Retinoic acid-induced modulation of rat liver transglutaminase and total polyamines *in vivo*

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The effect of a single intraperitoneal injection of retinoic acid on liver transglutaminase (EC 2.3.2.13) activity and total putrescine, spermidine and spermine was studied. The results demonstrate that: (1) transglutaminase activity is increased over control values as early as 4–6 h after treatment, reaching a maximum (2-fold increase) at 12 h and returning to control values at 36 h; (2) the retinoic acid-induced form of enzyme is the soluble tissue transglutaminase; (3) actinomycin D treatment does not completely inhibit the early (6 h) increase of activity, while suppressing that at 12 h; (4) the immunoassay of the soluble transglutaminase shows that, 6 h after treatment, there is no increase in the protein, whereas at 12 and 24 h a significant increase is observed; (5) putrescine, but not spermidine and spermine, increases (5–7-fold) 6 and 18 h after the retinoic acid treatment. The possibility also that the expression of soluble transglutaminase is modulated *in vivo* by retinoic acid and the relationship to polyamine levels are discussed.

INTRODUCTION

Transglutaminases are a class of enzymes that catalyse a Ca^{2+} -dependent acyl-transfer reaction in which γ -carboxamide groups of peptide-bound glutamine residues serve as acyl donors (Folk, 1980). In contrast with their limited donor-substrate specificity, transglutaminases have a broad specificity for acceptor substrates. Primary amino groups of several compounds may function as acceptor substrates *in vitro*, leading to the covalent cross-linking of proteins via ϵ -(γ -glutamyl)lysine isopeptide linkages or to the covalent incorporation of biogenic amines (polyamines and histamine) into proteins (Folk & Finlayson, 1977; Folk, 1980; Lorand & Conrad, 1984; Beninati *et al.*, 1985; Fesus *et al.*, 1985).

Transglutaminase activity has been demonstrated in various cells and in biological fluids (Folk, 1980). Extracellular transglutaminases play important roles in the stabilization of proteins by ϵ -(γ -glutamyl)lysine cross-links in reactions such as fibrin-clot polymerization and formation of the seminal plug in rodents (Folk & Finlayson, 1977; Folk, 1980; Lorand & Conrad, 1984; Williams-Ashman, 1984). The physiological role of intracellular transglutaminases is still poorly understood, and in only few cases have the natural substrates for these enzymes been identified (Rice & Green, 1979).

The intracellular membrane-bound transglutaminase has been shown to play an important role in the stabilization of plasma membrane during the terminal differentiation of keratinocytes (Peterson & Wuepper, 1984; Lichti & Yuspa, 1985). In this process the enzyme catalyses the covalent cross-linking of soluble cytoplasmatic substrates into a large polymer, the so-called 'cornified envelope' (Rice & Green, 1979; Thacher & Rice, 1985). Moreover, a high- M_r substrate for the membrane-associated liver transglutaminase has been identified in rat liver plasma membrane (Slife *et al.*,

1986). The biological roles and the substrates of the soluble form of the enzyme, 'tissue transglutaminase', which is largely distributed in eukaryotic cells (Chung, 1975), are completely unknown.

It is not known how the different forms of intracellular transglutaminases are related to each other or whether they are the product of the same gene. Catalytic and immunogenic differences for the soluble and particulate enzymes have been demonstrated (Lichti *et al.*, 1985; Thacher & Rice, 1985). The sensitivity to vitamin A and its derivatives is particularly interesting (Lichti & Yuspa, 1985; Rubin & Rice, 1987). The intracellular level of tissue transglutaminase is highly enhanced upon exposure of different cell lines, both normal and neoplastic, to retinoic acid (Scott *et al.*, 1982; Murtaugh *et al.*, 1983; Davies *et al.*, 1985; Maddox & Haddox, 1985; Mehta & Lopez-Berestein, 1985), whereas the level of epidermal particulate enzyme is not increased and, in some cases, is even inhibited after retinoid treatment (Thacher *et al.*, 1985; Jetten & Shirley, 1986).

In an attempt to address the above points further, we have studied the expression of soluble rat liver 'tissue transglutaminase' *in vivo* after intraperitoneal injection of vitamin A and its derivative, retinoic acid. Since we have recently demonstrated the presence of diamines and polyamines covalently bound to protein in rat liver (Beninati *et al.*, 1985; Piacentini & Beninati, 1988) and that the level of total putrescine correlates with changes of transglutaminase activity (Piacentini *et al.*, 1986a,b), the effect of these compounds on total diamine and polyamine levels has also been monitored.

EXPERIMENTAL

Chemicals

[1,4(n)- ^3H]Putrescine dihydrochloride (26.3 Ci/mmol) was from Amersham International (Amersham, Bucks.,

U.K.). Instagel II was from Packard (Zurich, Switzerland). Dithiothreitol, unlabelled putrescine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride, *NN'*-dimethylcasein and bovine serum albumin were purchased from Fluka (Buchs, Switzerland). All-*trans*-retinoic acid, retinol and actinomycin D were from Sigma (St. Louis, MO, U.S.A.). Other chemicals were of reagent grade and used without further purification.

Animals

Male Wistar rats, weighing 250–300 g, were used. The animals were kept at 20–22 °C on a 12 h-light/12 h-darkness regime and fed *ad libitum* with a standard diet. Rats were injected with 20 mg of retinoid/kg dissolved in olive oil. At different time intervals the rats were anaesthetized with sodium thiopental (Farmotal; Farmitalia, Milan, Italy; 100 mg/kg) and the livers were perfused *in situ* with cold 0.25 M-sucrose through the portal vein. The homogenate (25%, w/v) was prepared in 0.25 M-sucrose, pH 7.0, containing 1 mM-EDTA. In some experiments subcellular fractionation was performed as follows. The whole homogenate was centrifuged at 600 *g* for 10 min; after removing the supernatant, the resulting pellet was washed twice by rehomogenizing it in the same medium and by re-centrifuging at 600 *g* for 10 min. The final pellet was suspended in 0.25 M-sucrose containing 1 mM-EDTA, pH 7.0 (N), whereas the pooled 600 *g* supernatant (E) was centrifuged at 150 000 *g* for 90 min in a 50Ti rotor (LS-50 Beckman ultracentrifuge). The supernatant was collected (S) and the resulting pellet was resuspended in 0.25 M-sucrose, pH 7.0, containing 1 mM-EDTA (P). All manipulations were carried out at 0–4 °C.

Enzyme assays and chemical determinations

Transglutaminase activity was measured by the method of Folk & Cole (1966), with some modifications, by detecting the incorporation of [³H]putrescine into *NN'*-dimethylcasein. The incubation mixture contained 50 mM-Tris/HCl buffer, pH 8.3, 5 mM-CaCl₂, 10 mM-dithiothreitol, 30 mM-NaCl, 2.5 mg of *NN'*-dimethylcasein/ml, 0.2 mM-putrescine, containing 1 μ Ci of [³H]-putrescine and 0.2–0.5 mg of protein in a final volume of 0.3 ml. After 20 min of incubation at 30 °C in a shaking water bath, 100 μ l of the incubation mixture was spotted on to a Whatman 3MM filter paper moistened with 20% trichloroacetic acid. The incorporation of putrescine into *NN'*-dimethylcasein was proportional to the amount of the homogenate protein up to 0.6 and 1.0 mg for the soluble and particulate fractions respectively. Free [³H]putrescine was eliminated by washing with large volumes of cold 5% (w/v) trichloroacetic acid containing 0.2 M-KCl, and filters were transferred into counting vials containing 5 ml of Instagel. The concentration of transglutaminase protein in homogenates was determined by a recently developed sandwich enzyme-linked-immunosorbent-assay (e.l.i.s.a) system (Fesus & Arato, 1986) using purified guinea-pig liver transglutaminase as standard. The assay is specific for tissue transglutaminase and sensitive enough to quantify nanogram amounts of the enzyme in the presence of unrelated proteins in concentration of several milligrams per ml. One enzyme unit is defined as the amount of enzyme that incorporates 1 nmol of putrescine to *NN'*-dimethylcasein/h. Marker-enzyme activities for different

subcellular fractions were assayed as previously reported (Sartori *et al.*, 1983). Protein was determined as described by Lowry *et al.* (1951), with bovine serum albumin as standard.

Total putrescine, spermidine and spermine were determined as dansyl derivatives in 2 ml samples of liver total homogenate after hydrolysis in 6 M-HCl for 16 h at 110 °C. The dansylation procedure was essentially that described by Seiler (1970), with 1,6-diaminohexane as internal standard and toluene instead of benzene to extract the derivatives from the reaction mixture. The chromatography was performed as described by Van Rooijen *et al.* (1984) on a 4.6 mm $\times$ 250 mm column packed with Hypersil ODS (5 μ m particle diameter), with a Beckman h.p.l.c. apparatus equipped with a 450 data system/controller.

RESULTS

In order to study the effect *in vivo* of vitamin A and its derivative, retinoic acid, on transglutaminase, we choose the rat liver as an experimental model on the basis of the following considerations: (i) liver is known to have very high level of transglutaminase activity, of which the soluble form accounts for more than 85% (Barnes *et al.*, 1985; Piacentini *et al.*, 1986a); (ii) in the liver the transglutaminase reaction products, ϵ (γ -glutamyl)lysine and γ -glutamyl di- and poly-amine derivatives, have been demonstrated (Beninati *et al.*, 1985; Fesus *et al.*, 1987; Piacentini & Beninati, 1988); (iii) liver is the main organ concerned with the metabolism and storage of retinoids (Blomhoff *et al.*, 1984, 1985). Fig. 1 shows the time course of rat liver transglutaminase activity after the intraperitoneal injection of retinol or retinoic acid. No difference between control and retinol-treated animals was observed at any time interval tested. Statistically significant increases in total transglutaminase activity were detected in the livers of retinoic acid-treated rats. In fact, the transglutaminase specific activity is increased

Fig. 1. Effect of retinoids on rat liver transglutaminase

Animals were injected with olive oil alone (control; ●) or oil containing retinoic acid (20 mg/kg; ○) or retinol (20 mg/kg; □) and killed at the indicated time intervals. Rat livers were perfused with, and homogenized in, 0.25 M-sucrose containing 1 mM-EDTA. Enzymic assays and total protein assays were carried out on aliquots of total homogenates as outlined in the Experimental section. Values are the means of duplicate determinations on six different groups of animals ($\pm$ S.D.). Significance: **P* < 0.01 versus control.

Table 1. Effect of retinoic acid on the intracellular distribution of transglutaminase, marker enzymes and total protein in rat liver

Animals were intraperitoneally injected with olive oil alone (control, 'C') or plus retinoic acid (20 mg/kg, 'RA') and killed 12 h later. Subcellular fractionation of liver homogenates and total protein and enzymic assays were carried out as reported in the Experimental section. Fractions: E, cytoplasmic extract, i.e. supernatant after the 600 g pellet; N, 600 g for 10 min pellet; P, 150 000 g for 90 min pellet; S, 150 000 g for 90 min supernatant. Absolute values are given as mg/g wet wt. of liver for protein and as units/g for enzyme activities. Values are the means ($\pm$ S.D.) of duplicate determinations in three different groups of animals.

Enzyme or protein	Values ...	Absolute E + N	Percentage			Recovery (%)
	Fraction ...		N	P	S	
Protein						
C		180.7 $\pm$ 21	54.0 $\pm$ 2	24.1 $\pm$ 3	20.4 $\pm$ 3	100.8 $\pm$ 9
RA		173.4 $\pm$ 24	56.6 $\pm$ 5	28.3 $\pm$ 6	20.8 $\pm$ 3	106.1 $\pm$ 8
Glucose-6-phosphatase						
C		19.5 $\pm$ 4	19.5 $\pm$ 2	74.6 $\pm$ 8	6.0 $\pm$ 1	101.0 $\pm$ 5
RA		23.0 $\pm$ 4	20.5 $\pm$ 2	73.7 $\pm$ 7	5.8 $\pm$ 3	100.5 $\pm$ 7
5'-Nucleotidase						
C		8.0 $\pm$ 0.2	47.0 $\pm$ 4	51.8 $\pm$ 2	1.1 $\pm$ 0.2	100.0 $\pm$ 6
RA		8.6 $\pm$ 0.4	48.7 $\pm$ 5	50.5 $\pm$ 3	1.5 $\pm$ 0.2	102.0 $\pm$ 4
Alkaline phosphatase						
C		0.3 $\pm$ 0.02	21.0 $\pm$ 5	46.8 $\pm$ 4	23.0 $\pm$ 6	92.0 $\pm$ 7
RA		0.3 $\pm$ 0.07	31.2 $\pm$ 5	46.1 $\pm$ 2	15.3 $\pm$ 4	94.4 $\pm$ 6
Acid phosphatase						
C		6.0 $\pm$ 1	15.3 $\pm$ 2	65.2 $\pm$ 9	16.9 $\pm$ 1	99.6 $\pm$ 3
RA		6.1 $\pm$ 1	23.6 $\pm$ 2	68.0 $\pm$ 5	11.0 $\pm$ 1	102.4 $\pm$ 8
Transglutaminase						
C		392.0 $\pm$ 49	35.8 $\pm$ 4	6.0 $\pm$ 1	70.0 $\pm$ 9	111.0 $\pm$ 9
RA		880.5 $\pm$ 55	18.5 $\pm$ 3	4.5 $\pm$ 1	84.7 $\pm$ 9	108.9 $\pm$ 9

Table 2. Effect of actinomycin D (ACT) at different dosages on retinoic acid (RA)-induced rat liver transglutaminase activity

Animals were treated as indicated in Table 1 and killed 12 h later. In the experiments with actinomycin D, rats were first injected with actinomycin D (200 μ g/kg) and 6 h later with a further dose of actinomycin D alone or plus retinoic acid. Control animals were injected with olive oil alone. Values are the means ($\pm$ S.D.) for four experiments.

Treatment	Transglutaminase activity (nmol/h per mg of protein)
Control	3.2 $\pm$ 0.7
RA (20 mg/kg)	6.0 $\pm$ 0.8
ACT (400 μ g/kg)	2.0 $\pm$ 0.4
ACT (200 μ g/kg) + RA	3.3 $\pm$ 0.8
ACT (400 μ g/kg) + RA	2.8 $\pm$ 0.7
ACT (600 μ g/kg) + RA	2.4 $\pm$ 0.6

(about 75%) over the control values as early as between 4 and 8 h after treatment. The highest activity is observed 12 h after treatment, with an increase of 100% with respect to the corresponding control values. The activity is still higher than controls until 24 h, reaching control values at 36 h. The retinoic acid-induced effect is therefore reversible and dose-dependent (results not shown).

Since two forms of transglutaminases have been described in rat liver, namely the soluble 'tissue transglutaminase' and the particulate enzyme localized in the

plasma membrane (Barnes *et al.*, 1985; Slife *et al.*, 1986), the next step in the present study was to characterize which form is induced by the retinoic acid treatment. Table 1 reports the subcellular distribution of liver transglutaminase activity and of some enzymes, marker of cell organelles, in control (C) and retinoic acid-treated (RA) animals 12 h after treatment. Data clearly indicate that retinoic acid-induced activity is largely (85%) recovered in the soluble phase of liver cell lysate, suggesting that it likely represents the 'tissue transglutaminase'. This notion is further supported by the observation that the increased activity was paralleled by higher concentration of enzyme protein (Table 3), detected by using an e.l.i.s.a. system specific for tissue transglutaminase (Fesus & Arato, 1986). The slight increase in the absolute activity in the N and P fractions, which mainly contain the particulate form of transglutaminase, may be explained on the basis of contamination by the soluble form of the enzyme. In fact these fractions had not been washed before the assay, so that small amounts of absorbed or loosely bound soluble enzymes could still have been present. However, definite conclusions, excluding activation of the particulate form of transglutaminase, cannot be drawn.

It is noteworthy that no significant difference is present for other enzymic activities tested, either in terms of absolute values or subcellular-distribution patterns. Moreover, no morphological alteration in liver cells on retinoic acid treatment has been observed by studies utilizing both optical and electron microscopy (results not shown).

The evidence that the intraperitoneal injection of retinoic acid determines an increased level of soluble transglutaminase activity in liver cells raises the question

Table 3. Time course of the effect of actinomycin D on the level of rat liver transglutaminase induced by retinoic acid treatment

Animals were intraperitoneally injected with olive oil alone (control) or oil containing retinoic acid (20 mg/kg); in the experiments with actinomycin D, rats were first injected with actinomycin D (200 µg/kg) and 6 h later with a second dose of actinomycin D (400 µg/kg) alone or plus retinoic acid (20 mg/kg). After the latter injection the animals were killed at the reported time intervals. Abbreviations: n.d., not determined; *a*, transglutaminase specific activity (nmol/h per mg of protein); *b*, transglutaminase amount (ng/mg of protein). Data are means of duplicate determinations on three different animals.

Treatment	Time after treatment (h) ... Parameter ...	Transglutaminase level					
		6		12		24	
		<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>	<i>a</i>	<i>b</i>
Control		2.1	4.0	3.2	5.1	2.5	5.3
Actinomycin D		1.5	n.d.	2.0	3.5	2.4	n.d.
Retinoic acid		3.7	4.2	6.0	13.2	4.3	10.3
Actinomycin D + retinoic acid		2.4	n.d.	2.5	3.0	3.0	n.d.

Fig. 2. Effect of retinoic acid on rat liver putrescine, spermidine and spermine levels

Animals were injected intraperitoneally with olive oil alone (control; ●) or oil plus retinoic acid (20 mg/kg; ○) and killed at the indicated time intervals. Aliquots (2 ml) of total liver homogenates were hydrolysed in 6 M-HCl for 16 h at 110 °C. Total di- and poly-amine analysis was performed by an h.p.l.c. procedure as described in the Experimental section. (a) Putrescine; (b) spermidine; (c) spermine. Values are the means of four determinations in different groups of animals ($\pm$ S.D.). Significance * $P < 0.01$ versus control.

of whether this activity is due to activation of the enzyme already present in the organ or to synthesis *de novo*. In order to address this question, enzyme activity was measured in animals exposed to different doses of actinomycin D. Table 2 shows that the induction of tissue transglutaminase 12 h after treatment is prevented by exposing liver cells to actinomycin D. The retinoic acid-induced increase of transglutaminase was completely abolished by actinomycin D at concentrations greater than 200 µg/kg body wt. In Table 3 the action of actinomycin D on the retinoic acid-induced transglutaminase at different time intervals after treatment is shown. The amount of liver transglutaminase protein, quantified by an e.l.i.s.a. method utilizing specific anti-(tissue transglutaminase) antibodies, is also shown. It is noteworthy that, 6 h after retinoic acid treatment, the increased transglutaminase activity is not paralleled by an increase of enzyme protein. Thus it seems that an activation of pre-existing enzyme molecules may mediate the early response to retinoic acid treatment. At 12 h the parallel increase of both activity and transglutaminase protein together with the inhibition of the enzyme level in the presence of actinomycin D demonstrate that, at this time, retinoic acid elicits neosynthesis of tissue transglutaminase in liver cells. Moreover the decrease (about 40%) in enzyme protein 12 h after actinomycin D treatment suggests that, *in vivo*, the half-life of liver transglutaminase is slightly longer than 12 h. Finally, the results obtained at 24 h indicate that the induction of an increased transglutaminase level is still apparent, although at this time actinomycin D seems less effective as an inhibitor of the phenomenon.

Since polyamines have been demonstrated to be natural substrates for liver transglutaminase (Beninati *et al.*, 1985) and their level varies under conditions where fluctuations in transglutaminase activity occur (Piacentini *et al.*, 1986a,b; Schindler, 1986), we investigated the time-course behaviour of total putrescine, spermidine and spermine contents in the liver of retinoic acid-treated animals (Fig. 2). Two definite increases (5- and 7-fold) in total putrescine concentration (6 and 18 h after the injection of retinoic acid) were observed. A less evident variation in spermidine content was found. There was no change in spermine concentration.

DISCUSSION

The data reported here clearly demonstrate that a single intraperitoneal injection of retinoic acid to rats induces a significant increase of liver transglutaminase activity. The same effect is not observed with retinol. This effect of retinoic acid has been already observed in a variety of cell lines (Scott *et al.*, 1982; Murtaugh *et al.*, 1983; Davies *et al.*, 1985; Lichti *et al.*, 1985; Maddox & Haddox, 1985; Jetten & Shirley, 1986; Mehta & Lopez-Berestein, 1986; Rubin & Rice, 1986; Goldman, 1987). However, this is the first report showing that such a modulation also takes place *in vivo*. Our data indicate that the induced activity is the soluble form of the enzyme 'tissue transglutaminase', as previously demonstrated in a large number of cell cultures (Davies *et al.*, 1985; Mehta & Lopez-Berestein, 1986; Rubin & Rice, 1987) and in many cell types (Scott *et al.*, 1982; Birckbichler *et al.*, 1983; Davies *et al.*, 1985; Korner & Bachrach, 1985; Mehta & Lopez-Berestein, 1986; Murtaugh *et al.*, 1986). The bulk of the increase in transglutaminase is due to neosynthesis of the enzyme protein, as evidenced by the experiments with actinomycin D and the immunoassay of the specific protein. However, an early activation of the enzyme seems to take place during the first few hours after retinoic acid injection. Transglutaminase activation phenomena have been previously reported in Chinese-hamster ovary and melanoma cells stimulated by retinoid treatments (Scott *et al.*, 1982) and in glioma cells treated with isoprenaline (Korner & Bachrach, 1985).

Despite the fact that the effects of retinoids have been studied intensively both *in vivo* and *in vitro*, there still exists considerable uncertainty regarding the mode of their action as well as their plasmatic transport and cellular uptake [for reviews, see Lotan (1980) and Sherman (1986)]. There is also some question as to whether retinoic acid may be generated intracellularly from retinol, although some cell lines have been shown to convert retinol to retinoic acid (Sherman, 1986). On the basis of retinol failing to induce liver transglutaminase, it is possible to hypothesize that either intraperitoneally injected retinol does not penetrate liver cells or retinol is not converted into retinoic acid in liver cells. That retinoic acid is the active form leading to the induction of the enzyme *in vivo* finds support also from experiments *in vitro* (Scott *et al.*, 1982; Davies *et al.*, 1985). The effect of retinoic acid on liver soluble transglutaminase seems not to be due to unspecific alterations of cellular organelles (i.e. plasma membrane, lysosomes) or of liver metabolism, since neither morphological nor biochemical analysis indicates any appreciable difference from control animals. Thus the induction of liver transglutaminase seems to be a specific response to retinoic acid, as hypothesized from cell-culture studies (Davies *et al.*, 1985). It is noteworthy that, although the basal level of transglutaminase is high in liver cells, retinoic acid elicits a 2-fold increase in enzyme. Similar increases have been detected on exposure, to this vitamin A derivative, of cell lines whose basal enzyme level may be considered elevated (specific activity over 0.5 nmol/h per mg of protein) (Scott *et al.*, 1983; Birckbichler *et al.*, 1983). These cells show a biphasic response to retinoic acid treatment, with both activation and neosynthesis of transglutaminase and a similar time course with respect to what we observed concerning liver transglutaminase (Scott *et al.*, 1982; Korner & Bachrach, 1985).

It is well known that liver contains many different cell types, i.e. parenchymal, endothelial, Kupffer and stellate cells (Blomhoff *et al.*, 1985). At present we do not know whether all these cells are involved in the response to retinoic acid. Thus it is not possible to exclude the possibility that a dramatic increase in macrophage-like cells, such as Kupffer cells, might account for the 2-fold increase detected in liver total homogenate. In fact, in mouse peritoneal macrophages and in rat bone-marrow-derived mononuclear phagocytes, retinoic acid induces a very high enzyme activity, which can reach levels 100-fold greater than those in unstimulated cells (Moore *et al.*, 1984; Goldman, 1985).

Although retinoids have been reported to inhibit ornithine decarboxylase activity, so far the number of reports in which the effects of these compounds have been directly correlated with changes in polyamine metabolism is very limited (Schindler, 1986). Retinoid treatment of mouse epidermal (Piacentini *et al.*, 1986b) and HL60 cells was seen to be accompanied by a large increase in putrescine levels, which, in the latter instance, does not seem to be mediated by a concomitant increase of ornithine decarboxylase activity (Huberman *et al.*, 1981). This evidence, together with the notion that polyamines are natural substrates for liver transglutaminase (Beninati *et al.*, 1985; Piacentini & Beninati, 1988), have prompted us to investigate the behaviour of the total polyamine content in rat liver on retinoic acid treatment. Our data clearly show that the intraperitoneal injection of retinoic acid induces large increases in the putrescine concentration in the liver, with peaks 6 and 18 h after treatment. This finding is in accordance with previous observations *in vivo*, where parallel increases in the transglutaminase activity and the total putrescine content without any concomitant elevation of ornithine decarboxylase activity, were observed (Piacentini *et al.*, 1986a). At present it is not possible to relate causally these time-dependent increases of both transglutaminase activity and putrescine, although it is tempting to hypothesize an influence of polyamine levels on the expression of tissue transglutaminase.

In conclusion, the data reported here indicate that retinoic acid is a powerful inducer of tissue transglutaminase expression in rat liver. Our present knowledge of retinoid action is inadequate in enabling us to fully understand the role that such regulation may have in different aspects of cell behaviour. However, the intriguing evidence that retinoids elicit similar effects on transglutaminase both *in vivo* and *in vitro* confirms the modulation of this enzyme as a selective and rapid response of cells to retinoic acid.

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