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Kupffer Cell Function in Thyroid Hormone-Induced Liver Oxidative Stress in the Rat

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The influence of thyroid hormone (L-3, 3', 5-triiodothyronine, T_3) on Kupffer cell function was studied in the isolated perfused rat liver by colloidal carbon infusion. Rates of carbon uptake were determined from the influent minus effluent concentration difference and the flow rate, and the respective carbon-induced respiratory activity was calculated by integration of the area under the O_2 curves during carbon infusion. In the concentration range of 0.2 to 2.0 mg of carbon/ml, livers from euthyroid rats exhibited a sigmoidal-type kinetics of carbon uptake, with a Vmax of 4.8 mg/g liver/min and a concentration of 0.82 mg/ml for half-maximal rate; carbon-induced O_2 uptake presented a hyperbolic-type kinetics, with a Vmax of 4.57 μmol of O_2/g liver and a Km of 0.74 mg of carbon/ml, which significantly correlates with the carbon uptake rates. Light-microscopy showed that carbon was taken up exclusively by non-parenchymal cells, predominantly by Kupffer cells. Thyroid calorigenesis was found in parallel with increased rates of hepatic O_2 consumption and thiobarbituric acid reactive substances (TBARS) formation, glutathione (GSH) depletion, and higher sinusoidal lactate dehydrogenase (LDH) efflux compared to control values. In the concentration range of 0.25 to 0.75 mg/ml, carbon infusion did not modify liver LDH efflux in control rats, while it was significantly enhanced in T_3-treated animals. In this latter group, higher carbon concentrations (1 and 1.3 mg/ml) led to loss of viability of the liver. At 0.25 to 0.75 mg of carbon/ml, both the rates of carbon uptake and the associated carbon-induced respiratory activities were significantly increased by T_3 treatment, effects that were abolished by pretreatment of the rats with gadolinium chloride ($GdCl_3$). In addition, $GdCl_3$ decreased by 50% the changes induced by T_3 in hepatic GSH content and TBARS formation. It is concluded that hyperthyroidism enhances Kupffer cell function, correlated with the increased number of liver macrophages observed histologically, which may represent an alternate source of reactive O_2 species to that induced in parenchymal cells, thus contributing to the enhanced oxidative stress status developed.

Keywords: Thyroid hormone-induced liver oxidative stress, lipid peroxidation, glutathione, liver lactate dehydrogenase efflux, Kupffer cell function, free radicals

INTRODUCTION

Kupffer cells are macrophages residing in the sinusoids of the liver in close contact with endothelial cells, and may reach into the space of Disse and near the hepatocytes.[1] In the nor-

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mal rat liver, the Kupffer cell population makes up about 30% of the sinusoidal cells, with a mean absolute number of 14 to 20 $\times$ 10^6 cells/g liver.[2] They are usually observed in greater number in periportal areas than in central zones,[2,3] performing the clearance of the blood from the splanchnic area of particulate or immunologic active materials, as well as migrating tumor cells.[1,4] Liver macrophages stimulated with phagocytosable particles release several chemical mediators related to cell killing,[5] cell movement and attachment,[6] and to the regulation of biochemical functions of hepatocytes.[7] Under these conditions, Kupffer cells show a respiratory burst activity with generation of reactive O_2 species (ROS),[8,9] which are considered of importance for their bactericidal and tumoricidal effects, and in the cytotoxicity to hepatic endothelial and parenchymal cells.[1,4]

Previous studies by our group have shown that rats made hyperthyroid by L-3, 3', 5-triiodothyroinine (T_3) administration exhibit a significant increment in the oxidative stress status of the liver, as a result of the acceleration of tissue respiration.[10] This effect of thyroid hormone is characterized by a drastic increase in the hepatic production of ROS at microsomal, mitochondrial, and peroxisomal sites,[11,12] coupled to a diminution in some key antioxidant defenses (superoxide dismutase, catalase, glutathione (GSH))[13] and a subsequent enhanced lipid peroxidation response.[11,13–16] Despite the prooxidant condition imposed on the liver by hyperthyroidism, recent light microscopy studies revealed an absence of hepatocellular injury, except for the appearance of Kupffer cell hyperplasia in the form of focal aggregates within the hepatic lobule.[17] This feature induced by T_3 treatment correlates with the enhanced hepatic *opsonized* zymosan-induced luminol-amplified chemiluminescence, compared to control values.[17] The latter effect does not seem to be due to neutrophil infiltration in the liver, as shown histologically and by the lack of changes in hepatic myeloperoxidase activity in T_3-treated rats over values observed in euthyroid animals.[17] In addition to thyroid hormone-induced hypermetabolic state of the liver, a similar condition is elicited by the administration of a large dose of ethanol leading to a swift increase in O_2 uptake and ethanol metabolism, which was shown to largely depend on Kupffer cell activity.[18] In view of these findings, this study was designed to evaluate the influence of T_3 treatment on Kupffer cell function. For this purpose, uptake of colloidal carbon by the isolated perfused liver from control and T_3-treated animals was studied. Data obtained were correlated with the magnitude of the simultaneous respiratory activity elicited by particle infusion, assessed in continuously O_2 uptake measurements.

MATERIALS AND METHODS

Animals

Male Sprague Dawley rats (Instituto de Salud Pública, Santiago, Chile) weighing 170–225 g were fed *ad libitum* and received a single intraperitoneal injection of either T_3 (0.1 mg/kg body weight) or equivalent volumes of T_3 diluent (0.1 N NaOH) (controls). Twenty-four hours after treatment, the weight of the animals [controls, 195 $\pm$ 5 (n = 41) g; T_3-treated rats, 186 $\pm$ 7 (n = 26)] and the respective liver weight/body weight ratios [controls, 4.08 $\pm$ 0.08 (n = 41) g/100 g; T_3-treated rats, 3.95 $\pm$ 0.12 (n = 26)] were comparable. At this time, serum T_3 levels (measured by the Gamma Coat™ [^{125}I] T_3 Radioimmunoassay Kit; Baxter Healthcare Corp., Cambridge, MA), the rectal temperature of the animals (measured with a thermocoupla Cole-Parmer model 8112–20; Cole-Parmer Instrument Co., Chicago, IL), and the rate of O_2 consumption of the liver (measured as described bellow) were determined as parameters of thyroid calorigenesis.

Liver Perfusion, Colloidal Carbon Uptake, and Carbon-Induced Respiratory Activity

Animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally), and perfusate (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, and 10 mM glucose, equilibrated with an O_2:CO_2 mixture (19:1, vol/vol) to give pH 7.4) was pumped into the liver via a cannula placed in the portal vein. Perfusions were carried out at constant flow rates [controls, 3.29 ± 0.09 (n = 35) ml/g liver/min; T_3-treated rats, 3.75 ± 0.10 (n = 18); not significant] and temperature (36–37°C), without recirculation of the perfusate. O_2 consumption was determined polarographically[17] in the effluent perfusate collected via a cannula placed in the vena cava and allowed to flow past a Clark-type oxygen electrode. Livers were allowed to equilibrate for 15 min, and perfusate samples were taken every 5 min for the measurement of LDH activity[19] (one unit of LDH activity corresponds to 1 μmol/min at 25°C). Rates of sinusoidal LDH efflux (mU/g liver/min) were calculated by multiplying the perfusate activity by the respective perfusion flow; at the end of perfusion, liver samples were taken to determine LDH activity in the tissue.[19]

For determination of colloidal carbon uptake by perfused livers, suspensions of India ink (Rotring R-591217, Hamburg, Germany) were prepared, dialysed, and diluted as described by Cowper *et al.*[20] Carbon was infused between 30–45 min of perfusion, and perfusate samples were taken every 5 min in the presence and absence of the liver to measure the absorbance of colloidal carbon at 623 nm[20] (specific extinction coefficient of 0.97 $[mg/ml]^{-1}$) (Fig. 1A). Rates of carbon uptake were calculated from influent minus effluent concentration differences, referred to the perfusion flow (Fig. 1A). The respiratory activity induced upon colloidal carbon infusion was assessed by the integration of the area under the O_2 uptake curves between 30–45 min, and expressed as μmol O_2/g liver (Fig. 1B); similar calculations were done for the sinusoidal efflux of LDH, and results were expressed as U/g liver (Fig. 1C). These parameters were determined in livers from control rats and T-treated animals, as well as in separate groups of rats pretreated with gadolinium chloride ($GdCl_3$) (10 mg/kg body weight by tail vein injection) 24 h before experiments, and livers were perfused with the buffer solution described above containing 50 μg/ml $GdCl_3$. This procedure was adopted to obtain maximal effects, as the *in vivo* pretreatment with $GdCl_3$ chloride does not inactivate all Kupffer cells.[21]

Biochemical Parameters Related To Liver Oxidative Stress and Morphological Studies

Rates of hepatic thiobarbituric acid reactants (TBARS) formation[17] and GSH content[22] were determined in separate groups of control rats and T_3-treated animals, without and with $GdCl_3$ pretreatment, as indexes related to oxidative stress in the liver. For this purpose, livers obtained from rats under sodium pentobarbital (50 mg/kg) anesthesia were perfused *in situ* with 200 ml of a cold solution containing 140 mM KCl and 10 mM potassium phosphate buffer pH 7.4, to remove blood. Protein content of liver homogenates was determined according to Lowry *et al.*[23] Microscopy studies were performed in liver samples from groups (n = 6) of control rats and animals given T_3 after perfusion with colloidal carbon, fixed in 10% formaldehyde, paraffin embedded, and sections were stained with hematoxylin-eosin. Kupffer cell counting was performed in 4 different fields in each liver section and average values were expressed as number of cells/mm^2.

Chemicals and reagents used, were obtained from Sigma Chemical Co. (St. Louis, MO).

Statistical Analysis

Values shown are means ± SEM for the number of separate experiments indicated. The statistical

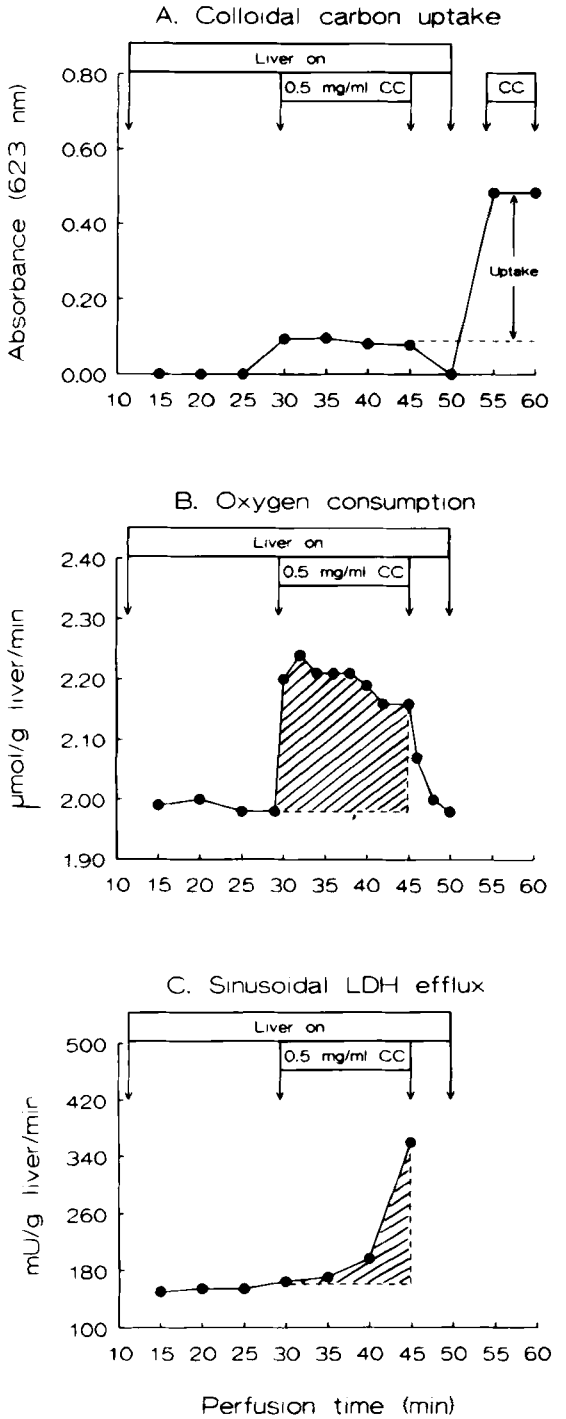

FIGURE 1 Representative study of the effect of colloidal carbon (CC) infusion on uptake of carbon (A) and O_2 (B) and on the sinusoidal lactate dehydrogenase (LDH) efflux (C) by the isolated perfused liver from a control rat.

A) The rate of carbon uptake (1.41 mg/g liver/min) was calculated as described in Methods. B) Carbon-induced O_2 uptake (2.46 µmol/g liver) (and C) total LDH efflux (3.1 U/g liver) were calculated from 30 to 45 min perfusion (shaded area) as described in Methods.

significance of differences between mean values was assessed either by Student's t test for unpaired data or by two-way ANOVA and the Newman-Keuls' test as indicated.

RESULTS

Rat livers perfused in vitro with a colloidal carbon containing buffer exhibited a significant uptake of the particles, with a concomitant respiratory response (Fig. 1). The light microscopy examination of the livers from control rats (Fig. 2A) and T_3-treated animals (Fig. 2B) revealed that most of the carbon particles were present in aggregates in Kupffer cells and to a small extent in the endothelial cells, while they were absent in parenchymal cells. Using this experimental set up, the rate of carbon uptake and the associated O_2 uptake were studied in perfused livers from euthyroid rats as a function of the particle concentration. The rate of colloidal carbon uptake was found to increase in the concentration range of 0.2 to 2.0 mg/ml, exhibiting a sigmoidal-type kinetics with a Vmax of 4.8 mg/g liver/min (Fig. 3A). Half-maximal rates were observed at a carbon concentration of 0.82 mg/ml, as analyzed by the Hill equation (Fig. 3A), which showed two components with Hill coefficients of 1.56 and 8.32 respectively. Carbon-induced O_2 uptake, assessed as the integrated O_2 consumption during carbon stimulation (Fig. 1B), exhibited a hyperbolic-type kinetics, with a Vmax of 4.57 μmol/g liver and a Km of 0.74 mg/ml, calculated by the double-reciprocal plot analysis (Fig. 3B).

Thyroid hormone administration to rats, involving enhanced serum T_3 levels, developed a calorigenic response evidenced by the significant increase in the rectal temperature of the animals, with a drastic enhancement (40%) in the rate of hepatic O_2 consumption, over control values (Table I). These adaptive changes were observed in parallel with a 47% diminution in the content of the major hepatic antioxidant GSH (net decrease of 3.17 μmol/g liver) and a 97% increase in the rate of TBARS formation (net increase of 1.34 nmol/mg protein/h) (Table I). T_3 treatment, however, elicited a 27% decrease in the hepatic content of GSH over control values in animals pretreated with $GdCl_3$ (controls, 6.33 ± 0.60 ($n = 7$) μmol/g liver; T_3-treated rats, 4.62 ± 0.25 ($n = 7$); net decrease of 1.71 μmol/g liver; $P < 0.05$), representing a 46% reduction in thyroid hormone-induced liver GSH depletion. In addition, hyperthyroidism led to a 73% increase in liver TBARS formation compared to control values in rats pretreated with $GdCl_3$ (controls, 0.90 ± 0.11 ($n = 5$) nmol/mg protein/h; T_3-treated rats, 1.56 ± 0.15 ($n = 5$); net increase of 0.66 nmol/mg protein/h; $P < 0.05$), leading to a 50% diminution in T_3-induced liver lipid peroxidation. Thyroid hormone administration resulted in Kupffer cell hyperplasia (Kupffer cell count: 128 ± 4 ($n = 6$) cells/mm^2 in control rats and 179 ± 2 ($n = 6$) in T_3-treated animals; $P < 0.01$ assessed by the Student's t test for unpaired data) and hypertrophy, as revealed by the light microscopy examination of the livers of T_3-treated animals (Fig. 2B), compared to those from euthyroid rats (Fig. 2A), performed after perfusion with colloidal carbon under comparable particle concentration, temperature, and flow rate conditions.

Infusion of different concentrations of colloidal carbon (0.25 to 1.3 mg/ml) into perfused livers from euthyroid rats produced minimal changes in the integrated sinusoidal efflux of LDH, compared to perfusions carried out in the absence of particle stimulation (Fig. 4). In fact, LDH release amounted to 0.2% and 0.9% of the total LDH activity in the liver, at colloidal carbon concentrations of 0.25 and 1.3 mg/ml, respectively. As previously reported,[17,24] in the absence of Kupffer cell stimulation by carbon infusion, T_3-treated rats presented higher values of liver LDH efflux over control values, measured in comparable conditions to those used in carbon perfusion experiments (Fig. 4). In the concentration range of 0.25 to 0.75 mg/ml, car-

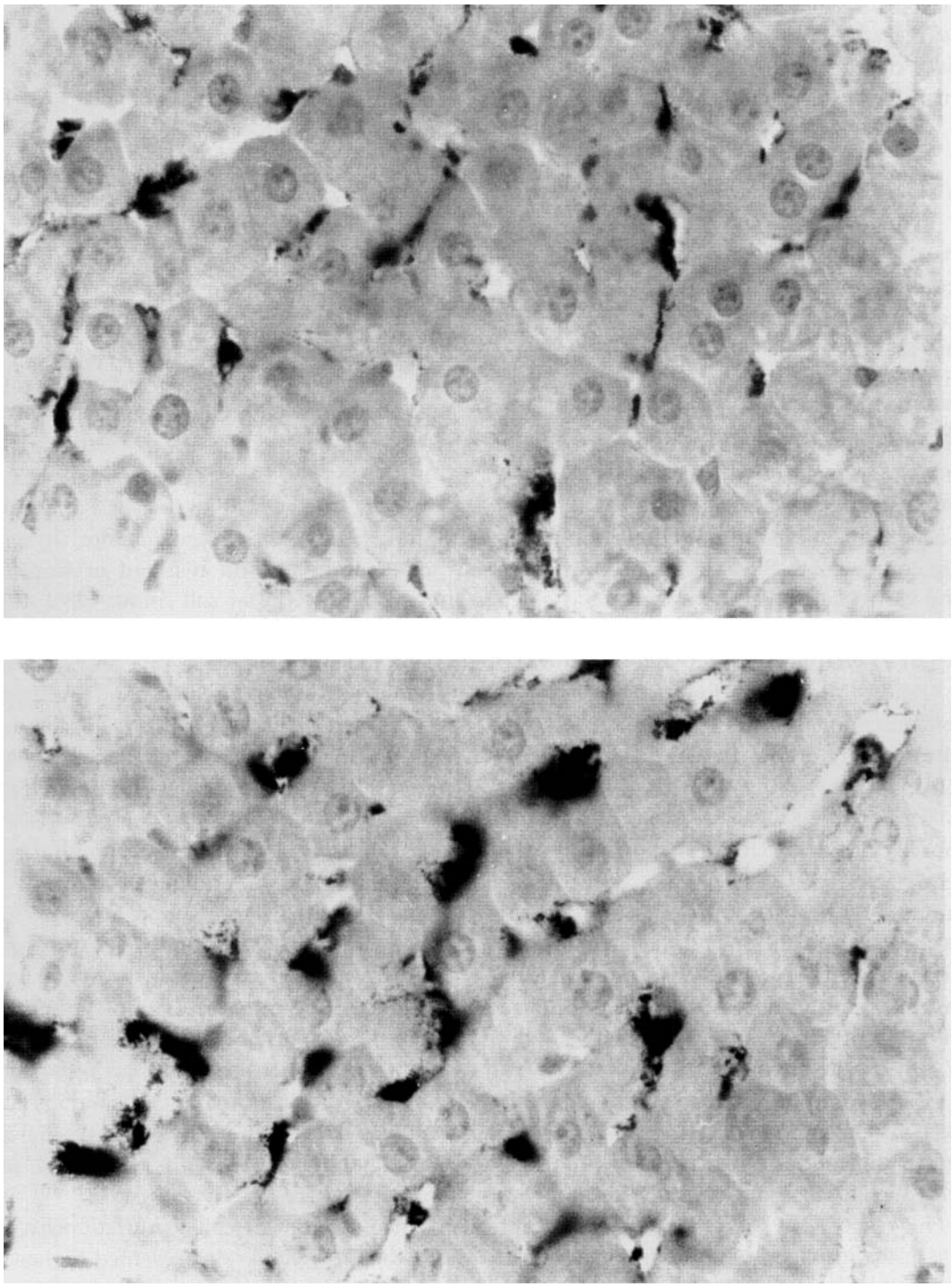

FIGURE 2 Structural characteristics of liver parenchyma in a control rat (A) and in a T_3-treated animal (B), perfused in vitro with colloidal carbon (0.5 mg/ml) at comparable flow rates for 15 min, as described in Materials and Methods and in Figure 1. (hematoxylin-eosin, magnification × 600)

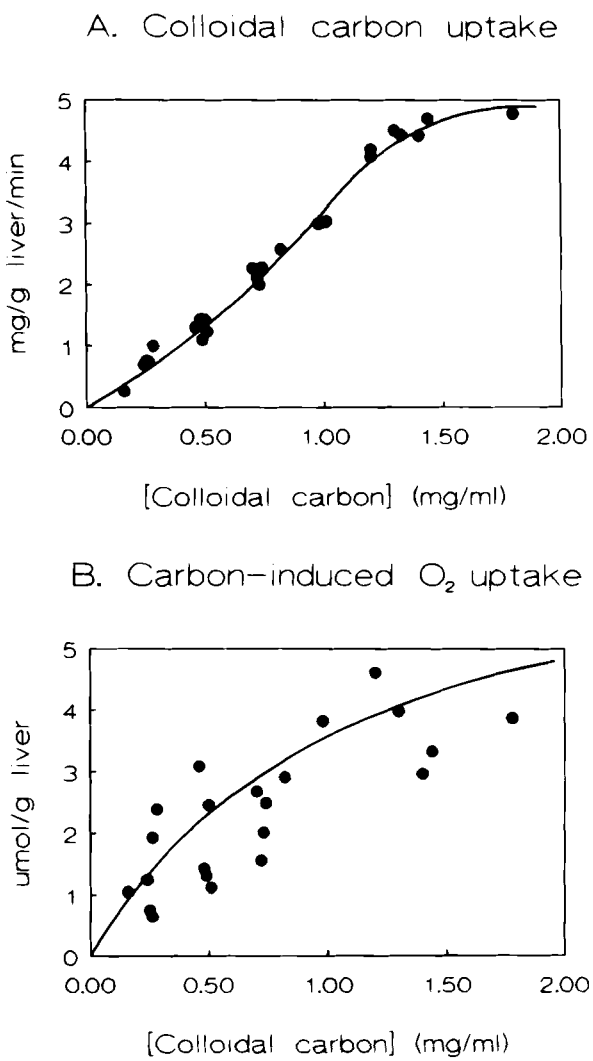

FIGURE 3 Rate of colloidal carbon uptake (A) and carbon-induced O_2 uptake (B) as a function of carbon concentration in the isolated perfused livers from control rats.

Data (n = 25) concerning the rate of carbon uptake (A) were analyzed according to the Hill equation, and revealed two components with Hill coefficients of 1.56 (regression line: Y = 0.136 + 1.56X; r = 0.97: P < 0.001) and 8.32 (regression line: Y = 0.161 + 8.32X; r = 0.97; P < 0.001). Carbon-induced O_2 uptake values (n = 22) (B), calculated as described in Materials and Methods and in Figure 1B, were analyzed according to the Lineweaver-Burk equation (regression line: Y = 0.219 + 0.162X; r = 0.75; P < 0.05).

bon infusion significantly increased LDH efflux by the liver of T_3-treated rats, compared either to control livers perfused with similar carbon concentrations or to livers from hyperthyroid rats perfused in the absence of colloidal carbon (Fig. 4). In fact, LDH release amounted to 7.3, 6.9, and 4.4% of the total hepatic LDH activity, at 0.25, 0.5, and 0.75 mg of carbon/ml, respectively. Livers from hyperthyroid rats perfused with higher carbon concentrations exhibited a further and drastic elevation in LDH efflux, corresponding to 15.8% and 22.3% of the total activity in the tissue, at carbon concentrations of 1.0 and 1.3 mg/ml, respectively (Figure 4). Under these conditions, the initial increase in liver O_2 uptake was followed by a decrease far bellow the basal val-

TABLE I Parameters related to thyroid calorigenesis and liver oxidative stress in control rats and T_3-treated animals

Parameters	Control rats	T_3-treated rats	P
Serum T_3 (ng/dl)	72 ± 5 (6)†	156 ± 14 (6)	0.001
Rectal temperature (°C)	37.9 ± 0.05 (37)	38.8 ± 0.05 (22)	0.05
Liver O_2 uptake (μmol/g liver/min)	1.89 ± 0.03 (31)	2.63 ± 0.07 (14)	0.01
Liver TBARS formation (nmol/mg protein/h)	1.38 ± 0.08 (10)	2.72 ± 0.22 (4)	0.01
Liver GSH content (μmol/g liver)	6.72 ± 0.20 (10)	3.55 ± 0.49 (4)	0.001
TBARS/GSH ratio	0.21 ± 0.01 (10)	0.77 ± 0.07 (4)	0.01

†Results represent the means ± SEM for the number (n) of animals indicated in parentheses. The significance of the differences between mean values was assessed by the Student's t test for unpaired results.

ues observed prior to carbon infusion (data not shown). These observations indicate that excessive Kupffer cell stimulation by high carbon concentrations leads to the loss of liver viability in the hyperthyroid state, probably due the preexisting enhanced oxidative stress status of the organ imposed by thyroid calorigenesis (Table I). In view of these findings, Kupffer cell function in euthyroid and hyperthyroid rats was assessed by carbon infusion into perfused livers in the 0.25 to 0.75 mg/ml concentration range. As can be seen in Figure 5, both the rate of colloidal carbon uptake (Fig. 5A) and the associated O_2 consumption (Fig. 5B) were significantly enhanced by T_3 treatment over control values, at the three carbon concentrations considered. Furthermore, all the increase in colloidal carbon uptake (80%) (Fig. 6A) and in the associated respiratory activity (89%) (Figure 6B) elicited by the infusion of 0.5 mg of carbon/ml in the liver of T_3-treated rats relative to controls was abolished by $GdCl_3$ pretreatment.

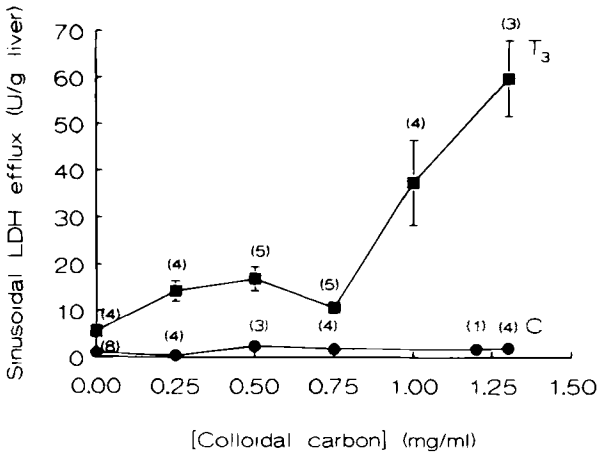

FIGURE 4 Sinusoidal efflux of lactate dehydrogenase (LDH) from isolated perfused livers from control rats (C) and T_3-treated animals (T_3) as a function of carbon concentration.

Values shown correspond to the means ± SEM (n), calculated by integration of the area of the LDH release curves from 15 to 45 min of carbon infusion (Fig. 1C). At all carbon concentrations studied, values for T_3-treated rats were significantly different ($P < 0.05$) compared to both control animals or hyperthyroid rats whose livers were studied in the absence of carbon infusion (zero carbon concentration). At zero carbon concentration, values for control rats were significantly different to those in T_3-treated animals ($P < 0.05$). Hepatic LDH activity measured after perfusion without carbon: controls, 230 ± 12 (n = 8) U/g liver; T_3-treated rats, 229 ± 11 (n = 4). Liver LDH activity in control animals measured after perfusion with 0.25, 0.50, 0.75, and 1.3 mg of carbon/ml, 192 ± 17 (n = 4) U/g liver, 230 ± 22 (n = 3), 292 ± 29 (n = 4), and 213 ± 4 n = 4), respectively. Hepatic LDH activity in T_3-treated rats measured after perfusion with 0.25, 0.50, 0.75, 1.0, and 1.3 mg of carbon/ml, 195 ± 10 (n = 4) U/g liver, 246 ± 11 (n = 5), 241 ± 20 (n = 5), 237 ± 6 (n = 4), and 268 ± 28 (n = 3), respectively.

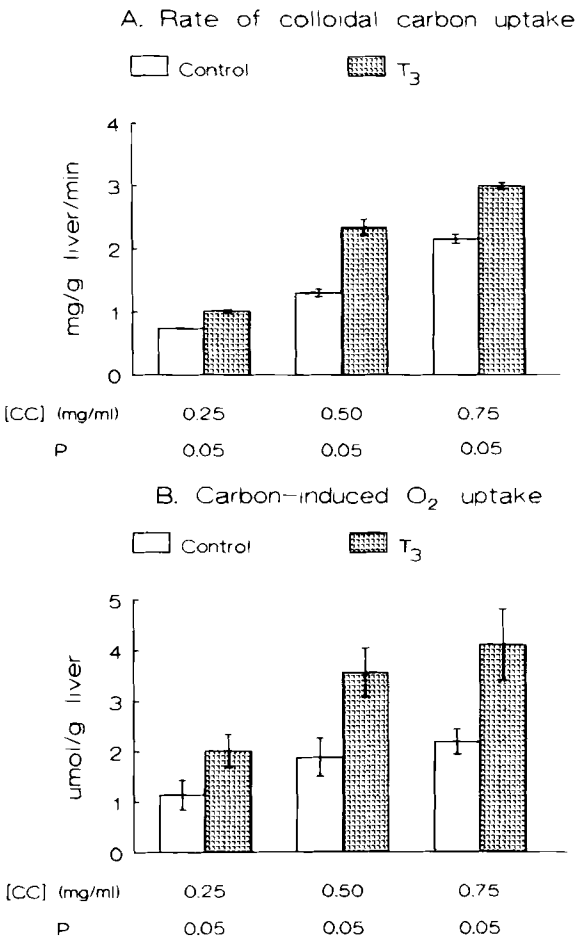

FIGURE 5 Rate of colloidal carbon (CC) uptake (A) and the respective carbon-induced O_2 consumption (B) in isolated perfused livers from control animals and T_3-treated rats.

Values shown correspond to the means ± SEM for 4–5 animals calculated as described in Figure 1A and 1B.

DISCUSSION

Kupffer cell function was assessed by the infusion of colloidal carbon in the isolated perfused rat liver. Carbon particles, characterized by their chemical inertness, are rapidly taken up by liver macrophages with an adequate permanency, which allows their readily visualization.[25] Carbon uptake in this system is known to be mediated through its interaction with specific binding sites present in the plasma membrane of Kupffer cells, with the subsequent internalization of the particles.[4] The process exhibited a sigmoidal saturation curve with two components that suggest the participation of two cellular types. At low concentrations, colloidal carbon is likely to be taken up by a highly phagocytic subpopulation of Kupffer cells, while at high concentrations the process may involve, in addition, endothelial cells and/or different subpopulations of Kupffer cells exhibiting lower phagocytic capacities.[1,26,27] Carbon phagocytosis was found to occur concomitantly with an enhanced O_2 consumption by the perfused liver, parameters that were significantly reduced $GdCl_3$ pretreatment, rare earth metal known to specifically block

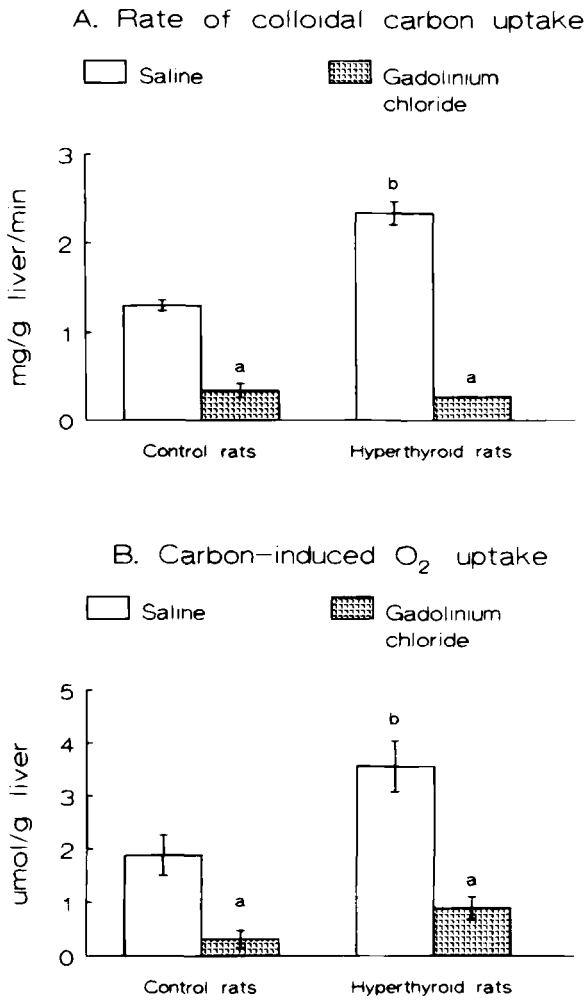

FIGURE 6 Effect of gadolinium chloride ($GdCl_3$) pretreatment on the rate of colloidal carbon uptake (A) and the respective carbon-induced O_2 consumption (B) in isolated perfused livers from control rats and T_3-treated animals.

Rats were given 10 mg/kg $GdCl_3$ as described in Methods. Values shown correspond to the means ± SEM for 4–5 animals per experimental group, calculated as described in Figure 1A and 1B, using a colloidal carbon concentration of 0.5 mg/ml. a) $P < 0.05$ compared to saline pretreatment; b) $P < 0.05$ compared to control rats.

Kupffer cell phagocytosis[28] by selective destruction of liver macrophages.[21] Furthermore, in the range of carbon concentrations employed, the rate of carbon uptake and the associated O_2 consumption were found to be significantly correlated ($r = 0.83$; $P < 0.01$), pointing out the adequacy of the experimental design used to assess these aspects of Kupffer cell function.

Thyroid calorigenesis involving a drastic increase in the rate of O_2 consumption by the liver, led to GSH depletion and enhancement in the rate of TBARS formation, an index indicative of free radical-mediated lipid peroxidative processes. Accordingly, the TBARS formation/GSH content ratio of the liver exhibited a 2.7-fold increase in T_3-treated animals over control values (Table I), parameter that constitutes a partial indication of the oxidative stress status of the liver.[17] The effects of hyperthyroidism on hepatic GSH content and TBARS formation were reduced by 46% and 50% by GdCl pretreatment, respectively, suggesting a significant involvement of Kupffer cells in T_3-

induced liver oxidative stress. In these conditions, T_3 administration to rats significantly enhanced Kupffer cell function, as evidenced by the increased rates of colloidal carbon uptake and the associated O_2 consumption. At the different carbon concentrations used (0.25, 0.5, and 0.75 mg/ml) (Fig. 5) the ratios between the amount of O_2 consumed to that of carbon taken up (calculated by integration of the carbon uptake rates during the 15 min of particle infusion) in control and hyperthyroid rats were comparable, with average values of 0.092 ± 0.010 ($n = 13$) μmol of O_2/mg of carbon and 0.105 ± 0.010 ($n = 14$) in control and T_3-treated rats, respectively. This observation strongly suggest that the enhancement in Kupffer cell function by hyperthyroidism is primarily related to the increased number of the liver macrophages observed histologically, although alternate mechanisms are also possible. In fact, the enhancement in the $GdCl_3$-sensitive O_2 uptake induced by carbon infusion in the liver of T_3-treated animals (Fig. 6B) could represent an increased respiratory burst activity of Kupffer cells conditioned by the induction of the enzymatic processes involved, namely, NADPH oxidase and nitric oxide synthase.[4,9] This suggestion is based on the existence of nuclear binding proteins for thyroid hormone in Kupffer cells,[29] whose interaction is known to enhance the synthesis of enzymatic systems related to redox processes.[30] In line with this view, thyroid hormone-induced respiratory burst in rat polymorphonuclear leukocytes seems to be primarily related to the adaptive increase in NADPH oxidase activity, with the observed higher myeloperoxidase activity playing a contributory role.[31] Also, T_3-induced $GdCl_3$-sensitive liver O_2 consumption may be due to an elevation in the mitochondrial respiration of Kupffer cells[20] for energy supply needed for the enhanced carbon phagocytosis observed (Fig. 5A). In addition to these direct mechanisms that could be exerted at the Kupffer cell level, an indirect stimulatory effect on the O_2 consumption of hepatocytes may occur,[32] possibly mediated by eicosanoids that are effectively released from Kupffer cells activated by colloidal carbon.[4] However, these aspects need to be evaluated in isolated Kupffer cells to gather further insight on the mechanisms responsible for the increased $GdCl_3$-sensitive O_2 uptake observed in the liver of hyperthyroid rats. Thyroid hormone-induced Kupffer cell activity may represent an alternate source of liver ROS generation[8,9] to that induced in parenchymal cells,[11,12] which was suggested to play a contributory role in the increased susceptibility of the liver to the toxic effects of lindane.[17] In addition, activation of Kupffer cells seems to be of importance in the pathogenesis of liver injury after acetaminophen,[33] carbon tetrachloride,[34] and galactosamine[35] intoxication, as well as in that induced by ischemia-reperfusion[36,37] or by chronic alcohol consumption.[38] Conversely, damaged parenchymal cells may produce factors that recruit liver macrophages into the injured areas,[38,39] pointing out to a reciprocal Kupffer cell-hepatocyte interaction in the development and progression of cytotoxicity.

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