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INFLUENCE OF THYROID HORMONE ADMINISTRATION ON HEPATIC GLUTATHIONE CONTENT AND BASOLATERAL γ-GLUTAMYLTRANSFERASE ECTOACTIVITY IN THE ISOLATED PERFUSED RAT LIVER

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Abstract—The effect of thyroid hormone administration on liver glutathione (GSH) content and γ-glutamyltransferase activity in the isolated perfused liver was studied for a period of 1–7 days in fed rats following a single dose of 0.1 mg 3,5,3′-L-triiodothyronine (T_3)/kg. T_3 elicited an early and transient calorigenic response, together with GSH depletion at 1 day after treatment. Recovery of hepatic GSH content and enhancement in total basolateral γ-glutamyltransferase activity occurred in parallel 2–3 days after T_3 treatment, parameters that were normalized in the 4- to 7-day time interval studied. The increase in total basolateral γ-glutamyltransferase activity by T_3 at early times after treatment was due mainly to increments in its transpeptidation mechanism, and was characterized by increments in the apparent maximum velocities without changes in the apparent Michaelis constant (K_m) for the substrate γ-glutamyl-*p*-nitroanilide. Data presented suggest that the elevation in sinusoidal γ-glutamyltransferase activity could be related to the recovery of hepatic GSH content after depletion by T_3 treatment, by supplying the precursors for intracellular GSH synthesis, an effect that seems to be mediated by enhanced synthesis of the enzyme.

Acceleration of energy metabolism in several mammalian tissues represents one of the major functions of thyroid hormones, determining the calorigenic state of the individual [1]. In the liver, enhancement in the rate of oxygen consumption by thyroid hormone administration has been associated with increments in electron flux through microsomal and mitochondrial electron-transport systems [2], with a concomitant increase in the rate of superoxide radical generation in these subcellular fractions [3, 4]. In parallel with these effects, hyperthyroidism in the rat elicited a diminution in some antioxidant protective mechanisms of the hepatocyte [5], inducing an oxidative stress condition in the tissue [2, 3, 5] with a consequent lipid peroxidative response [3, 5–7]. These observations support the contention that the lipid peroxidative potential of tissues is strongly dependent on the extent of the basal metabolic rate of the individuals, which is related to the energy metabolism of the tissues, as previously established for different mammalian species [8].

Depletion of hepatic reduced glutathione (GSH†), a major antioxidant molecule affording intracellular protection [9], is a concomitant of the hyperthyroid state, both in experimental animals [5, 10] and in humans [11]. In the hyperthyroid rat, elevations in the sinusoidal GSH efflux from the liver and in the hepatic capacity to degrade the tripeptide are the major mechanisms involved in the attainment of a low steady state of GSH, as the increased GSH use is not balanced by an elevation in GSH synthesis [12]. The increase in GSH degradation observed in the liver of hyperthyroid rats was evidenced by a marked enhancement in the activity of γ-glutamyltransferase, measured in whole tissue homogenates [12]. This enzymatic activity, which is located primarily in the canalicular membranes of the hepatocytes [13], has recently been demonstrated histochemically in the sinusoidal pole of the liver cell in normal adult rat and guinea pig liver [14]. Furthermore, basolateral γ-glutamyltransferase ectoactivity was shown to be functionally active when assessed in liver perfusion experiments, possibly degrading GSH of plasmatic and hepatic origin [15]. Considering the impermeability of liver cells towards the pre-formed tripeptide [16], sinusoidal γ-glutamyltransferase was suggested to play a role in providing the precursors for intracellular GSH synthesis *de novo*, a role that may be of particular relevance in conditions of GSH depletion [17]. Thus, the experiments described in the present work were conducted to evaluate the recovery of hepatic GSH content after depletion by thyroid hormone administration, in relation to possible changes in γ-glutamyltransferase activity at the basolateral level. For this purpose, GSH content in liver homogenates and sinusoidal γ-glutamyltransferase activity in the isolated perfused liver were measured for a period of 1–7 days in rats given a single dose of 3,5,3′-L-triiodothyronine (T_3).

MATERIALS AND METHODS

Animals. Female Sprague–Dawley rats (Instituto

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† Abbreviations: GSH, reduced glutathione; T_3, 3,5,3′-L-triiodothyronine; γ-GpNA, γ-glutamyl-*p*-nitroanilide; p-NA, *p*-nitroaniline; Gly-Gly, glycyl-glycine; and LDH, lactate dehydrogenase.

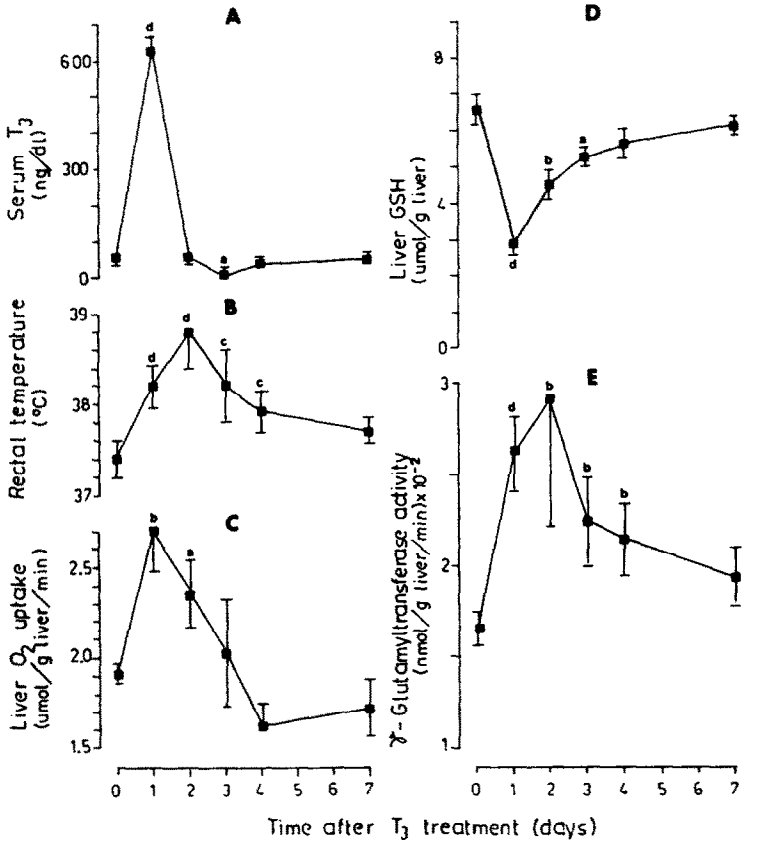

Fig. 1. (A) Serum T_3 levels, (B) rectal temperature of the animals, (C) hepatic oxygen uptake assessed in the isolated perfused liver, (D) liver GSH content, and (E) total γ-glutamyltransferase activity in liver homogenates, after the administration of a single dose of T_3 (0.1 mg/kg) to fed rats and control animals (time zero). Results shown are the means ± SEM for 4–12 rats per experimental time. Statistical significance: (a) $P < 0.05$, (b) $P < 0.02$, (c) $P < 0.002$, and (d) $P < 10^{-5}$.

de Salud Pública, Santiago) weighing 150–270 g were fed *ad lib.* and received a single intraperitoneal injection of either 0.1 mg T_3/kg body weight or equivalent volumes of T_3 diluent (0.1 N NaOH) (controls). Animals were studied 1, 2, 3, 4, or 7 days after hormone treatment, conditions in which the weight of the rats [controls, 220 ± 7 g (N = 28). T_3-treated rats after 1 day, 215 ± 8 (N = 22); 2 days, 218 ± 10 (N = 14); 3 days, 217 ± 9 (N = 15); 4 days, 215 ± 7 (N = 17); 7 days, 222 ± 8 (N = 15)] and the respective liver/body weight ratios [controls, 3.4 ± 0.1 g liver/100 g body wt. T_3-treated rats after 1 day, 3.7 ± 0.2; 2 days, 3.6 ± 0.3; 3 days, 3.8 ± 0.2; 4 days, 3.8 ± 0.3; 7 days, 3.9 ± 0.3] were not significantly different. No significant changes in the weight of control rats and T_3-treated animals were observed in the first 24 hr [at time zero: controls, 220 ± 7 g (N = 28); T_3-treated rats, 219 ± 5 (N = 22). At 24 hr: controls, 219 ± 7 (N = 28); T_3-treated rats, 215 ± 8 (N = 22)], indicating the lack of influence of the hormone treatment on the food intake of the animals. Criteria for assessment of the thyroid status included serum T_3 levels [measured by the GammaCoat™ [^{125}I]T_3 Radioimmunoassay Kit; assay sensitivity limit of 9.0 ng of T_3/dL, intra-assay variation of 3.7% at 33 ng of T_3/dL and 2.5% at 290 ng/dL, and between-assay variation of 4.2%) (Baxter Healthcare Corp., Cambridge, MA)], the rectal temperature (measured with a thermocouple Cole–Parmer 8112-20, Cole–Parmer Instrument Co., Chicago, IL), and the hepatic rate of O_2 consumption (measured polarographically in the isolated perfused liver) [18].

Liver perfusion and basolateral γ-glutamyltransferase activity. Livers obtained from rats under Nembutal anesthesia (50 mg/kg body wt, i.p.) were perfused as described previously [12], using a solution containing 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 D-glucose, equilibrated with an O_2:CO_2 mixture (19:1 v/v) to give pH 7.4, at constant flow rates of 2.9 to 3.8 mL/g liver/min. Perfusions were carried out at a buffer temperature of 35.8 ± 0.2° (N = 29) without recirculation of the perfusate. The assessment of viability of the preparations included the continuous determination of O_2 uptake [18], together with efflux of lactate dehydrogenase (LDH) [19] (one unit of LDH activity represents 1 μmol/min at 25°) and protein [20] into the caval perfusate, measured at 10-min intervals,

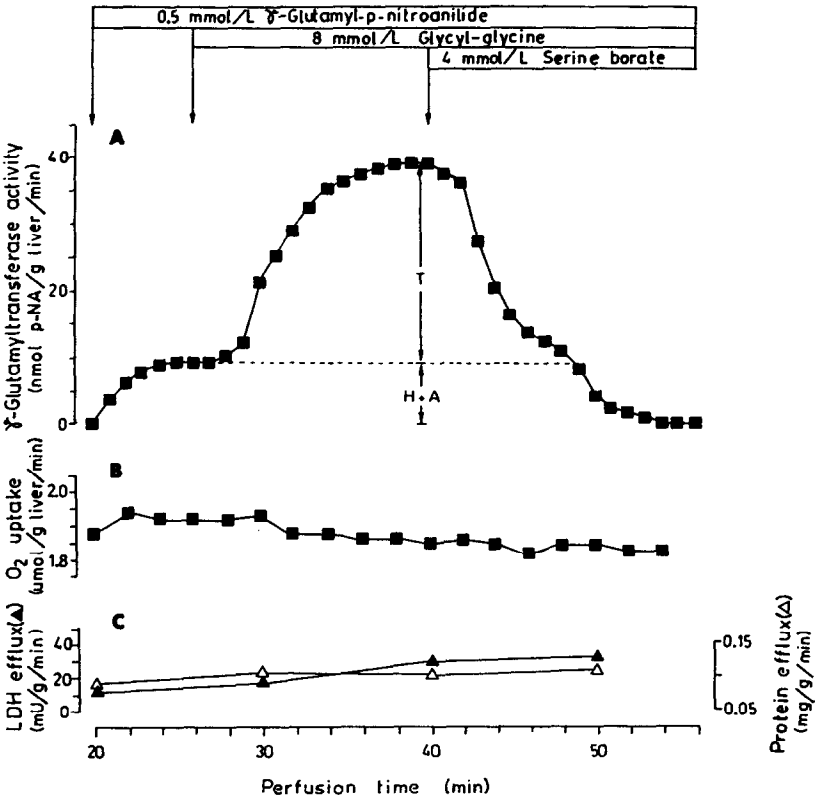

Fig. 2. Representative study of the assessment of (A) γ-glutamyltransferase activity in the isolated perfused liver from a naive fed rat, and the effect of serine borate. Enzyme activity corresponds to the rate of formation of p-NA in the caval perfusate after addition of γ-GpNA alone [representing the reactions of hydrolysis (H) plus autotranspeptidation (A)] or γ-GpNA plus Gly-Gly [representing the transpeptidation reaction (T)]. Assessment of viability of the liver perfusion included measurements of oxygen uptake (B) and sinusoidal efflux of LDH and protein (C).

in relation to the respective values found in the tissue [12]. After an equilibration period of 20 min to allow the recovery of the livers from surgery, the chromogenic γ-glutamyltransferase substrate γ-glutamyl-*p*-nitroanilide (γ-GpNA) (concentration range of 0.08 to 0.83 mM) was infused directly into the portal perfusate by means of a variable-speed infusion pump Sage-351 (Orion Research Inc., Cambridge, MA), at rates corresponding to 1.8 to 9.1% of the perfusion flow. Formation of *p*-nitroaniline (p-NA) was measured spectrophotometrically at 405 nm in caval perfusate aliquots taken every minute, until a plateau was reached. At this time, a buffer containing 8 mM glycyl-glycine (Gly-Gly), an acceptor of the γ-glutamyl moiety of γ-GpNA, was introduced in the perfusion system (void volume of 44 mL, with a transit time of 2.1 min at a flow rate of 20 mL/min), and measurements of p-NA were carried out until a second plateau was obtained (see Fig. 2A). Basolateral γ-glutamyltransferase activity corresponds to the rate of p-NA production (nmol/g liver/min) calculated by multiplying the change in absorbance at 405 nm by the perfusion flow (mL/g liver/min), divided by the absorption coefficient of p-NA (9.9 $cm^2/\mu mol$) [15, 17]. Each liver perfusion allowed the use of 3–5 different γ-GpNA concentrations, and the apparent kinetic parameters V_{max} and K_m were calculated from Lineweaver–Burk plots using linear regression analysis.

Glutathione content and γ-glutamyltransferase activity in liver homogenates. Total GSH equivalents and total γ-glutamyltransferase activity were determined by the methods of Tietze [21] and Tate and Meister [22], respectively, as described elsewhere [12].

Statistical analysis. Results are expressed as means ± SEM. Statistical comparisons were performed by one-way analysis of variance for unequal size groups. All reagents and chemicals used were obtained from the Sigma Chemical Co. (St. Louis, MO).

RESULTS

Administration of a single dose of 0.1 mg T_3/kg to fed rats resulted in a marked elevation in serum T_3 levels 1 day after hormone administration, compared with control animals (time zero; Fig. 1A). Circulating T_3 levels were normalized by 4 days

following treatment, after a significant diminution at 3 days (Fig. 1A), which probably reflects the inhibitory effect of the given T_3 on thyroid-stimulating hormone secretion, exerted at both hypothalamic and pituitary levels [23]. Concomitantly, the rectal temperature of the animals (Fig. 1B) and the rate of O_2 consumption of the liver (Fig. 1C) exhibited similar time-dependent changes, thus confirming the development of an early and transient T_3-induced calorigenic state. In these conditions, T_3 elicited a drastic diminution in the content of hepatic GSH 1 day after treatment, with a subsequent recovery being observed afterwards, leading to values similar to those in control rats in the 4- to 7-day time interval studied (Fig. 1D). In parallel with these changes in liver GSH content, T_3-treated animals exhibited an enhanced total γ-glutamyltransferase activity, measured in liver homogenates, which presented a maximal response at 2 days after treatment, returning towards control values at the end of the experimental period studied (Fig. 1E).

The evaluation of the basolateral activity of γ-glutamyltransferase was performed in the isolated perfused rat liver (Fig. 2A), under suitable conditions of viability of the organ (Fig. 2, B and C). This was evidenced by continuous determination of liver O_2 uptake in the different experimental groups studied, which remained approximately constant through all the perfusion period (see Fig. 2B). Furthermore, average values of fractional sinusoidal efflux of lactate dehydrogenase in the T_3 groups studied were not significantly different from those in control rats (Table 1), whereas fractional protein effluxes in T_3-treated groups were significantly lower than control values (Table 1), thus indicating the adequacy of perfusion [24]. The experimental design employed allowed the estimation of the rate of the hydrolysis plus autotranspeptidation reactions and that of the transpeptidation process, catalyzed by γ-glutamyltransferase. This was achieved by using a continuous and constant influx of the enzyme substrate γ-GpNA to the perfused liver, in the absence and presence of the γ-glutamyl acceptor Gly-Gly, respectively (Fig. 2A), as proposed in earlier studies [15, 17]. In agreement with previous work by Speisky and Israel [25], formation of p-NA by perfused livers from control rats using 0.6 mM γ-GpNA in the presence of 8.0 mM Gly-Gly was abolished by 2.0 mM GSH, added to the portal perfusate (data not shown). These data suggest that, at hepatic GSH levels in the range of 3.0 μmol/g liver (T_3-treated rats 1 day after treatment; Fig. 1D) to 6.6 μmol/g liver (controls rats; Fig. 1D), utilization of γ-GpNA by extrasinusoidal γ-glutamyltransferase activities would be drastically inhibited, with negligible contribution of p-NA to the caval perfusate. In these conditions, formation of p-NA derived from γ-GpNA by the perfused rat liver was suppressed by infusion of serine borate (Fig. 2A), a reversible γ-glutamyltransferase inhibitor [26].

The hydrolytic plus autotranspeptidation activities of γ-glutamyltransferase assayed in perfused livers from control rats and T_3-treated animals at 1–7 days after treatment are shown in Fig. 3. It can be observed that saturation conditions were not achieved in the range of 0.08 to 0.83 mM γ-GpNA, with comparable slope values of the V_0 versus γ-GpNA concentration plots being found for the different experimental groups, as determined by linear regression analysis. In the presence of variable concentrations of γ-GpNA (0.08 to 0.83 mM) at a fixed Gly-Gly concentration of 8 mM, the transpeptidation activity of basolateral γ-glutamyltransferase in perfused livers from controls and T_3-treated rats at 1 day after treatment exhibited Michaelis–Menten-type kinetics (Fig. 4A). Regression analysis of the Lineweaver–Burk plots showed apparent K_m values for γ-GpNA of 0.35 and 0.34 mM in controls and T_3-treated rats, respectively, with apparent V_{max} values of 52.5 and 105.2 nmol p-NA/g liver/min, respectively (Fig. 4A; Table 2). Total basolateral γ-glutamyltransferase activity, comprising the hydrolytic, autotranspeptidation, and transpeptidation reactions, exhibited a similar kinetic pattern (Fig. 4B). The kinetic data obtained for basolateral γ-glutamyltransferase in the different experimental groups indicate that T_3 administration to rats markedly enhanced either the total or the transpeptidation activities of the enzyme for up to 3 days after treatment, returning towards control values at the end of the experimental period studied (Table 2). These findings were observed together with minor changes in the respective apparent K_m values for γ-GpNA, as well as in those of V_{max} for the hydrolytic plus autotranspeptidation reactions, obtained by the difference between the estimated apparent V_{max} values for total and transpeptidation activities (Table 2).

DISCUSSION

GSH plays a key role in detoxification processes in the hepatocyte, acting either as a free-radical scavenger, in the reduction of peroxides, or in the conjugation of electrophilic metabolites of xenobiotics, thus preventing cell injury [16, 27]. In this respect, γ-glutamyltransferase was suggested to be related to cellular protection by its participation in the removal of the γ-glutamyl moiety of GSH-conjugates in the formation of mercapturic acids for further elimination [26, 27], or by allowing the replenishment of hepatic GSH stores after depletion [17]. Data presented in this work show that the administration of a single dose of T_3 to fed rats elicited a depletion of GSH and an enhancement of total γ-glutamyltransferase activity in liver homogenates, in concomitance with the development of a calorigenic state in the animals and increased oxidative capacity of the liver. These results are in agreement with previous data obtained in an animal model using similar doses of T_3 given for up to 3 consecutive days, in which a liver oxidative stress related to thyroid calorigenesis was demonstrated [3, 5, 12].

Recovery of hepatic GSH content in T_3-treated animals was observed at 2–3 days after hormone administration in concomitance with marked increases in total γ-glutamyltransferase activity, assessed in the isolated perfused rat liver (Fig. 5). These findings suggest that an enhanced degradation of GSH by the basolateral activity of γ-glutamyl-

Table 1. Assessment of viability of isolated perfused livers from control rats and T_3-treated animals at different periods after hormone treatment*

Parameters†	Experimental groups					
	Control rats (6)	T_3-treated rats				
		1 day (7)	2 days (4)	3 days (4)	4 days (4)	7 days (4)
LDH efflux (mU/g liver/min)	64.5 ± 5.3	93.7 ± 13.7	82.3 ± 8.7	74.7 ± 10.1	74.4 ± 11.1	63.6 ± 5.2
Liver LDH activity (U/g liver)	295 ± 33	287 ± 26	391 ± 61	349 ± 23	390 ± 26	336 ± 32
Fractional LDH efflux (%/hr)	1.3 ± 0.3	1.9 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.1 ± 0.1	1.1 ± 0.1
Protein efflux (mg/g liver/min)	0.213 ± 0.009	0.057 ± 0.006‡	0.099 ± 0.020‡	0.116 ± 0.042‡	0.092 ± 0.019‡	0.066 ± 0.012‡
Liver protein content (mg/g liver)	193.2 ± 14.4	163.2 ± 11.5	200.3 ± 12.4	197.3 ± 5.7	228.4 ± 71.3	253.0 ± 65.0
Fractional protein efflux (%/hr)	6.6 ± 0.6	2.1 ± 0.2‡	3.0 ± 0.5‡	3.5 ± 1.0‡	2.4 ± 1.2‡	1.6 ± 0.5‡

* Animals were given a single i.p. dose of 0.1 mg T_3/kg or T_3 diluent (controls) and were studied at the indicated days after hormone administration.

† Sinusoidal efflux of LDH or protein was calculated by multiplying the values of LDH activity (mU/mL) or protein concentration (mg/mL) in the caval perfusate by the respective perfusion flow (mL/g liver/min). The corresponding fractional efflux values were estimated by dividing those of the net efflux by the respective LDH activity or protein content in the liver. Values shown are means ± SEM for the number of perfusions indicated in parentheses.

‡ $P < 0.05$, compared with control rats.

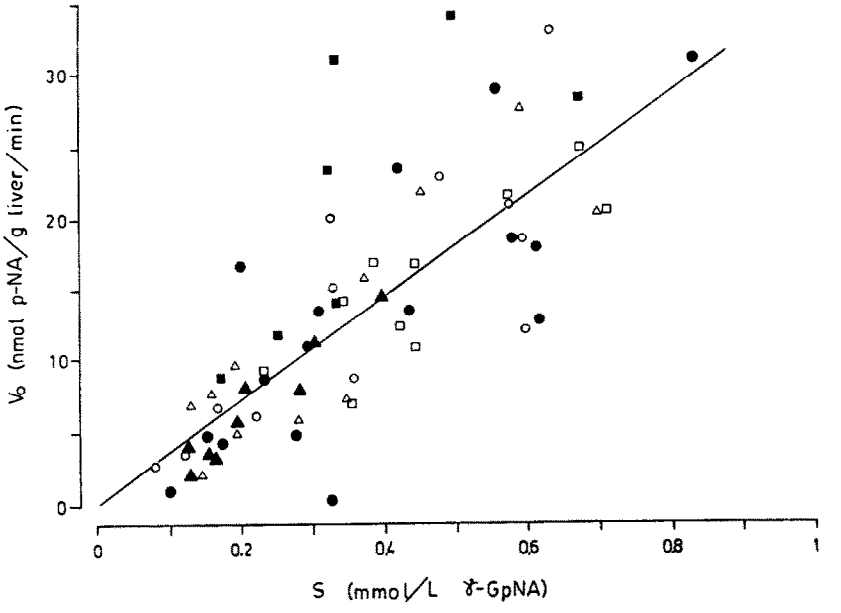

Fig. 3. Basolateral γ-glutamyltransferase activity in the absence of peptide acceptor (hydrolysis plus autotranspeptidation reactions) as a function of substrate, γ-GpNA concentration, in perfused livers from control rats (●) and T_3-treated animals after 1 (○), 2 (■), 3 (△), 4 (□), and 7 (▲) days of hormone administration. Regression line: $Y = 35.6\times$ ($r = 0.77$; $P < 10^{-6}$).

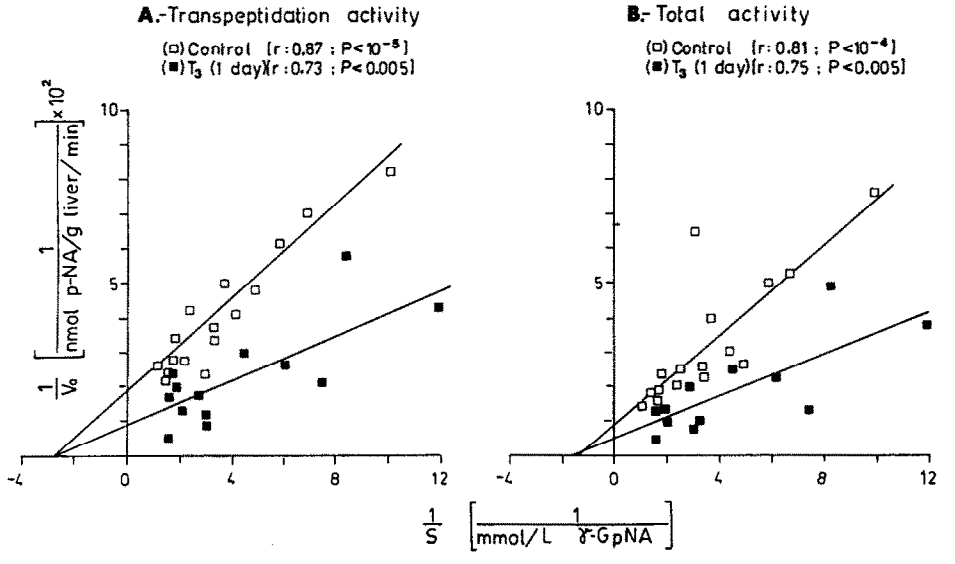

Fig. 4. Lineweaver–Burk plots of basolateral γ-glutamyltransferase activity for the transpeptidation reaction (A) and total activity including hydrolysis, autotranspeptidation and transpeptidation reactions (B), in perfused livers from control rats and T_3-treated animals after 1 day of hormone administration.

transferase may occur in the liver of rats subjected to thyroid hormone treatment, a condition that also determines an increased availability of the tripeptide for the enzyme, as evidenced by the significant increment in the rate of sinusoidal GSH efflux from the liver [12]. The subsequent uptake of the GSH precursors would favor the intracellular synthesis of the tripeptide, thus leading to the normalization of hepatic GSH levels observed at 4–7 days following T_3 administration (Fig. 5). This suggestion is supported by the existence of the required amino acid transport systems in the hepatocyte cell

Table 2. Effect of T_3 administration on the kinetic parameters of basolateral γ-glutamyltransferase in the isolated perfused rat liver*

Experimental groups†	Total activity		Transpeptidation activity		Hydrolytic plus autotranspeptidation activities
	app V_{max}	app K_m	app V_{max}	app K_m	app V_{max}
Control rats	106.4	0.69	52.5	0.35	53.9
T_3-treated rats					
1 day	156.4 (47%)‡	0.56	105.2 (100%)‡	0.34	51.2 (−5%)‡
2 days	169.5 (59%)	0.61	100.0 (91%)	0.36	69.5 (30%)
3 days	182.8 (72%)	0.65	105.3 (100%)	0.37	77.5 (43%)
4 days	124.6 (17%)	0.64	64.7 (23%)	0.37	60.1 (12%)
7 days	110.7 (4%)	0.65	60.5 (15%)	0.42	50.2 (−7%)

* Values of apparent V_{max} (nmol pNA/g liver/min) and apparent K_m for γ-GpNA (mmol/L) were calculated by linear regression analysis of Lineweaver–Burk plots, for hydrolytic (H) plus autotranspeptidation (A) plus transpeptidation (T) reactions (total activity), as well as for the transpeptidation process alone; H + A = total activity − T (see Fig. 2A).
† Animals were studied at the indicated days after T_3 (0.1 mg/kg) treatment or T_3 diluent administration (controls).
‡ Numbers in parentheses correspond to the percentage change relative to control values.

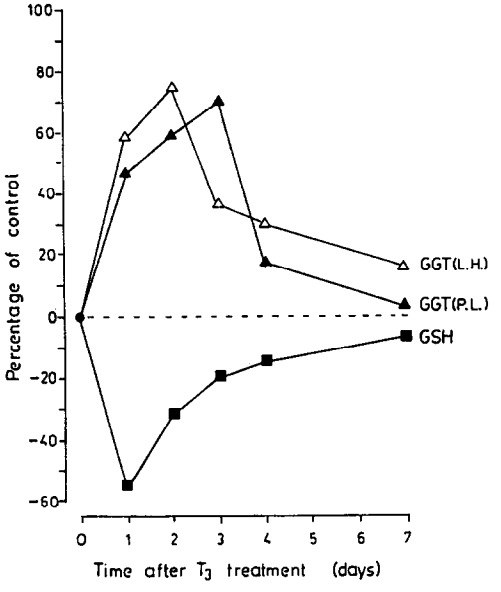

Fig. 5. Time course of the changes in liver GSH content and total γ-glutamyltransferase activity in liver homogenates [GGT(L.H.)] and in the isolated perfused liver [GGT(P.L.)], after T_3 administration in the rat. Values are expressed as percentages of control values [control values: liver GSH content, 6.52 ± 0.37 μmol/g liver (N = 12); total GGT activity in liver homogenates, 165.0 ± 9 nmol/g liver/min (N = 12); total GGT activity in the isolated perfused rat liver, 106.4 nmol/g liver/min (Table 2)].

membrane [28, 29]. In fact, evidence has been provided for the Na^+-dependent uptake of glycine (systems Gly and A), L-glutamate (system for anionic amino acids), and L-cysteine (system ASC) by rat liver plasma membrane vesicles [28, 29]. In addition, recent studies in the perfused rat liver have revealed the existence of two transport systems for L-cysteine, the rate-limiting amino acid for GSH synthesis [27], namely, a high-affinity system in acinar zone III and a low-affinity system uniformly distributed in the hepatic acinus [30]. Although thyroid hormones have been shown to affect amino acid transport systems in different animal tissues [28], their effects on those of the adult rat liver remain to be elucidated.

In the different experimental groups studied, the values of total basolateral γ-glutamyltransferase activity, assessed in liver perfusion experiments, were correlated significantly with those obtained in liver homogenates ($r = 0.77$; $P < 0.05$). At 2 days after T_3 administration, the enhancement in total basolateral γ-glutamyltransferase activity (net increase of 63.1 nmol/g liver/min; Table 2) corresponded to 50% of that observed in the total activity of the enzyme in liver homogenates (net increase of 127.0 nmol/g liver/min; Fig. 1E). This would suggest that the remaining increment could correspond to an enhancement in γ-glutamyltransferase activity at the canalicular level, where the transport systems for L-glutamate [31] and L-cysteine [32] seem to predominate in relation to the basolateral domain of the liver cell. The enhancement in total basolateral γ-glutamyltransferase activity elicited by T_3 at early times after administration was due primarily to increments in the transpeptidation mechanism, rather than to changes in the hydrolytic and autotranspeptidation processes, catalyzed by the enzyme. Furthermore, the changes in the apparent V_{max} of the total and transpeptidation activities of basolateral γ-glutamyltransferase found in the liver of T_3-treated rats at different times after hormone treatment were observed together with comparable values in the apparent K_m for γ-GpNA, which were similar to that in control animals. These findings suggest that the enhancement in basolateral γ-glutamyltransferase activity found in the liver at early times after T_3 administration is related to an induction phenomenon triggered by the hormone, as seen after chronic alcohol consumption in the rat [25]. In the liver, the level of γ-glutamyltransferase is known to be modulated by development, xenobiotics, various

pathological situations, and glucocorticoids [33]. However, further studies on the molecular biology of the enzyme under the influence of thyroid hormones are required, to understand the mechanism of the adaptive increase observed.

In summary, thyroid hormone administration induced an enhancement of the basolateral activity of γ-glutamyltransferase in rat liver, which was correlated with the recovery of hepatic GSH levels after depletion of the tripeptide. The effect was due mainly to an increase in the transpeptidation activity of γ-glutamyltransferase and seemed to be mediated by enhanced synthesis of the enzyme. This increment in liver sinusoidal γ-glutamyltransferase activity could be of importance as a mechanism of cell protection against T_3-induced oxidative stress [2–7, 12], by converting circulating GSH into its constituent amino acids for the synthesis of intracellular GSH, either in the liver or in extrahepatic tissues [34].

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