Videla, L.A. and Boveris, A. (1988) Chemiluminescent and respiratory responses related to thyroid hormone-induced liver oxidative stress. *Free Rad. Res. Commun.* 5, 77-84.
- 5 Landriscina, C., Petragallo, V., Morini, P. and Marcotrigiano, G.O. (1988) Lipid peroxidation in rat liver microsomes. I. Stimulation of the NADPH-cytochrome P-450 reductase-dependent process in hyperthyroid state. *Biochem. Int.* 17, 385-393.
- 6 Marzoev, A.I., Kozlov, A.V., Andryushchenko, A.P. and Vladimirov, Y.A. (1982) Activation of lipid peroxidation in liver mitochondria of hyperthyroid rabbits. *Bull. Exp. Biol. Med.* 93, 269-272.
- 7 Tribble, D.L., Aw, T.Y. and Jones, D.P. (1987) The pathophysiological significance of lipid peroxidation in oxidative cell injury. *Hepatology* 7, 377-386.
- 8 Fernández, V., Simizu, K., Barros, S.B.M., Azzalis, L.A., Pimentel, R., Junqueira, V.B.C. and Videla, L.A. (1991) Effects of hyperthyroidism on rat liver glutathione metabolism: related enzymes' activities, efflux, and turnover. *Endocrinology* 129, 85-91.
- 9 Gores, G.J., Kost, L.J. and LaRusso, N.F. (1986) The isolated perfused rat liver: conceptual and practical considerations. *Hepatology* 6, 511-517.
- 10 Estabrook, R.W. (1967) Mitochondrial respiratory control and polarographic measurement of ADP/O ratios. *Methods Enzymol.* 10, 41-47.
- 11 Bergmeyer, H.U. and Bernt, E. (1974) Lactate dehydrogenase. In: H.U. Bergmeyer (Ed.), *Methods of Enzymatic Analysis*, Academic Press, New York, Vol. 2, pp. 574-579.
- 12 Videla, L.A., Villena, M.I., Salgado, C., Canales, P. and Lissi, E.A. (1987) Antioxidant capacity of desferrioxamine in biological systems. *Biochem. Int.* 15, 205-214.

- 13 Fauré, M., Lissi, E.A. and Videla, L.A. (1990) Antioxidant capacity of allopurinol in biological systems. *Biochem. Int.* 21, 357–366.
- 14 Keberle, H. (1964) The biochemistry of desferrioxamine and its relation to iron metabolism. *Ann. NY Acad. Sci.* 119, 758–768.
- 15 Chalmers, R.A., Kromer, H., Scott, J.T. and Watts, R.W.E. (1968) A comparative study of the xanthine oxidase inhibitors allopurinol and oxipurinol in man. *Clin. Sci.* 35, 353–362.
- 16 Nordstrom, G., Seeman, T. and Hasselgren, P.O. (1985) Beneficial effect of allopurinol in liver ischemia. *Surgery* 97, 679–683.
- 17 Szilagyi, A., Lerman, S. and Resnick, R.H. (1983) Ethanol, thyroid hormones and acute liver injury: is there a relationship? *Hepatology* 3, 593–600.
- 18 Sir, T., Wolff, C., Soto, J.R., Perez, G. and Armas-Merino, R. (1987) Relationship between hepatic levels of glutathione and sulphobromophthalein retention in hyperthyroidism. *Clin. Sci.* 73, 235–237.